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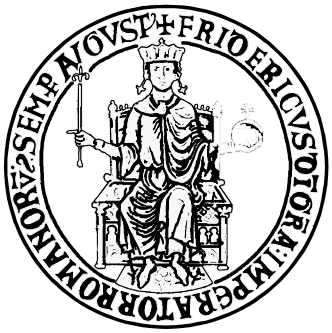

PHARMACOLOGICAL CHARACTERIZATION OF PARACETAMOL: NEW THERAPEUTIC APPROACH TO POSTOPERATIVE PAIN

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"Nessuno che ti abbia preso del tempo si sente tuo debitore.
Eppure il tempo è l'unica cosa che nemmeno l'uomo più generoso
del mondo sarà mai in grado di restituirti."
(Seneca)

Alla mia famiglia

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LIST OF ABBREVIATIONS:

- Adenosin triphosphate (ATP)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Arachidonic acid (AA)
- Aspartate aminotransferase (AST)
- Bradykinin (BK)
- Calcitonin – gene- related – peptide (CGRP)
- Central nervous system (CNS)
- Chronic postsurgical pain(CPSP)
- Cicloxygenase (COX)
- Cyclooxygenase 2 (COX-2)
- Cytochrome P (CYP)
- Dorsal root gangliar (DRG)
- Endovenous (IV)
- Fatty acid amide hydrolase (FAAH)
- Glutamate (Glu)
- International normalized ratio (INR)
- Intracerebroventricular (ICV)
- Intramuscolar (IM)
- Intrathecal (IT)
- Leukotrienes (LTs)
- N-acetyl-p-benzoquinone imine (NAPQUI)
- Nerve growth factor (NGF)
- Neurokinin A (NA)
- Neutral endopeptidase (NEP)
- Nitric oxide (NO)
- N-methyl- D- Aspartate (NMDA)
- Non steroidal antinflammatory drugs (NSAD)
- No-observed-adverse-effect level (NOAEL)

- Noradrenaline (NA)
- Norepinephrine (NE)
- Oral (OS)
- Paracetamol for oral administration (PARA)
- Patient-controlled analgesia (PCA)
- Peroxidase (POD)
- Phospholipase A_2 (PLA_2)
- Prostacyciclins (PGI_2)
- Prostaglandin G_2 (PGG_2)
- Prostaglandin H_2 (PGH_2)
- Prostaglandins (PGS)
- Serotonin (5- HT)
- Substance P (SP)
- Supersaturated aqueous solution of paracetamol for spinal administration (SIN)
- Thromboxanes (TXS)
- World health organisation (WHO)

ABSTRACT

Acetaminophen, commonly known as paracetamol, is an active ingredient possessing analgesic and antipyretic activity widely used in medical practice to alleviate acute and chronic pain and to reduce the body temperature when this exceeds physiological values. Paracetamol, conversely to the majority of commonly used analgesic drugs, is not an NSAIDs, since it is completely devoid of antiaggregant and anti-inflammatory activity.

The most common pharmaceutical form is the solid one as tablet, granule form or suppositories. Moreover, solution containing paracetamol for IV infusion can also be found on the market. These are formulations indicated for short-term treatment of medium pain, in particular of the type experienced following a surgical intervention. IV administration is reserved for cases in which is needed to treat pain and/or hyperthermia urgentely or when other administration routes are not available. Paracetamol administration by alternative methods is still yet to be extensively explored and essentially no specific applications have been found in analgesic therapy.

Acute postoperative pain is a normal response to surgical intervention and is a cause delayed recovery and discharge after surgery as well as increased risk of wound infection and respiratory/cardiovascular complications. Untreated acute

pain leads to reduced patient satisfaction and increased morbidity and mortality and also places a burden on the patient and health system finances. Acute pain that becomes intractable and persists is considered as CPSP. CPSP can have a significant impact on the patient's quality of life and daily activities, including disturbances of sleep and affective mood. In clinical field, paracetamol is principally used as an antipyretic in the treatment of febrile states. Recently, much attention was focused on spinal administration of paracetamol, in order to overcome the possible hepatotoxicity after oral administration. The administration of injectable solutions by spinal administration generally presents limitations. First restriction is that drug is perfused in a defined and confined space in which a limited amount of solution can be infused.

Our aim has been to verify effect of a new supersaturated aqueous solution of paracetamol (SIN) at different doses (100-500 μg/it) after IT administration in an animal model of postoperative pain. Mechanical hyperalgesia was evaluated by mechanical stimuli using the Randall-Selitto analgesimeter for rats. Hyperalgesia was assessed on incised paw 2, 4, 24, 48, 72 h after spinal administration.

Data showed that SIN administration produced a significant antihyperalgesic effect, in dose- and time- manner. In particular, the highest dose (500 μg) produced a significant analgesic effect until 72 h after surgery.

Moreover, knowing the marked analgesic effect of paracetamol following oral administration, and considering the use of this drug as a premedication before surgery, we investigated the combination of oral and spinal routes administrations of PARA and SIN using inactive and active doses (PARA 200 and 500 mg/kg and SIN 100 and 500 µg respectively). Surprisingly, a synergic effect was obtained after oral and intrathecal combination of inactive doses; in fact PARA 200 mg/kg/os and SIN 100 µg/it produced a prolonged analgesic effect up to 24 h after administration.

Despite its medical use is consolidated by many years, paracetamol mechanism of action is still poorly understood. Our results indicated that in paracetamol-induced analgesia cannabinergic, opioidergic and serotoninergic systems are involved.

Finally, it is well known that orally high doses of paracetamol could cause perilobular hepatotoxicity, which is the main limit to use this drug, especially in fasting patients before chirurgical surgery. It very poor the knowledge about the possible toxicity of paracetamol after intrathecal administration. We examined if single or repeated SIN administration by spinal catheter showed physiological and/or morphological modification of cauda equina or nerve bundles of the lumbosacral spinal cord sections. Both acute (500µg) or repeated (200-500 µg

for 7 days) administration of SIN resulted in a mild degree of toxicity with little or no degeneration of nerve fibers and there was no difference between vehicle- and SIN-treated rats. Furthermore, we observed macroscopically, whether SIN administration for 7 days produced liver toxicity. No significant alteration of margins and sizes was observed in vehicle- and SIN-treated rats.

In conclusion, during this my PhD, we evaluated the pharmacological and toxicological profile of a new supersaturated aqueous solution of paracetamol; our data confirm the efficacy of this drug in a postoperative pain model, offering a new therapeutic approach based on its spinal administration.

1 - PAIN PERCEPTION

Pain is a complex and polyhedric universe, it is difficult to view only through a definition; it is a perception of pain that can also profoundly influence lives of patients.

Recognition and management of pain continues to be one of the most commonly encountered clinical situations for practitioners. Pain has a considerable impact on biological, psychological, sociological and economical welfare of patient that cannot be underestimated. On a global scale pain impact has far to reaching effects upon social structure, function and economic welfare of society as a whole (Breivik H. et. al; 2006). Pain medicine has evolved over recent years into a large specialty area, being recognized as its own discipline within Australia in 2005.

Pain is the sensation that warns about a possible or real damage to tissues. We use the word pain to denote any sensation that hurts. Yet there are several distinct types of pain, which have different mechanisms and biological functions.

Unpleasant sensation of hurt, discomfort, or distress acts in two main ways: one, is a useful response of the organism, an early-warning system that promotes survival in a hostile and dangerous environment, and two, is an expression of

pathological change in nervous system. Former pain is beneficial, or “good” pain, while latter is “bad” pain, responsible for causing persistent suffering in millions of patients, with a substantial cost to society due to lost work, disability, and medical expenses (Clifford J. et al. 2000). More precisely, the International Association for the Study of Pain defines pain as “*an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*” and underlies that pain “*is unquestionably a sensation in a part or parts of the body, but it is also always unpleasant and therefore also an emotional experience*” (Macintyre PE. et al. 2010).

Pain is vital to avoid dangerous situations, protect the human body and allow healing processes to occur. Due to its importance as key mechanism of body defence and protection, pain has evolved as an intricate interplay between sensory and cognitive mechanisms, distinct from the classical senses: it is inherently variable and multifaceted, it is a discriminative sensation, an affective motivation, a potent autonomic drive and a reflexive motor stimulus (Craig A. D. 2003; Perl E.R. 2011). Unlike other senses such as vision, hearing, and smell, pain has an urgent and primitive quality, a quality responsible for the affective and emotional aspect of pain perception. Moreover, the intensity with which pain is felt is affected by surrounding conditions, and the same stimulus can produce

different responses in different individuals under similar conditions (Tracey I. et al. 2007; Wiech K. et al. 2008).

Pain cannot be described as a sensory phenomenon, but it has been considered as a composition of two part, the first defined “nociception”, which allows the reception and transport of stimuli to CNS, that are harmful for the organism, and a part of the experiential (the real experience of pain), which is the mental state of the perception of an unpleasant sensation.

Only in Europe, for example, epidemiological studies have revealed the 19% of population suffers from chronic pain, with a greater prevalence in women or in adult aged between 41 and 50 years (figure 1). Furthermore, it has been reported a varying prevalence rates for this chronic condition among counties, ranging from 12 to 30% (figure 1.1), but also within the same country. In Italy, for example, the prevalence is above 32% in the northern part of the country and less than 22% in the southern part (Breivik H. et al. 2006).

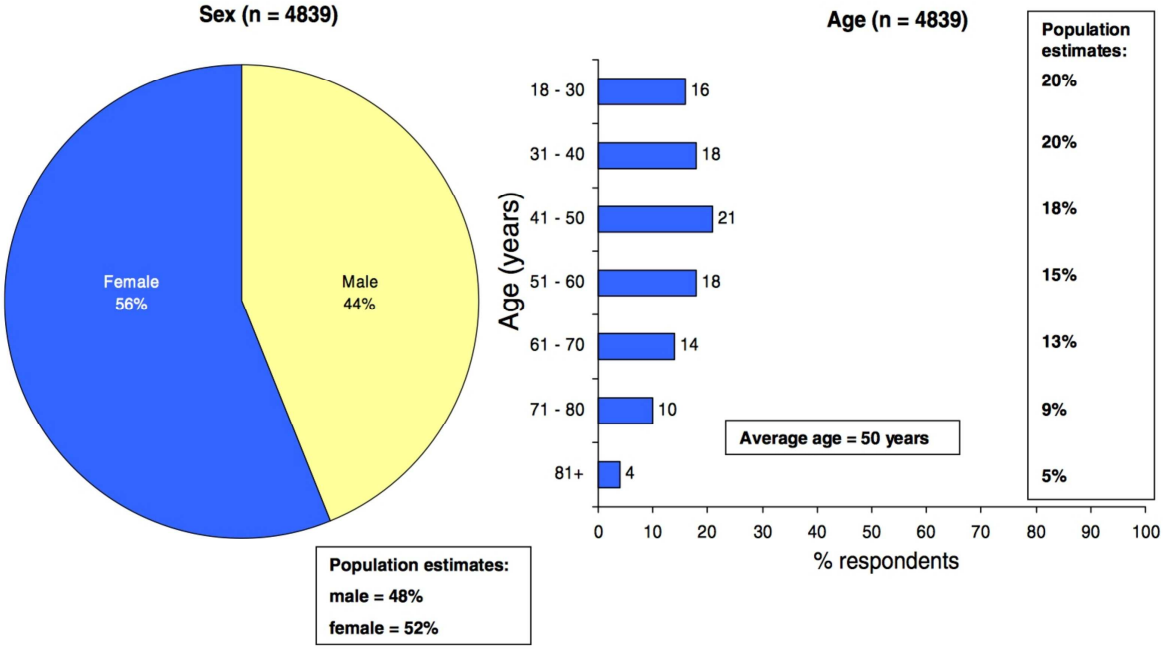


Fig.1 Age and sex of 4839 responders suffering from chronic pain as described under Fig. 1. Population estimates are from US Census Bureau International Database (IDB), Summary of Demographic Information. October 2002.

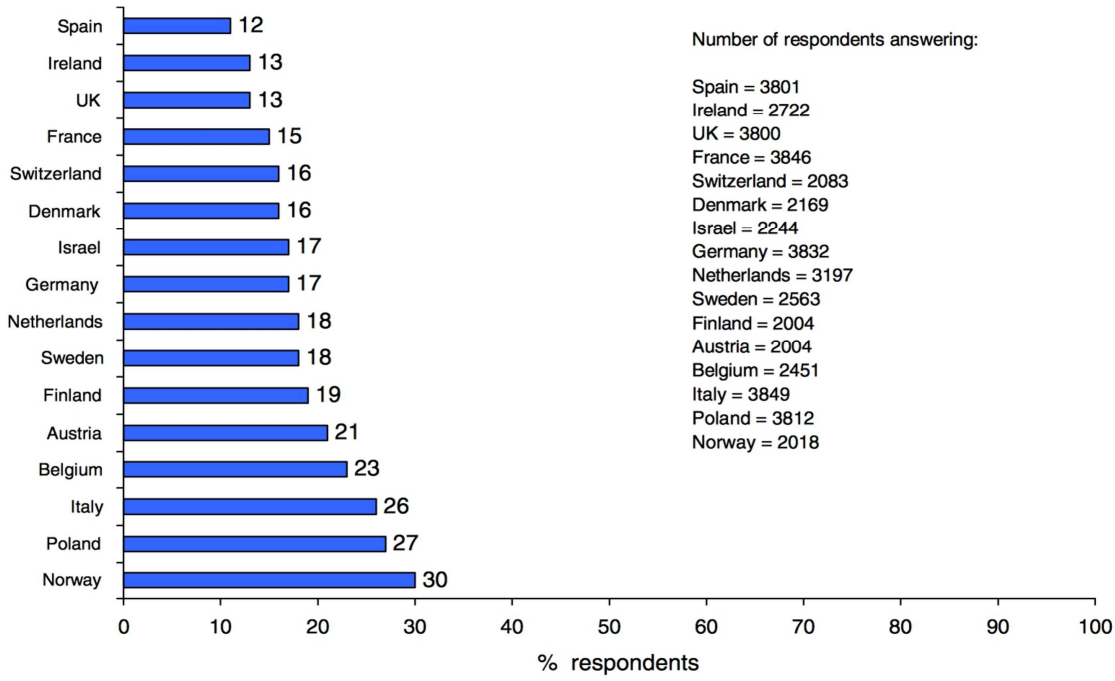


Fig. 1.1 Prevalence of chronic pain among 46,394 adults (>18 years) in 15 European countries and Israel responding to a computer-aided telephone screening interview. Chronic pain was defined as pain lasting more than 6 months, having pain during the last month, several times during the last week, and last experienced pain having an intensity 5 or more on a Numeric Rating Scale: 1 (no pain) to 10 (worst pain imaginable).

The needs of chronic pain sufferers are still largely unmet, creating an enormous emotional and financial burden to sufferers, careers and society. Improvements in our ability to diagnose chronic pain and develop new treatments are needed, together with robust and less subjective "readouts" of pain experience. Brain imaging techniques have provided novel insights into functional, anatomical and chemical changes in the human nervous system that allow to define new approaches that may assist current drug development efforts. Our knowledge about mechanisms of pain perception, especially in pathological conditions, is still far from be clear and complete, as well the processes involved in fine modulation of pain perception to adapt the appropriate behavioural responses to the surrounding environment are not completely understood (Borsook D. et al. 2006; Borsook D. et al. 2007; Borsook D. et al. 2011).

Multiple options are available for the clinical management of pain, most of which are usually pointed on pharmacological therapy. Use of these medications and the literature surrounding them, can often be conflicting, confusing and poorly understood. As an area of medicine there are continuous attempts to develop more effective analgesics that are easy to administer, safe and economically viable. As we continue to deepen our understanding of pain physiology, it can be hoped that this will allow for further research and development into treatments

that can improve quality of life for both the individual and society as a whole (Stephan A. et al. 2014)

The research for new treatments of pain is important for several reasons:

- to understand basic mechanisms of pain and neurobiological phenomena related to it;
- for clinical research, to formulate more accurate and efficient therapeutic drawings for patients;
- for public health, to reduce the costs of pain therapy;
- to improve the quality of life of suffering patients.

1.1 – PainTransmission

Pain is a complex experience that involves not only the transduction of noxious environmental stimuli, but also cognitive and emotional processing of brain. Progress has been made in identifying cortical loci that process pain messages, but far greater advances have been made in understanding the molecular mechanisms whereby, primary sensory neurons detect pain-producing stimuli, a process referred to nociception. These insights have predominantly arisen from the analysis of sensory systems in mammals, as well as from studies of invertebrates. Of course, invertebrate organisms do not experience pain *per se*,

but they have transduction mechanisms that enable them to detect and avoid potentially harmful stimuli in their environment.

These signalling pathways can be regarded as the evolutionary precursors of nociceptive processing in vertebrates, and genetic studies have facilitated the identification and functional characterization of molecules and signalling pathways that contribute to detection of noxious stimuli in animals (David J. et al. 2001).

Nearly a century ago, Sherrington proposed the existence of the nociceptor, which has the task of recognizing pain stimuli, which can be chemical, mechanical or thermal. A primary sensory neuron is activated by stimuli capable of causing tissue damage (Sherrington C. et al. 1906). According this model, nociceptors have characteristic thresholds or sensitivities that distinguish them from other sensory nerve fibers. In fact, electrophysiological studies have shown, the existence of primary sensory neurons that can be excited by noxious heat, intense pressure or irritant chemicals, but not by innocuous stimuli such as warming or light touch (Burgess P.R. et al. 1967).

Pain is unique among sensory modalities in that electrophysiological recordings of single primary sensory fibers have been made in awake humans, allowing simultaneous measurement of psychophysical responses when regions of the

head and body are stimulated (Weidner C et al. 1999). Fibers that innervate regions of the head and body arise from cell bodies in trigeminal and DRG, respectively, and can be divided into three main groups based on anatomical and functional criteria (Fig. 1.2).

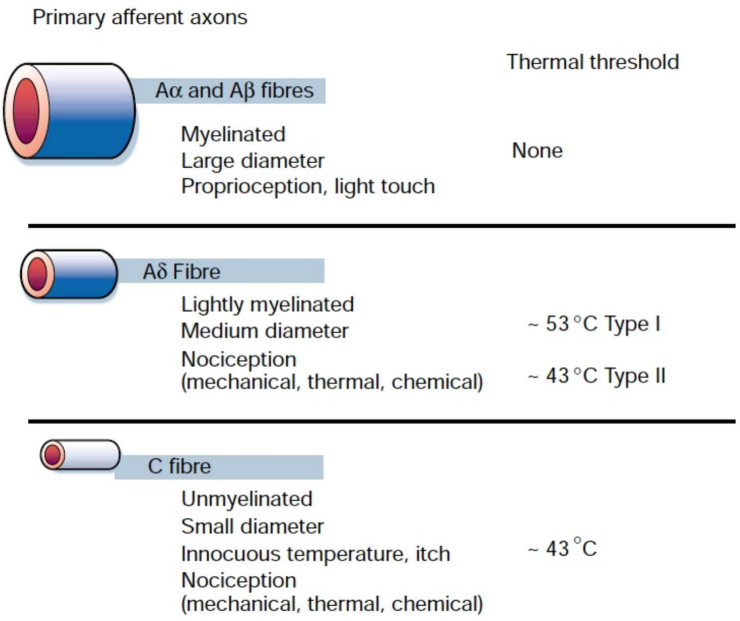


Fig.1.2-Different nociceptors detect types of pain. Peripheral nerves include small-diameter (Aδ) and medium-to large diameter (Aα, β) myelinated afferent fibres, as well as small-diameter unmyelinated afferent fibres (C).

Fibers type *A* have a large diameter, are myelinated, and have the highest conduction velocity of all nerves in the body. Myelin is a fatty white substance that surrounds the axon of some nerve cells, forming an electrically insulating layer and is essential for the right functioning of nervous system. The main purpose of a myelin layer (or sheath) is to increase the speed at which impulses

propagate along the myelinated fiber. Along unmyelinated fibers, impulses move continuously as waves, but, in myelinated fibers, they "hop" or propagate by saltatory conduction. Myelin decreases capacitance and increases electrical resistance across the cell membrane. Thus, myelination helps to prevent the electric current from leaving the axon. It has been suggested that myelin permits larger body size by maintaining agile communication between distant body parts (Hartline DK., 2008). Most, but not all *A* fibers, detect innocuous stimuli applied to skin, muscle and joints and thus do not contribute to pain. Indeed, stimulation of large fibers can reduce pain, as occurs when they are activated by rubbing hand (Djouhri L. et al. 1998). By contrast, small- and medium-diameter cell bodies give rise to most of nociceptors, including unmyelinated slowly conducting ***C* fibers** and thinly myelinated, more rapidly conducting **Aδ fibers**, respectively. It has long been assumed that A and C nociceptors mediate 'first' and 'second' pain, respectively namely rapid or acute pain and delayed, more diffuse, dull pain evoked by noxious stimuli (Basbaum A. et al. 2000).

There are two main classes of A nociceptors (Raja S.N. et al. 1999): Aβ and Aδ, both responding to intense mechanical stimuli, but can be distinguished by their differential responsiveness to intense heat or tissue injury. Most of C fiber nociceptors are polymodal, responding to thermal and mechanical noxious

stimuli, while others are mechanically insensitive, but respond to noxious heat (Raja S.N. et al. 1999).

Spinal dorsal horn receives sensory information from primary afferent Aδ and C fibers after nociceptive stimuli (Figure 1.3) (Braz J. et al. 2014 ;Todd AJ. et al. 2010; Prescott SA. et al. 2014). Terminals of C and Aδ fibers are concentrated in the superficial dorsal horn, and activate projection neurons and excitatory interneurons (Figure 1.3). On the contrary, the terminals of Aβ fibers are concentrated in the deeper dorsal horn, and mainly target excitatory and inhibitory interneurons (Figure 1.3) and projection neurons that are in the same area. Although Aβ fibers polysynaptically link to projection neurons in the superficial dorsal horn, the link is considered to be normally strongly repressed by inhibitory interneurons. Therefore, under normal conditions, Aβ fibers do not activate nociceptive projection neurons, not provoking pain.

Unraveling the mechanisms of pain hypersensitivity caused by nerve damage is therefore essential for the development of new therapeutic drugs for neuropathic pain.

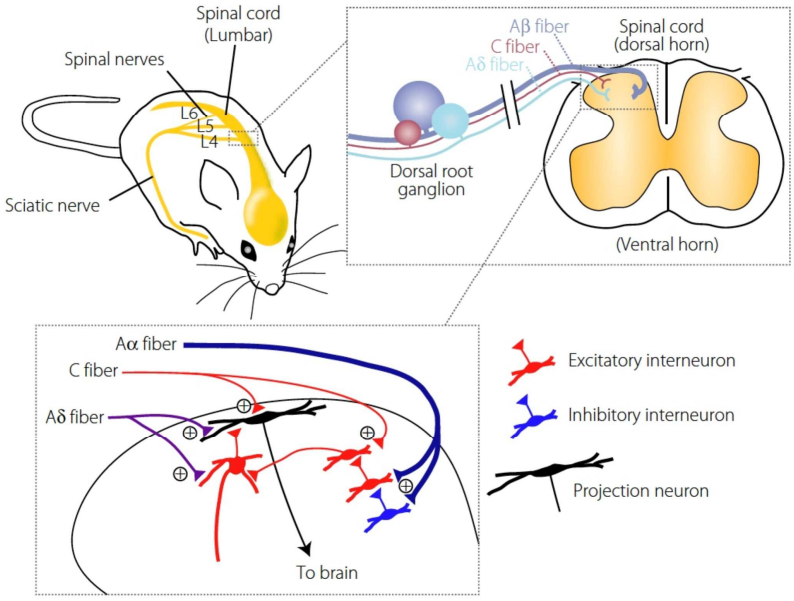


Fig 1.3 Schematic illustration of primary afferent sensory fibers and neuronal circuits in the dorsal horn. The dorsal root ganglion contains cell bodies of primary afferent neurons that transmit sensory information from the periphery to the spinal dorsal horn. Nociceptive information is mainly mediated by Aδ and C fibers, and innocuous mechanical information is mediated by Aβ fibers. C and Aδ fibers terminate in the superficial dorsal horn, and activate projection neurons and excitatory interneurons. The terminals of Aβ fibers are concentrated in the deeper dorsal horn, and connect to excitatory and inhibitory interneurons.

1.2 Neurochemistry of nociceptors

All sensory systems must convert environmental stimuli into electrochemical signals. Nociception is unique because individual primary sensory neurons of 'pain pathway' have the remarkable ability to detect a wide range of stimulus modalities, including those of physical and chemical nature. Compared with sensory neurons of other systems, nociceptors have therefore be equipped with a diverse repertoire of transduction devices. At the same time, markedly different

stimuli of chemical (capsaicin and acid) or physical (heat) variety can excite nociceptors by activating a single receptor, enabling the cell to integrate information and respond to complex changes in the physiological environment. Primary afferent nociceptors are also unique in the extent to which their receptive properties can be modulated. Thus, nociceptors not only signal acute pain, but also contribute to persistent and pathological pain conditions (allodynia) that occur in the setting of injury, wherein pain is produced by innocuous stimuli (Basbaum A. et al. 2000; Raja S. N. et al. 1999; Schmidt R. F. et al. 1995;Gebhart G. F. et al. 1996; Snider W. D. et al. 1998;Hökfelt T. et al. 1994; Woolf C. J. et al. 2000; Basbaum A. et al. 1999).

Allodynia can result from two different conditions: increased responsiveness of spinal cord ‘pain’ transmission neurons (central sensitization), or lowering of nociceptor activation thresholds (peripheral sensitization). With central sensitization, pain can be produced by activity in non-nociceptive primary sensory fibers. Peripheral sensitization is produced when nociceptor terminals become exposed to products of tissue damage and inflammation, referred to collectively as the ‘inflammatory soup’ (Fig. 1.4). Such products include extracellular protons, AA and other lipid metabolites, 5-HT, BK, nucleotides and NGF, all of which interact with receptors or ion channels on sensory nerve

endings. Nociceptors can release peptides and neurotransmitters (for example, SP, CGRP and ATP) from their peripheral terminals when activated by noxious stimuli, they are able to facilitate production of the inflammatory soup by promoting the release of factors from neighbouring non-neuronal cells and vascular tissue, a phenomenon known as ***neurogenic inflammation*** (Woolf CJ. et al. 1999). As early as 1910, it was recognized that the application of mustard oil to the conjunctival sac in experimental models produces inflammation that can be blocked by sensory nerve ablation (Bruce A.N. et al. 1910; Bruce A.N. et al 1913). SP, NA and CGRP are now known to coexist in sensory neurons and to have potent vasodilatory properties (Tanaka D.T. et al. 1985; Uddman R. et al. 1988). Direct stimulation of sensory nerves produces vasodilatation (Hinsey JC. et al. 1939; Jancso-Gabor A. et al. 1970), which can be blocked by depletion of SP with capsaicin (Gasparovic I. et al. 1964; Chahl LA. et al. 1988). The sensory fibers involved in neurogenic inflammation have been identified as C-fibers with a slow velocity of 1-2 m/sec (Ehrlanger J. et al. 1929). Progress has been made in understanding the regulation of neurogenic inflammation (Nadel JA. et al. 1991). A cell-surface enzyme, NEP, downregulates neurogenic inflammation by degradating SP. In the lung this enzyme is inhibited by cigarette smoke, viral infections, and toluene diisocynate, whereas corticosteroids increase NEP.

Neurogenic inflammation is now a well-defined physiological mechanism by which mediators are directly released from sensory nerves to produce-vasodilatation, edema, and other manifestations of inflammation. The nerve fibers have been identified as slow velocity C-fibers, and the regulation of neurogenic inflammation has been studied.

In addition to SP and CGRP, other substances such as Glu and PGs are synthesized and released from small diameter sensory neurons. The release of glutamate from central terminals of sensory neurons is well documented, but its peripheral actions and potential role in neurogenic inflammation are still to be determined. Evidence also suggests that sensory neurons contain cyclooxygenases and are capable of synthesizing proinflammatory prostaglandins (Vasko et al., 1994). Because glutamate and prostaglandin receptors are localized on small diameter sensory neurons (Carlton et al., 2001; Donaldson et al., 2001;Southall MD. et al. 2001), it is intriguing to speculate that these substances have autocrine as well as paracrine actions when released. The questions remain as to what other potential mediators of neurogenic inflammation are released from capsaicin-sensitive sensory neurons and whether other types of sensory neurons contribute to the inflammatory symptoms.

Injury heightens our pain experience by increasing the sensitivity of nociceptors to both thermal and mechanical stimuli. This phenomenon results, in part, from production and release of chemical mediators from the primary sensory terminal and from non-neural cells (for example, fibroblasts, mast cells, neutrophils and platelets) in the environment (Zhang J. et al. 2006) (Fig. 1.4). Some components of the inflammatory soup (for example, protons, ATP, serotonin or lipids) can alter neuronal excitability directly by interacting with ion channels on the nociceptor surface, whereas others (for example, bradykinin and NGF) bind to metabotropic receptors and mediate their effects through second-messenger signaling cascades (Prescott SA. et al. 2014).

Considerable progress has been made in understanding the biochemical basis of such modulatory mechanisms.

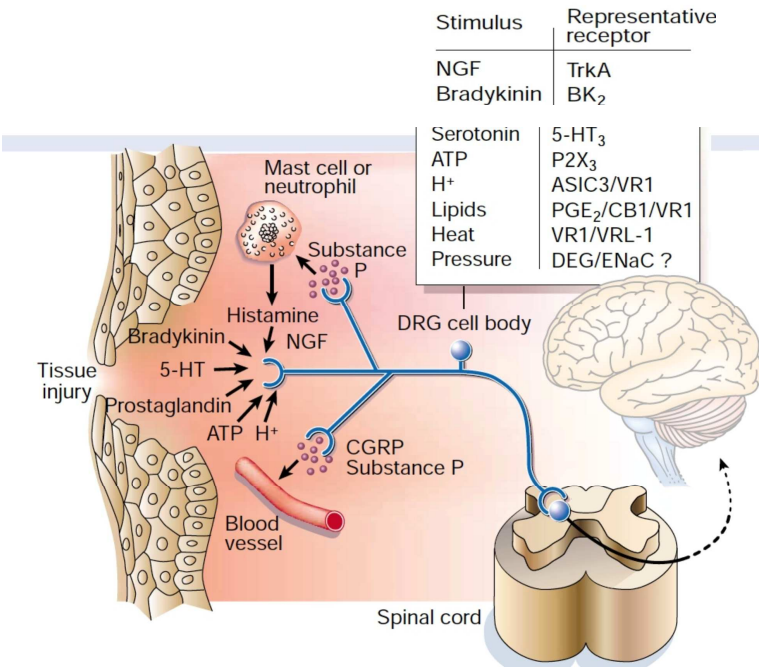


Fig.1.4 The molecular complexity of the primary afferent nociceptor is illustrated by its response to inflammatory mediators released at the site of tissue injury. Some of the main components of the 'inflammatory soup' are shown, including peptides (bradykinin), lipids (prostaglandins), neurotransmitters (serotonin (5-HT) and ATP) and neurotrophins (NGF). The acidic nature of the inflammatory soup is also indicated. Each of these factors sensitize (lower the threshold) or excite the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Examples of these factors and representative molecular targets are indicated in the box. Activation of the nociceptor not only transmits afferent messages to the spinal cord dorsal horn (and from there to the brain), but also initiates the process of neurogenic inflammation. This is an efferent function of the nociceptor whereby release of neurotransmitters, notably substance P and calcitonin gene related peptide (CGRP), from the peripheral terminal induces vasodilation and plasma extravasation (leakage of proteins and fluid from postcapillaryvenules), as well as activation of many non-neuronal cells, including mast cells and neutrophils. These cells in turn contribute additional elements to the inflammatory soup.

1.3 Pain classification

Even though the experience of pain varies from one person to an other, it is possible to categorize different kind of pain; clinically, we can distinguish two different types of pain, with specific characteristics of duration and therapeutic responsiveness: acute and chronic pain.

Acute pain appears suddenly and allows the individual to prevent more damage to the body. It is normally localized, lasts for a few days and decrease with healing. Causes inducing pain are usually clear: surgery, trauma, infectious disease in place or tissue damage. However tissue damage causes the release of potassium ions, bradykinin (BK) and serotonin (5-HT) [Rosland, J.H. et al. 1990] 5-HT is responsible of vasodilation and edema. BK activates C fibers receptors and PLA2/COX cascade that synthesize many eicosanoids (PGs, PGI_2, LTs) responsible of pain amplification. Currently, treatment options for acute pain control are varied and effective in most cases. Whatever the origin, acute pain produces defense and security reactions, including:

• mood swings (depression, anxiety, fear)

• modifications in the autonomic nervous system (changes in heart rate and blood pressure, nausea, vomiting, sweating)

• tendency to change posture.

Chronic pain oppresses hundreds of millions people in the world and alters their physical, emotional health and working conditions. It is long-lasting, often determined by the persistence of the damaging stimulus and/or by phenomena of self-maintaining, which retain the nociceptive stimulation even when the initial cause is limited. This kind of pain is characterized by a major emotional and psycho-relational components, strongly limits physical and social performance of patient and is often linked to chronic diseases (rheumatic, bone, cancer, metabolic). Chronic pain is hard to treat, requires a comprehensive and frequently multidisciplinary therapeutic interventions, managed with high level of expertise and specialization. From an etiopathogenetic standpoint, pain can be classified in: nociceptive, neuropathic and psychic.

Nociceptive pain: is the process whereby a stimulus noxious (thermal, mechanical or chemical) is perceived in periphery by nociceptors (peripheral nerves), next transmitted to the CNS. It is a transient pain, proportional to noxius stimuli and disappear at the end of this one. It can be classified in:

- ***"Superficial",*** as in case of injuries or minor burns, when triggered nociceptors activation on skin, looks like an acute pain and is well localized.

- ***"Somatic"***, when is caused by nociceptors stimulation in tendons, ligaments, bones, blood vessels and muscles; it is a dull ache.
- ***"Visceral",*** as in endometriosis, intestinal obstruction or metastatic cancer; is a throbbing pain, piercing, hard to locate, generalized or reported (perceived in areas distant from the damaged area, often superficial) frequently accompanied by nausea, vomiting and feeling unwell.

Post-operative pain can be classified as nociceptive pain, associated with changes in peripheral and CNS, in which psychological component may have a variable weight: is therefore a complex syndrome that requires a multimodal treatment.

Neuropathic pain: is a chronic disease resulting from dysfunction of the nervous system often due to peripheral nerve injury. Hypersensitivity to sensory stimuli (mechanical, thermal or chemical) is a common source of pain in patients and ion channels involved in detecting these stimuli are possible candidates for inducing and/or maintaining the pain.

Neuropathic pain is a multifactorial condition caused by damage or dysfunction of the nervous system resulting in loss of afferent sensory function, hyperalgesia and allodynia (Campbell JN. et al. 2006). Hyperalgesia is accentuated responses to painful stimuli while allodynia is pain in response to normally innocuous

stimuli, and spontaneous pain. The sites of injury are often peripheral nerves of DRG sensory neurons, which have an inherent plasticity. Changes in the biological properties and functions of neurons result in long-lasting hyperalgesia and/or allodynia that continue well after healing of the initial damage. The bases for these pathological conditions are gene expression changes in transmitters, receptors and ion channels that ultimately result in distorted connectivity, structure or survival of the neurons (Woolf CJ. et al. 2000).

It is more frequently described as a feeling of electric shock, burning or tingling continuous, and is associated with diseases such as diabetes mellitus, AIDS, Herpes Zoster, multiple sclerosis, but also to physical trauma of the spine, amputation (limb ghost), stroke and as a side effect of some chemotherapy. (Treede RD. et al. 2008)

Psychic pain: it is physical pain caused, increased or prolonged by emotional or behavioral factors. It can be seen in patients with mental disorders, but more frequently it accompanies events such as social rejection, the pain of love or the loss of a loved one, and is manifested as headache, backache or stomach ache. People who suffer of this pain are often stigmatized, because doctors tend to treat psychic pain as “not real” and therefore not in need of appropriate therapy, further exacerbating the mental state of the patient.

Table 1
Types of pain

Nociceptive Pain	• Normal processing of stimuli that damages normal tissues • Responds to opioids
Somatic	• Pain arises from bone, joint, muscle, skin, or connective tissue • Aching, throbbing • Localized
Visceral	• Arises from visceral organs • Tumor: localized pain • Obstruction of hollow viscus: poorly localized
Neuropathic Pain	• Abnormal processing of sensory input by PNS or CNS
Centrally generated	• Deafferentation pain: injury to PNS or CNS (eg, phantom pain) • Sympathetically maintained pain: dysregulation of autonomic nervous system (eg, complex regional pain syndrome I and II)
Peripherally generated	• Painful polyneuropathies: pain is felt along the distribution of many peripheral nerves (eg, diabetic neuropathy) • Painful mononeuropathies: associated with a known peripheral nerve injury (eg, nerve root compression, trigeminal neuralgia)

Fig. 1.5 Pain assessment and classification

Everyone reacts in a unique way to a painful stimulus, based on past experiences and its pain threshold, and each person is able to assess, according to its parameter, how strong its pain and therefore is able to quantify by a measurement. It is important that everyone learn to measure pain (Carpenter JS. et al. 1995).

Pain is measured through the use of official scales validated by international clinical trials. Intensity is the parameter less efficient, because it rests on subjective nature of pain perception (Fig. 1.6).

The scales, validated by international clinical trials, include (“Pain Intensity

Instruments". National Institutes of Health-Warren Grant Magnuson Clinical Center. July 2003):

- *Visual Analogue Scales (VASs)*: VAS is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain. From the patient's perspective this spectrum appears continuous ± their pain does not take discrete jumps, as a categorization of none, mild, moderate and severe would suggest. It was to capture this idea of an underlying continuum that the VAS was devised.

Operationally a VAS is usually a horizontal line, 100 mm in length, anchored by word descriptors at each end, as illustrated in Fig. 1. The patient marks on the line the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the patient marks.

- *Scale VDS* . It is a one-dimensional scale that offers a succession of adjectives (None, Very soft, Feeble, Moderate, strong, very strong) that patient can choose the one that best characterizes their status.

- *Numerical Rating Scales (NRSs)*: NRS is an 11-point scale where 0 is no pain

and 10 the worst imaginable pain and is preferred by most patients (Hjermstad MJ. et al. 2011). There is, however, a discrepancy between patients and healthcare professionals regarding how the ratings from the pain assessment should be interpreted (van Dijk FM. et al., 2012). Several studies have described and compared the use of different pain scales (Hjermstad MJ. et al. 2011), but no study has described how patients perceive the use of a pain scale in postoperative care. Knowledge of patients' different perceptions can facilitate healthcare professionals' possibilities to meet individual needs (Sjöström B. et al. 2002)

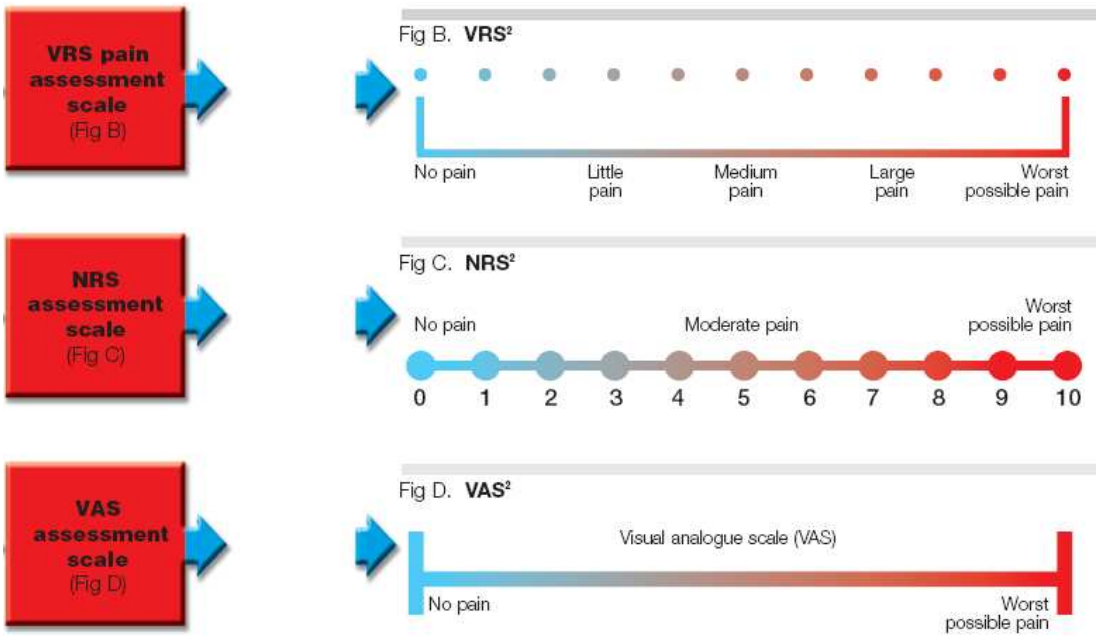


Fig. 1.6 Representation of pain scales

2 – POST OPERATIVE PAIN

Effective control and management of post-operative pain are clearly of primary concern to the patient and also of importance to the surgeon, because of potential adverse effects of the physiologic response to pain from surgery. Inadequate treatment of postoperative pain continues to be an important clinical problem, not only leading to worse outcomes in the immediate postoperative period but also an increased risk for persistent postoperative pain.

An estimated 25 million inpatient surgeries and an additional 35 million ambulatory surgeries are performed annually in the USA (Hall MJ. et al. 2010; Cullen KA. et al. 2009). Greater than 80% of surgical patients experience postoperative pain, and 39% experience “severe” to “extreme” postoperative pain (Apfelbaum JL. et al. 2003). The mismanagement of postoperative pain, whether undertreatment or overtreatment, is associated with a variety of negative consequences, including cardiac alterations and increased risk of myocardial ischemia or infarction, thromboembolic and pulmonary complications, immune alterations, increased risk of persistent postoperative pain, impaired rehabilitation, increased length of stay and/or hospital readmission, decreased quality of life, and adverse events related to excessive analgesic use (Taylor S. et al. 2003; Lucas CE. et al. 2007; Gandhi K. et al. 2011; Lavand’homme P. et al.

2011; American Society of Anesthesiologists Task Force on Acute Pain Management 2012). Consequences of overtreatment are often overlooked but can be life-threatening. Indeed, an observational study of surgical patients found high rates of analgesic-induced oversedation in the first 12 postoperative hours, with dangerous levels of sedation occurring in 72.7% of patients on PCA (Taylor S. et al. 2003). A variety of new analgesic medications and techniques have been introduced to more effectively manage acute postoperative pain during the preoperative, intraoperative, and postoperative periods, all of which may contribute to the development of acute postoperative pain.

2.1 Pathophysiology of postoperative pain

Acute postoperative pain is a normal response to surgical intervention and causes delayed recovery and discharge after surgery as well as an increased risk of wound infection and respiratory/cardiovascular complications (Khan R. et al 2011). Untreated acute pain leads to reduced patient satisfaction and increased morbidity and mortality and also places a burden on the patient and health system finances. Acute pain that becomes intractable and persistent is considered as CPSP. CPSP can have a significant impact on the patient's quality of life and daily activities, including disturbances of sleep and affective mood (Butterworth

et al. 2013; Khan R. et al 2011). Acute postsurgical pain occurs secondary to inflammation from tissue trauma or direct nerve injury and can be classified as nociceptive or neuropathic. Tissue trauma releases local inflammatory mediators, which can produce hyperalgesia (increased sensitivity to stimuli in the area surrounding an injury) or allodynia (misperception of pain to non noxious stimuli). Other mechanisms contributing to hyperalgesia and allodynia include sensitization of the peripheral pain receptors (primary hyperalgesia) and increased excitability of CNS neurons (secondary hyperalgesia) (Kodali BS. et al. 2014). It is increasingly recognized that genetic factors should be considered within the context of the interacting physiologic, psychological, and environmental factors that influence responses to pain and analgesia. Pain control has traditionally used opioid analgesia to target central machanisms involved in the perception of pain. A multimodal approach recognizing the pathophysiology of surgical pain uses several agents to decrease pain receptor activity and diminish the local hormonal response to injury (Kodali BS. et al. 2014;American Society of Anesthesiologists Task Force on Acute Pain Management 2012). This approach lessens the dependence on a given medication and mechanism. For example, local anesthetics can directly block pain receptors activity, anti-inflammatory agents can decrease the hormonal response to injury, and drug such

acethaminophen, ketamine, clonidine, dexmedetomidine, gabapentin pregabalin can produce analgesia by targeting specific neurotransmitters (Kodali BS. et al. 2014).

2.2 Pain management approaches targeted at the postoperative period

Traditional pharmacological approaches to pain management in postoperative period include oral or intravenous administration of opioids and oral administration of paracetamol or NSAIDs. These approaches are associated with a variety of adverse events, including respiratory depression (Dahan A. et al. 2010), nausea and vomiting (Becker DE. et al. 2010), pruritus (Tey HL. et al. 2011)and constipation (Camilleri M. et al. 2011) with opioids, and gastrointestinal injury (Scarpignato C. et al. 2010), myocardial infarction or stroke (Trelle S. et al 2011), and acute renal failure (Harirforoosh S. et al. 2009) with NSAIDs. Accidental overdose and death also is not uncommon after opioid use (Porucznik CA. et al 2011). New opioids, drug delivery approaches and systems, and PCA techniques have been developed to enhance the analgesic effects of NSAIDs and opioids and to minimize the risk of adverse events.

2.2.1 Paracetamol

Oral, rectal, and parenteral paracetamol can be an effective component of multimodal anesthesia. Paracetamol significantly reduces pain intensity and spares opioid consumption after abdominal surgery. The analgesic effect is 30% less than that of NSAIDs, but side effects are fewer (Butterworth J. et al. 2013). Paracetamol can also be used in conjunction with an NSAIDs to improve postoperative analgesia and as an adjunct to PCA opioids to reduce morphine requirements (Elia N. et al. 2005; Remy C. et al. 2005). The primary concern with use of paracetamol is hepatotoxicity, which is most concerning in the elderly and patients who chronically consume alcohol [US Food and Drug Administration(FDA) 2005]. Even if paracetamol is one of the oldest and most used analgesics, the debate on its mechanism of action continues. Contrary to previous assumptions, the analgesia is most likely mediated centrally and may involve direct and indirect inhibition of central COX, but also the activation of the endocannabinoid system and spinal serotonergic pathways (Graham GG. et al. 2013).

The more recent availability of a paracetamol preparation for infusion has increased its usefulness, in particular in the perioperative setting (Tzortzopoulou A. et al. 2011). Perioperative administration reduces postoperative nausea and

vomiting, in particular if prophylactically given at induction of anesthesia (Apfel CC. et al. 2013).

With regard to adverse effects, concerns about hepatotoxicity with overdose, which is in 50% of cases unintentional, continue (Blieden M. et al. 2014) and has lead the FDA to enforce a reduced dose per tablet. However, in therapeutic doses below 4 g/day, hepatotoxicity is very unlikely to occur (Dart RC. et al. 2007); surprisingly, even excessive alcohol consumption seems to be no risk factor for paracetamol -induced hepatotoxicity (Graham GG. et al. 2013).

2.2.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs such as ibuprofen, ketorolac, naproxen and COX-2 inhibitors are effective analgesics in a variety of acute pain states and have a broad spectrum of anti-inflammatory and antipyretic effects (Macintyre PA. et al. 2010). Intravenous ketorolac is widely used during the perioperative period for short-term treatment of acute pain and as an adjunct to opioids for the treatment of moderate to severe postoperative pain. Maximal benefit occurs when the NSAIDs is continued for 3 to 5 days postoperatively (Elvir-Lazo O. et al. 2010). The addition of NSAIDs to systemic opioids diminishes postoperative pain intensity, reduces opioid requirements, and decreases opioid side effects, such as

postoperative nausea and vomiting and respiratory depression (Butterworth J. et al. 2013). NSAIDs are the key components of multimodal analgesia but are generally inadequate as the sole analgesic agent in control of severe postoperative pain. When used in combination with opioids, NSAIDs improve analgesia, decrease opioid consumption and its adverse effects, such as postoperative nausea, vomiting, and sedation (Macintyre et al. 2010).

NSAIDs increase the risk of gastrointestinal bleeding and postoperative bleeding, decreased kidney function, impaired wound healing, and risk of anastomotic leakage (Butterworth J. et al. 2013). Their use should therefore be guided by the type of surgery being performed and by consultation between the surgical and anesthesia teams. COX-2 inhibitors also reduce postoperative pain, with less risk of NSAID-related platelet dysfunction and bleeding, but are associated with cardiovascular risk in the perioperative period (Butterworth J. et al. 2013). The risk of adverse renal effects of nonselective NSAIDs and COX-2 inhibitors is increased in the presence of preexisting renal impairment, hypovolemia, hypotension, and use of other nephrotoxic agents and angiotensin-converting enzyme inhibitors.

2.2.3 Ketamine

Ketamine can be used as an antihyperalgesic in the perioperative period (Grosu I. et al. 2011). Although traditionally used intraoperatively, low-dose ketamine has increasingly been given for postoperative analgesia (Hurley R. et al. 2010). Perioperative subanesthetic doses have been shown to decrease the opioid requirements and decrease the reported pain intensity (Hurley R. et al. 2010). At the low doses used in the postoperative period, ketamine does not result in hallucinations or cognitive impairment that are often seen with high doses.

2.2.4 Local Anesthetics

Lidocaine patch is primarily used for allodynia relief (painful hypersensitivity) and chronic pain in postherpetic neuralgia. Onset is approximately 4 hours. Absorption is dependent on dose, application site, and time exposure. Time to peak effect of 5% transdermal lidocaine is approximately 11 hours after application of 3 patches. Lidocaine patches have been used successfully for the treatment of pain secondary to rib fractures, back pain, and orthopedic surgeries. On-Q pain relief system is a non-narcotic elastomeric pump that automatically and continuously delivers a regulated flow of local anesthetic to a patient's

surgical site or in close proximity to nerves, providing targeted pain relief for up to five days. Studies have suggested clinical benefit with use of this system after abdominal, gynecologic, and thoracic surgeries (Macintyre et al. 2010;Ventham NT. et al. 2013;Gebhardt R. et al. 2013). A meta-analysis of studies using the system after colorectal surgery via laparotomy (Karthikesalinigam A. et al. 2008) showed a reduction in pain with movement and decrease in total opioid consumption, but no decrease in length of stay or ileus. Definitive conclusions about the overall benefit of this approach await further study.

2.2.5 Opioid analgesics

Opioids remain the cornerstone of the management of surgical pain, despite their potential side effects, and can be given through IV, IM, oral or transdermal routes. IV opioids provide rapid and effective analgesia for patients with moderate to severe pain. Morphine is the prototypical opioid agonist and the standard for management of acute pain. It has moderate analgesic potency, slow onset, and intermediate duration of action. The half-life is 2 hours, and its duration of action is about 5 hours. The metabolites of morphine are excreted by the kidney and therefore the sedating effects can be prolonged in patients with renal failure (Gandhi G. et al. 2012). Hydromorphone is a semisynthetic opioid,

which is 4 to 6 times more potent than morphine; it is onset of action is more rapid than morphine, but short-acting. It is a better choice for patients with renal failure and has a lower incidence of pruritus and sedation than morphine. It is particularly useful in patients who are opioid tolerant.

Fentanyl is a synthetic opioid, which is 50 to 80 times more potent than morphine. It has a rapid onset of within 5-7 minutes, with a short duration of only about 1 hour. IV fentanyl can be particularly effective when rapid analgesia is needed, such as in the post-anesthesia care unit or intensive care unit. Transdermal fentanyl is an alternative to sustained-release oral morphine and oxycodone preparations. This patches have a drug reservoir, which is separated from the skin by a microporous rate-limiting membrane, and provide medication that last for 2 to 3 days. Meperidine lowers seizure threshold, has a dysphoric effect, and is not recommended for postoperative pain control. In addition, meperidine has a slower rate of metabolism in the elderly and in patients with hepatic and renal impairment, leading to accumulation of meperidine and its active metabolite normeperidine, and consequent risk for seizures.

Oxycodone is a potent opioid agonist, which is metabolized in the liver. In an experimental pain model, oxycodone was more effective than morphine for pain related to mechanical and thermal stimulation of the esophagus, suggesting that it

could be more effective than morphine for visceral pain.

Tramadol is an effective analgesic for mild to moderate pain and neurophatic pain. The risk of respiratory depression is less compared with other opioids, and significant respiratory depression has been reported only in patients with severe renal failure.

2.2.6 Antidepressants

Antidepressants are useful for patients with neurophatic pain, even when depression is not a diagnosis of the patient. The analgesic effects occur at lower doses than needed for antidepressant activity. Older tricyclic agents, such as amitriptyline and nortriptyline, which block the reuptake of 5-HT and NE, seem to be more effective than selective serotonin reuptake inhibitors (Butterworth J. et al. 2013). The onset of pain relief is usually not immediate and may take weeks to have a complete effect. Antidepressants work best for pain from nerve damage secondary to diabetes, peripheral neurophaty, spinal cord injury, stroke, and radiculopathy (Butterworth J. et al. 2013).

2.2.7 Anticonvulsants

Anticonvulsant medications are useful for patients with neurophatic pain as well as for suppressing postoperative pain (Melemeni A. et al. 2007). The most commonly used agents include gabapentin, phenytoin, carbamazepine, and clonazepam. Pregabalin is a newer agent, which has been approved for all forms of neuropathic pain (Butterworth J. et al. 2013). The synergism between gabapentin and opioids results in an opioid sparing effect (Melemeni A. et al. 2007). Procedures in which gabapentin use for postoperative pain relief has been studied include breast surgery, hysterectomy, spinal surgery, postamputation, orthopedic surgery, and post thoracotomy (Melemeni A. et al. 2007).

2.2.8 Corticosteroids

Corticosteroids when used as an adjuvant decrease opioid consumption and help reduce postoperative pain (Elvir-Lazo O. et al. 2010). Dexamethasone is the preferred corticosteroid, because it also reduces postoperative nausea and vomiting.

3.0 PARACETAMOL

Paracetamol (an international name used in Europe) and acetaminophen (an international name used in the USA) are two official names of the same chemical compound derived from its chemical name: N-acetyl-para-aminophenol (the segment “cet” inserted between “para” and “amino”) and N-acetyl-para-aminophenol. This drug has a long history and, as it often happens with important discoveries, it was found by chance. In the 80s of the 19th century, two young doctors at the University of Strasburg, in order to eradicate worms by mistake dispensed acetanilide to a patient instead of naphthalene (Fig. 3).

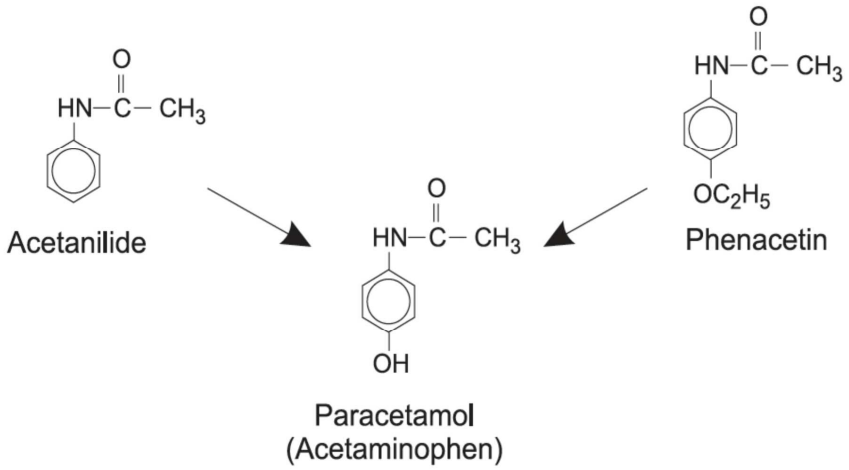


Fig.3 Chemical structure of analgesics - aniline derivatives. Phenacetin until the 80s of the 20th century was included in the composition of numerous mixtures.

They noticed that the drug had a small impact on intestinal parasites, however, it significantly decreased high temperature. Young doctors -Arnold Chan and Paul

Heppa - quickly published their discovery and acetanilide was introduced into medical practice in 1886 under the name of antifebrin (Chan A. et al. 1886). Soon it appeared that although the production of this drug was very cheap, acetanilide could not be used as an antipyretic medicament due to its high toxicity, the most alarming of which was methemoglobinemia. This resulted in a great deal of research on less toxic derivatives of acetanilide. Phenacetin and N-acetyl-p-aminophenol appeared to be the most satisfying compounds, which had been earlier synthesized by Harmon Northrop Morse in 1878 (Fig. 3) (Morse H.N. et al. 1878). The first clinical trials with those two acetanilide derivatives were performed by a German pharmacologist Joseph von Mering. On the basis of the obtained results, a faulty conclusion was drawn that paracetamol was characterized by high toxicity similar to acetanilide, therefore phenacetin was the first derivative to be introduced into medical practice in 1887. Phenacetin was widely used in analgesic mixtures until the time when it was associated with the development of analgesic nephropathy after a prolonged usage (von Mering J. et al 1893). In Poland, phenacetin was used as a component of very popular and available everywhere analgesic “tablets” with the “cross”. In fact, acethaminophen/paracetamol became popular half a year later in 1948 when Bernard Brodie and Julius Axelrod demonstrated that paracetamol was the main

active metabolite of acetanilide and phenacetin responsible for their analgesic and antipyretic action and that methemoglobinemia was induced by another metabolite, phenylhydroxylamine (Brodie B.B. et. al 1948). That discovery revolutionized the pharmaceutical market of analgesic drugs and since then paracetamol has started its staggering career.

3.1 Use of paracetamol

Paracetamol was introduced into pharmacological market in 1955 by McNeil Laboratories as a prescribed analgesic and antipyretic drug for children under its trade name Tylenol Children's Elixir (the name tylenol derives from its chemical name N-acetyl-p-aminophenol). One year later, 500-mg tablets of paracetamol were available over the counter in Great Britain under the trade name of Panadol, which were produced by Frederick Stearns & Co, the branch of Sterling Drug Inc. In Poland, paracetamol became available in 1961 and since then it has belonged to the one of the most frequently sold analgesic medications. There are about 100 preparations in the trade offer, which contain paracetamol alone or in combination with other active substances.

The paracetamol place on the WHO analgesic ladder, which precisely defines the rules for application of analgesic drugs, is impressive. This drug has been placed

on all three steps of pain treatment intensity. In different pains of moderate intensity, paracetamol as a weak analgesic together with NSAIDs or co-analgesics (e.g., caffeine) is a basic non-opioid analgesic (the first step of the analgesic ladder). When pain maintains or increases, paracetamol is used as an additional analgesic with weak (e.g., caffeine, tramadol) or strong (e.g., morphine, phentanyl) opioids from the second and third step of the analgesic ladder, respectively. Paracetamol, if efficient, is a recommended first choice oral analgesic to be used for a long time, e.g., in symptomatic treatment of slight and moderate pain occurring in osteoarthritis as well as in muscle or tendon pains. Moreover, it is a drug of choice in patients in whom application of NSAIDs are contraindicated, e.g., in the case of gastric ulcers, hypersensitivity to aspirin, impairments in blood coagulation, in pregnant women, nursing mothers and children with fever accompanying a disease (Leung L. et al. 2012). The use of paracetamol in children requires special care and maintain in an adequate dosage (based on age), which significantly differs from standard adult. The recommended dosage for children consider the metabolism of paracetamol, which determines the toxicity of the drug, especially hepatotoxicity (see below). In children, paracetamol metabolism changes with age: in younger children the sulfation pathway is dominated route of paracetamol elimination (which is

mature at birth); the glucuronidation pathway takes about two years to mature. The oxidation of paracetamol, which takes place mainly with the participation of the enzyme CYP2E1 in neonates is negligible, because the activity of CYP2E1 increases with age, reaching the adult value at age 1-10 years.

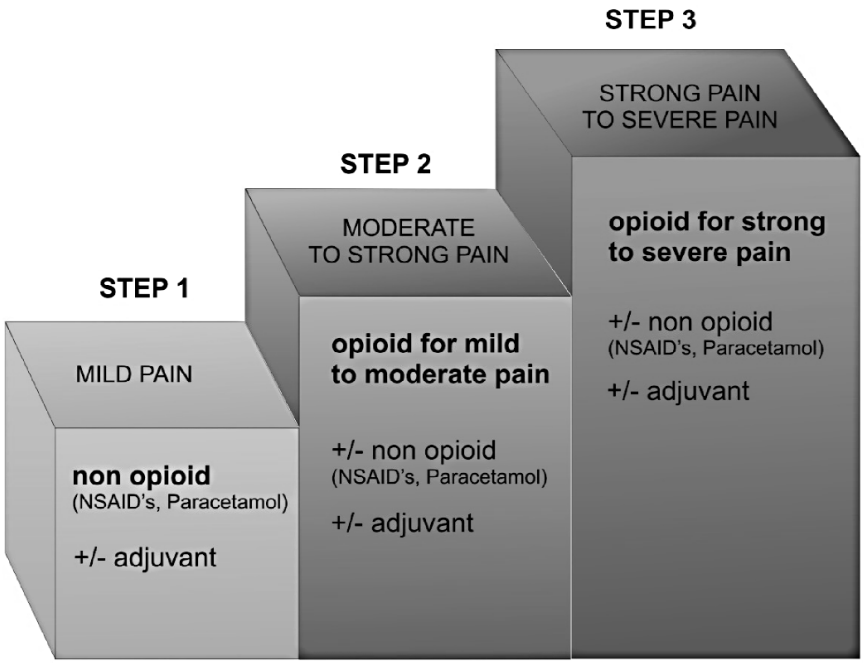


Figure 3.1. Paracetamol on the WHO analgesic ladder (the rules for using analgesics, which consider individual intensity of pain).

3.2 Mechanisms of action

More than 100 years after its synthesis, the mechanism of action of paracetamol remains unknown. In particular, it is still under discussion as to whether it acts

peripherally and/or centrally and which analgesic pathway is mainly affected by its administration (Smith HS. et al. 2009). Potential mechanisms include an inhibition of COX isoenzymes (Graham GG. et al. 2005), interaction with the endogenous opioid pathway (Raffa RB. et al. 2004), activation of the serotoninergic bulbospinal pathway (Roca-Vinardell A. et al. 2003) involvement of NO pathway (Bujalska M. 2004), and an increase in cannabinoid/ vanilloid tone (Ottani A. et al. 2006). As the analgesic actions of paracetamol resemble those of NSAIDs, the first effort to explain its mechanism of action was directed at demonstrating that paracetamol also inhibits COX. Flower and Vane showed that the antipyretic effect of paracetamol is related to the inhibition of PGs synthetase in the brain (Flower R. et al. 1972). In the 1990s a major advance in physiology and pharmacology was the discovery of the two COX isozymes (COX-1 and COX-2), which catalyze the conversion of AA to PGs, TXs, and PGI2 and represent the targets of NSAIDs. PGs are mediators of fever, pain and inflammation. Both of the COX enzymes have cyclooxygenase and peroxidase activity. The cyclooxygenase activity converts AA to PGG2, which is a hydroperoxide, and then the peroxidase part of the enzyme catalyzes the metabolism of PGG2 to PGH2 (Chandrasekharan NV. et al. 2004).

COX is sensitive to the local oxidation environment, which is influenced by organic peroxides and by reducing or oxidizing agents. A reducing agent is required to convert the COX enzyme from the active oxidized form (Fe^{4+}) to the inactive resting form (Fe^{3+}). In broken cell preparations, a phenol that is commonly added to the cells represents the reducing agent (Lucas R. et al. 2005). Paracetamol (para-acetyl-amino-phenol) is a substituted phenol; therefore, it acts as a reducing agent (Aronoff DM. et al. 2006). Although it has no affinity for the active site of COX, it blocks its activity by reducing the active oxidized form of the enzyme to an inactive form. In intact cells, when the levels of the substrate AA are low (less than 5 µmol/L), paracetamol is a potent inhibitor of PG synthesis, because it blocks the physiological regeneration of peroxidases; thus, the process is stopped. However, in broken cells, when the concentration of hydroperoxides is high, paracetamol is a weak inhibitor of PG synthesis (Ouellet M. et al 2001). The inhibitory effect of paracetamol on PGI2 production is completely blocked by butyl-hydroperoxide (Boutaud O. et al. 2002).This peroxide-dependent COX inhibition explains why paracetamol is not active at peripheral sites of inflammation where peroxide concentrations are high, whereas it is active in the brain where peroxide concentrations are low.

Paracetamol selectively inhibits COX activity in cells with a low oxidant status (endothelial cells), rather than cells with a high oxidant status (platelets) (Lucas R. et al. 2005). The selective inhibition of COX in CNS explains why paracetamol is not associated with gastric side effects and inhibition of platelet activity that are typically observed with NSAIDs. On the other hand, these findings support the hypothesis that paracetamol does not possess anti-inflammatory efficacy similar to NSAIDs, but rather it has only analgesic and antipyretic actions. However, due to the similarity of some of its in vivo effects to those of selective COX-2 inhibitors, some authors maintain that paracetamol has some anti-inflammatory activity; however, it clearly does not suppress the types of severe inflammation that accompany diseases such as rheumatoid arthritis (Graham GG. et al. 2005).

A second hypothesis posits that paracetamol acts by selectively inhibiting a particular isoform of the COX enzyme; this isoform, which was characterized and cloned in dog brain, was designated COX-3 (Chandrasekharan NV. et al. 2002). COX-3 is highly expressed in specific tissues, such as the brain and the heart. The presence of COX-3 could explain the pharmacological actions of paracetamol and other drugs that are weak inhibitors of COX-1 and COX-2 (Botting R. et al. 2005). However, COX-3 is simply a variant of COX-1 that is

derived from the same gene on chromosome 9 and retains intron 1. The retained intron sequence could alter folding and may affect the active site of the enzyme; this might lead to altered enzymatic properties, as shown by the lower potency (about 1/5) in generating PGE2 (Schwab JM. et al. 2003). Therefore, as COX-3 is unlikely to be the elusive target of paracetamol in human tissues, the mystery as to how paracetamol exerts an analgesic effect without affecting COX-1 and COX-2 remains unsolved. Recent findings have shown that the analgesic effect of paracetamol involves a “self-synergistic” interaction between spinal and supraspinal sites, with recruitment of endogenous opioid pathways. IT (spinal) administration of paracetamol in mice produced dose-related antinociception that was insensitive to the opioid antagonist naloxone, whereas ICV (supraspinal) administration had no effect. However, combined administration produced synergistic antinociception that was reversed when naloxone was given either spinally or subcutaneously (Raffa RB. et al. 2000). Moreover, each of the subtype-selective opioid receptor antagonists [beta]-funaltrexamine (μ), naltrindole (δ), and Norbinaltorphimine (κ) attenuated the site/site synergy produced by paracetamol; thus, each of the opioid receptor subtypes and endogenous pathways (endorphin, enkephalin, and dynorphin) were implicated to some degree in this synergy (Raffa RB. et al. 2004). As paracetamol does not

bind to opioid receptors (Raffa RB. et al 1996) and naloxone does not reverse its analgesic effect at a single site but only attenuates the spinal/supraspinal synergy (Raffa RB. et al. 2000) these findings support the hypothesis that the analgesic activity of paracetamol includes the activation of descending opioid pathways and a synergistic interaction at the level of the spinal cord. Many studies support the hypothesis that 5-HT participates in the central antinociceptive effect of paracetamol. 5-HT and NA are the two main neurotransmitters implicated in the endogenous descending pain inhibitory pathway, known as the "analgesic system", which originates at the level of the midbrain in the periaqueductal gray and in the magnus raphe nucleus that lies within the medulla (Coluzzi F. et al. 2005)

In rat brain, the antinociceptive action of paracetamol is associated with changes in the serotoninergic system. A significant down-regulation of 5-HT_{2A} binding sites in the frontal cortex in response to 5-HT release was demonstrated in rats after the administration of paracetamol; this indicates that the serotoninergic system plays a major role in the mechanism underlying analgesia produced by this drug (Srikiatkhachorn A. et al 1999).

The antinociceptive activity of intraperitoneally-administrated paracetamol in the hot-plate test in mice was increased by the selective blockade of 5-HT_{1A} and 5-

HT_{1B} receptors, whereas it was antagonized by the administration of selective agonists for these receptors (Roca-Vinardell et al. 2003). IV and oral administration of paracetamol in rats, following intraplantar injection of formalin, reduced nociceptive behaviors (biting and licking) in both phases of the typical nocifensive response to the test.

The antinociceptive activity of paracetamol was completely blocked by the IT administration of a 5-HT_{1A} receptor antagonist. Conversely, intraplantar injection of paracetamol failed to induce any anti-inflammatory effect and reduced nociceptive behavior only at high doses in the early phase of the test; this suggested a lack of relevant local activity (Bonnefont J. et al. 2003). The potent 5-HT_3 receptor antagonist tropisetron has been reported to reverse antinociceptive effect of paracetamol in the paw pressure test in rats (Pelissier T. et al. 1996). However, IT injection of other 5-HT_3 receptor antagonists, such as ondansetron and granisetron, was unable to block its activity. This suggested that a specific spinal tropisetron-sensitive receptor could be involved in the antinociceptive mechanism of action of paracetamol (Libert F. et al. 2004).All these findings reinforce the evidence for a centrally-acting component of paracetamol that involves the serotoninergic inhibitory descending pathway. Among various mechanisms proposed to account for the analgesic action of

paracetamol is the nitric oxide pathway. The L-arginine-NO pathway is activated by SP and NMDA, and its activation results in the facilitation of nociception transmission. Paracetamol inhibited SP-mediated hyperalgesia. Moreover, inhibitors of nitric oxide synthase activity produced antinociception and markedly increased the analgesic action of paracetamol (Bujalska M. et al. 2004). Recent investigations have demonstrated that analgesic effect of paracetamol is due to the indirect activation of cannabinoid CB_1 receptors (Bertolini A. et al. 2006). In brain and spinal cord, paracetamol, following deacetylation to its primary amine (p-aminophenol) and conjugation with AA by the action of FAAH, is converted to the bioactive metabolite N-acylethanolamine (AM404) (Hogestatt ED. Et al. 2005).

As it is an inhibitor of the cellular reuptake of anandamide (the first recognized endocannabinoid), AM404 can indirectly activate CB_1 receptors by increasing the levels of endogenous cannabinoids in the brain. Moreover, AM404 is a potent activator of vanilloid subtype 1 receptor (TRPV1) (Zygmunt PM. Et al. 2000). The antagonism of CB_1 receptor activity completely prevents the analgesic efficacy of paracetamol (Ottani A. et al. 2006). AM404 inhibits in a dose-dependent manner both COX-1 and COX-2, and because of the consumption of AA, it reduces the production of PGs (Zygmunt PM. et al. 2000) . This could

explain why paracetamol inhibits prostaglandin production in the brain. Moreover, besides inhibiting nociception, cannabinoids markedly lower body temperature via activation of CB_1 receptors. Therefore, the potential involvement of the cannabinoid system could also help explain antipyretic effect of paracetamol. Finally, the well-known effects of cannabinoids (relaxation, euphoria and feelings of wellness) are shared by aniline analgesics, such as paracetamol, acetanilide, and phenacetin (Bertolini A. et al. 2006).

3.3 Potential toxicity and safety profile

Paracetamol has been used safely and effectively for many years. At therapeutic doses, it is considered to be safer than NSAIDs, specially for chronic pain management (Courtney P. et al. 2002). Indeed it is currently recommended by several international guide-lines as the first line treatment for chronic conditions, such as osteoarthritis pain (Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines). However, in a small minority of patients paracetamol is responsible for life-threatening liver injury. This potential hepatotoxicity could still represent a perceived barrier to its use among some physicians.

The liver is the organ that is most affected by acute paracetamol toxicity. Damage to the liver following paracetamol ingestion is not due to the drug itself, but to the toxic metabolite NAPQI. Once absorbed, approximately 90% of paracetamol is metabolized by conjugation (mainly glucoronidation) via UDP-glucuronosyltransferase (isoform UGT1A6) and sulfation via two sulfotransferases (SULT1A1 and SULT1A3); the end-products are inactive metabolites that are eliminated in urine. A small fraction (5%) is eliminated unchanged. The remaining 5% is oxidized by the CYP2E1 subfamily of CYP450, which leads to the formation of NAPQI (Gelotte CK. et al. 2007). Other human CYP450 isoforms, including CYP1A2, CYP3A4, and CYP2A6, have been reported to form NAPQI in vitro, but their contributions *in vivo* seem negligible (Manyike PT. et al. 2000). Paracetamol is also oxidized by CYP2A6 to form inert catechols, such as methoxyparacetamol.

In the liver, NAPQI is quickly combined with the endogenous antioxidant glutathione to form non-toxic conjugates that are eliminated in the urine. However, after an overdose (when glutathione stores in the liver become depleted), free NAPQI begins to accumulate and causes centrilobular necrosis of the liver. Critical events that lead to cell death include the oxidation of enzymes, DNA fragmentation, and mitochondrial injury. Hepatic injury can be limited

through administration of N-acetylcysteine, which replenishes the levels of glutathione in the liver (Gelotte CK. et al. 2007). Risk factors that may predispose patients to paracetamol-induced hepatotoxicity are excessive dosing, increased CYP450 activation (as in patients treated with anticonvulsants and isoniazid, etc.), decreased gluthatione availability, and chronic severe ethanol abuse (Bertolini A, et al. 2006).

Paracetamol overdose remains a significant clinical problem, accounting for as many as 40% of acute liver failure cases in the United States and the United Kingdom. Furthermore, recent data suggest an increase in paracetamol intoxications in recent years. Besides suicide attempts, unintentional overdoses constitute at least half of paracetamol related hospitalizations. It is important to emphasize that the median dose ingested by individuals who developed acute liver failure was 24 g (equivalent to 48 tablets 500 mg) (Larson AM. et al. 2005). Risk factors include repeated dosing in excess of package labeling, use of multiple paracetamol containing products, simultaneous use or abuse of alcohol and narcotics, age, and comorbidities that include liver diseases and depression (Myers RP. et al. 2008). Conversely, when used at an appropriate dosage, paracetamol is a safe drug for both acute and chronic pain management. The maximum daily dosage is 4 g; this is consistent with the decline in analgesic

activity, which normally occurs over a period of 6 hours. The recommended dose for IV paracetamol injection in adults is 1 g.

The perception that paracetamol should be avoided in patients with chronic liver disease arose from an awareness of the association between massive paracetamol overdose and acute liver failure. However, there is no evidence in the literature of an increased risk of hepatotoxicity in these patients with the recommended doses. Alcoholic patients treated with the maximum recommended daily dose of paracetamol (4 g per day for three consecutive days) did not develop increases in serum transaminases or other measures of liver injury (Kuffner EK. et al. 2007). Therefore, paracetamol can also be used safely in patients with liver disease.

Paracetamol -induced nephrotoxicity occurs in 1-2% of patients with paracetamol overdose, and this becomes evident after hepatotoxicity. It can be differentiated from hepatorenal syndrome, which may complicate fulminant hepatic failure. The pathophysiology of renal toxicity in paracetamol poisoning has been attributed to CYP450 mixed function oxidase isoenzymes that are present in the kidney. The role of N-acetylcysteine therapy in the setting of paracetamol -induced renal failure is unclear. Paradoxically, glutathione conjugates have been implicated in the formation of nephrotoxic compounds (Mazer M. et al. 2008).

Generally, paracetamol is thought to have only minor effects on renal function, as it does not affect constitutively expressed COX-1.

In contrast to traditional NSAIDs, paracetamol is usually not considered to influence platelet function. However, recent investigations have shown that IV paracetamol is a weak inhibitor of platelet COX-1, with a dose-dependent antiaggregatory effect observed in healthy volunteers for at least 90 min after its administration (Munsterhjelm E. et al. 2005).

Paracetamol causes a mild degree of COX-1 inhibition when associated with parecoxib and it potentiates the antiaggregatory effects of aspirin and diclofenac (Galliard-Grigioni KS. et al. 2008). Platelet aggregation is more impaired by diclofenac than paracetamol, even when administrated at the loading dose of 3 g (Silvanto M. et al. 2007).

The antiaggregatory effect of paracetamol does not seem to be clinically relevant, and surgical bleeding attributable to paracetamol seems unlikely (Munsterhjelm E. et al. 2005). However, in chronic treatment, although paracetamol is considered the analgesic of choice in patients receiving anticoagulants, the combination of paracetamol and warfarin is not as safe, as is generally believed. A recent international study showed a significant increase in the INR and significant reductions in vitamin K-dependent clotting factors in patients

receiving a stable treatment of warfarin who received 4 g paracetamol per day for 14 days. These results suggest that an intensified INR monitoring in patients treated with oral anticoagulants and paracetamol is advisable (Mahel I. et al. 2006).

The identification of drug-drug interactions is an important aspect of patient care. Paracetamol is widely metabolized by UDP-glucuronosyl transferase (UGT) enzymes that play a key role in drug-drug interactions, as they catalyze the conjugation of various endogenous and exogenous substances. Experimental evidence indicates that ranitidine, propanolol, and cisapride inhibit paracetamol glucuronidation, whereas estrogen-containing oral contraceptives increase it. The effects of carbamazepine, phenytoin, phenobarbital, and rifampin on paracetamol glucuronidation remain to be determined.

4.0 AIM OF STUDY

Paracetamol is an active ingredient possessing analgesic and antipyretic activity.

In clinical field, paracetamol is principally used as an analgesic in mild and medium pain and as an antipyretic in the treatment of febrile states in adults and children.

The most common pharmaceutical form is the solid one as tablet, granule form or suppositories. Moreover, solution containing paracetamol for IV infusion can also be found on the market. These are formulations indicated for short-term treatment of medium pain, in particular of the type experienced following a surgical intervention. IV administration is reserved for cases in which is needed to treat pain and/or hyperthermia urgentely or when other administration routes are not available.

Paracetamol administration by alternative methods is still yet to be extensively explored and essentially no specific applications have been found in analgesic therapy. Recently, much attention was focused on spinal administration, in order to overcome the hepatotoxicity after oral administration of high doses.

However spinal administration of injectable solutions generally shows some limitations. First restriction is that drug is perfused in a defined and confined space in which a limited amount of solution can be infused.

In case of hypersaturated paracetamol solution, this limitation is overcome, since a therapeutically effective dose of paracetamol is dissolved in a lower volume compared to an unsaturated conventional solution.

In this regard, our aim has been to verify the effect of a supersaturated aqueous solution of paracetamol (SINTETICO; SIN) after spinal administration in a post-operative pain model. This solution was supplied by pharmaceutical company "Sintetica S.A". Moreover, knowing the marked analgesic effect of paracetamol following oral administration (PARA) and considering the use of this drug as a premedication before surgery, we investigated the efficacy of paracetamol combination by oral and spinal routes.

Furthermore, despite paracetamol medical use is consolidated by many years, its mechanism of action is still poorly understood. There are several hypothesis concerning the possible mechanism of action, showing that paracetamol has pleiotropic effects on several receptors. In fact, we studied the possible mechanism of the analgesic effect of paracetamol following spinal administration; in particular, on basis of literature's data, we evaluated the involvement of cannabinergic (CB_1 and CB_2), opioidergic (μ and κ) and serotoninergic ($5HT_3$) systems.

Finally, is well known that orally high doses of paracetamol could cause perilobular hepatotoxicity, which is the main limit to use this drug, particularly in fasting patients before surgery.

To date, paracetamol toxicity after spinal administration is still poorly known; so we examined if single or repeated administration showed physiological and/or morphological modification of cauda equina or nerve bundles of the lumbosacral spinal cord sections.

5.0 MATERIALS AND METHODS

5.1 Animals

The experiments were performed on Wistar Han rats (175–199 g rats Harlan, Italy) housed in the animal care facility of the Department of Pharmacy - University of Naples. Animals were housed in a room with controlled temperature (22±1°C), humidity (60±10%) and light (12 h per day); food and water were available *ad libitum*. Rats were randomly allocated to each experimental group. Each group was composed by at least 6 animals. Following surgery, rats were housed singly in cages containing clean soft bedding. All procedures involving rats were carried out in accordance with institutional guidelines and complied with Italian Ministry of Health Decree Law no.116 of 27 Jan 1992 and associated guidelines from European Communities Council Directive 86/609/EEC of 24 Nov 1986.

5.2 Drugs

SIN 3-5% (batch RD039, EXP. 05/2014-batch RD040, EXP. 05/2014) were synthesized in Sintetica laboratories (Mendrisio, Switzerland). This formulation is an paracetamol supersaturated injectable aqueous solution for

analgesic use by spinal administration, wherein said supersaturated injectable aqueous solution comprises paracetamol in a concentration ranging from 2 to 5 % w/v. The doses of SIN used were 100, 200,300 and 500 µg; 10 µl/it/rat and dissolved in sterile saline. SIN was administrated before incisional paw.

PARA was purchased from Sigma-Aldrich (Milan, Italy). It was dissolved in sterile saline. Drug was os administrated at the doses of 200, 300 and 500 mg/ kg (0.5 ml/rat), 15 min before surgery, but during combination with spinal route, acetaminophen was administrated 5 min before spinal injection.

Moreover, CB_1 and CB_2 antagonists (AM281/AM630), µ, δ and κ antagonists (Naloxone and Nor-Binaltorphimine) and $5HT_3$ antagonist (Tropisetron) were purchased from Tocris (Tocris Bioscience, Bristol, UK). All antagonists were administrated at the dose of 10 µg/ IT. Antagonists were injected by spinal route 5 min before SIN administration.

5.3 Spinal administration

Animals were anesthetized by inhaling enflurane/O_2 mixture and the anesthesia was maintained by a mask during the IT drug administration procedure. A foam block was placed under the animal's abdomen, in order to

produce a larger field for the needle insertion. After disinfecting the area with betadine, a 26G needle connected to a Hamilton syringe was introduced through the intervertebral space L4-L5. Puncture of the dura was followed by a marked tail flick, indicating the good practice of injection. The volume used for single spinal administration was 10 µl. The injection lasted 30-40 sec., Rats with tail movement or motor dysfunction in the hindlimbs following spinal injection have not been used for our experiments, and were sacrificed. Incisional paw model was made 5 min following spinal administration.

5.4 Intrathecal catheterization

The procedure of IT catheterization has been described earlier (Malkmus and Yaksh, 2004). Briefly, the animals were anesthetized with a mixture of ketamine and xylazine (respectively 100 and 5 mg/kg, intraperitoneal). The head was fixed in a stereotaxic frame. An incision was made over the back of the neck and scalp and the underlying muscle detached from the occipital crest. The muscle on either side of the external occipital crest was detached and retracted to expose about 3-4 mm^2 of the atlanto-occipital membrane. The membrane was incised by a needle, which led to the escape of cerebrospinal

fluid. The caudal edge of the cut was lifted and about 8.5 cm of 28G polyurethane catheter (Alzet 7741, Charles River, Lecco, Italy) was gently inserted into the IT space in the midline, dorsal to the spinal cord until the lumbar enlargement.

The out-dwelling part of the catheter (3 cm) was closed with a wire plug. The skin was sutured (polyamide 4-0 Ethicon). Animals showing motor abnormalities were euthanized. The rats were allowed to recover for 5 days. On the 3rd day, rats were observed for temporary hind limb paralysis after intrathecal 2% lidocaine (Xylocaine) injection (10μl). The placement of the catheter was also confirmed randomly by dissection at the end of the study.

5.5 Incisional pain

All rats were anesthetized with enflurane /O_2 mixture and anesthesia was maintained by a mask during the administration procedure. The left paw was disinfected with Betadine; a 1 cm longitudinal incision was made with a number 12 blade, through skin and fascia of the plantar aspect of the foot, starting 0,5 cm from the proximal edge of the heel and extending toward the toes. In all animals the plantaris muscle was elevated and incised longitudinally. The wound was closed with a 5-0 nylon thread. After surgery,

the animals were allowed to recover in their cages. The incisions were checked daily and any signs of wound infection or dehiscence excluded the animal from the study.

5.6 Paw edema and hyperalgesia by carrageenan

Initial paw volumes of all animals (before treatment) were measured using a plethysmometer apparatus (Ugo Basile, Milan, Italy). Paw edema was induced by a subplantar injection of 50 μl of saline containing 1% λ-carrageenan into the left hind paw.

SIN was spinal administrated before carrageenan challenge. Paw volume was measured at different time intervals by plethysmometer. The increase in paw volume was evaluated as the difference between the paw volume measured at each time point and the basal paw volume measured immediately before carrageenan injection.

5.7 Mechanical hyperalgesia

Latencies of paw withdrawal (g) was evaluated by mechanical stimuli using the Randall-Selitto analgesimeter for rats (UgoBasile, Varese, Italy).

Hyperalgesia was assessed on ispsilateral (incision) paw before (basal), 2, 4, 24, 48, 72 h after spinal administration. Each paw was tested twice per session. Cut-off force was set at 250 g.for rats and 100g for mice.

5.8 Perfusion and tissue fixation

Fixation by intracardiac perfusion is recommended for fixation of tissues which rapidly autolyse, such as nervous tissue or endocrine tissue. The rat is anesthetized with an intrperitoneal injection of Ketamine/Xylazine (see above). Once deep anesthesia is attained (absence of withdrawel reflex when the foot is firmly pinched with forceps), the rat is pinned in dorsal recumbancy. The chest is opened, and the right atrium incisioned with scissors. A 21G butterfly needle is placed in the left ventricle, and 120 ml of Phosphate Buffer Saline (PBS) 1X were flushed in over the course of about a minute. Thereafter, 120 ml of fixative (4% paraformaldehyde) is flushed in until rat body becomes stiff.

5.9 Spinal cord histological analysis

5.9.1 Decalcification

Each sample of column was then placed in 500 mL of electrolytic decalcifier for 30 h.

5.9.2 Processation

All samples were processed in paraffin wax and embedded on cutting surface, maintaining the orientation and the sequence of the samples during all working phases.

5.9.3 Cutting

Each block was cut to obtain 4 transversal slices of 5 µm thickness, far 200-250 µm one from each other. The slices were collected on slides progressively numbered starting from 1, as indicated in the drawn below. The total number of cross-sections collected from each animal was 20, that is 4 levels of cut per 5 spinal samples.

5.9.4 Staining

All slides were stained with haematoxylin and eosin according to the following procedure:

- Deparaffinize in three changes of xylene (each of 2 min).
- Hydrate in 100%ethyl alcohol for 2 min.
- Hydrate in 95% ethyl alcohol for 2 min.
- Hydrate in 70% ethyl alcohol for 2 min.
- Wash in distilled water for 3 min.
- Place in Mayer’s hematoxylin for 2 min.
- Wash in tap water for 10 min.
- Place in Eosin Y solution 5 wt. % in water for 2 min.
- Rinse in tap water for 4 sec.
- Dehydrate in two changes of 95% ethyl alcohol (each of 30 sec).
- Dehydrate in two changes of 100% ethyl alcohol (each of 30 sec).
- Clear in two changes of xylene (each of 1 min).
- Mount with resinous medium.

Spinal cord damage was graded on a scale of 0–3 as follows: grade 0, no edema and no injured nerve fibers; grade 1, edema and little or no nerve fiber

degeneration; grade 2, less than 50% of nerve fibers with degeneration; grade 3, more than 50% of nerve fibers with degeneration.

5.10 Statistical Analysis

All data were presented as % vs control group (animals operated that received only saline) of the mean of raw data and calculated by the formula: (T-C)/C X100:

- T= medium value (expressed in g) of analgesic effect evoked in rat treated with drug
- C= medium value (expressed in g) of effect evoked in control rat treated with vehicle (CTR)

Analysis of data was conducted using GraphPad Prism (GraphPad Software Inc., San Diego, CA).The significance of differences between groups was determined by two-way analyses of variance (ANOVA) followed by Bonferroni post hoc tests for multiple comparisons. The level of significance was set at $P< 0.05$.

6.0 RESULTS AND DISCUSSION

6.1 Dose-effect of SIN following spinal administration in a post-operative pain model and in carregeenan-induced paw edema

Previous results showed that pretreatment with paracetamol (100ug) spinal administration was associated with a significant decrease in hind limb motor dysfunction due to ischemic spinal cord injury 24 hours after ischemia/reperfusion in rats (Sahin M. et al. 2014). On basis of these data we evaluated the effect of SIN in a postoperative pain model.

Following incision of paw, operated animals showed signs of hyperalgesia if compared to basal data. In particular, in mechanical hyperalgesia experiments, single spinal treatment with SIN 100 μg/IT produced a significant antihyperalgesic effect only at 24 h, while no effect was observed at all other experimental time. SIN 200 μg/IT showed a significant antihyperalgesic effect from 2 to 48 h post dose, and the highest doses (SIN 300 μg/IT and 500 μg/IT) produced a more significant and prolonged effect until 72 h (Fig. 1; $^{**}p<0.01$ and $^{*}p<0.05$).

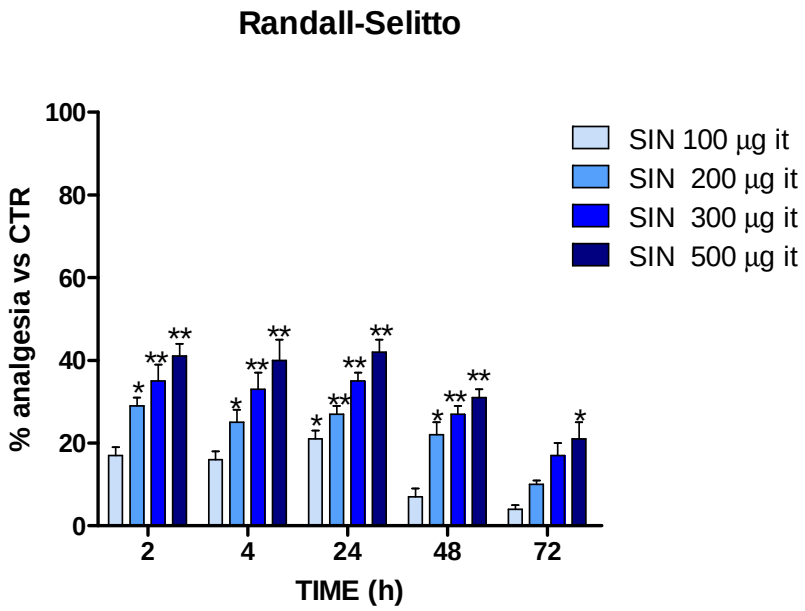


Fig. 1: Effect of SIN in incision paw-induced mechanical hyperalgesia. Rats received SIN 100-500 µg before incision paw. Mechanical hyperalgesia was assessed at 2, 4, 24, 48, 72 h after spinal administration. Data are shown as mean ± SEM of 6 animals per group and are presented as % analgesia vs CTR (*p<0.05 and ** p<0.01 vs CTR group).

Rezende RM and co-workers (2008), showed that subcutaneous pretreatment with paracetamol reversed hyperalgesia induced by λ-carrageenan. In this study paracetamol raised nociceptive thresholds also in non-inflamed paw.

Although bilateral anti hyperalgesia after paracetamol had been earlier noted (Alloui et al., 2002), it sharply contrasted with the unilateral (only in the inflamed paw) analgesia induced by systemically administered inhibitors of PGs biosynthesis (catalysed by both COX-1 or COX-2).

We also evaluated SIN efficacy after IT administration in carrageenan-induced paw edema in mice; edema was measured after 2, 4, 6 and 24 hours: 10 µg SIN did not reduce edema if compared to the control group mice, while the dose of 100 µg produced a slight edema reduction (27 % approximately) after 2 hours from induction (Fig.2). Similar data were also obtained in carragenan-induced hyperalgesia; in fact SIN 100 µg produced a slight effect on paw pressure by Randall-Selitto test (vehicle 45g vs SIN 75g, data not shown).

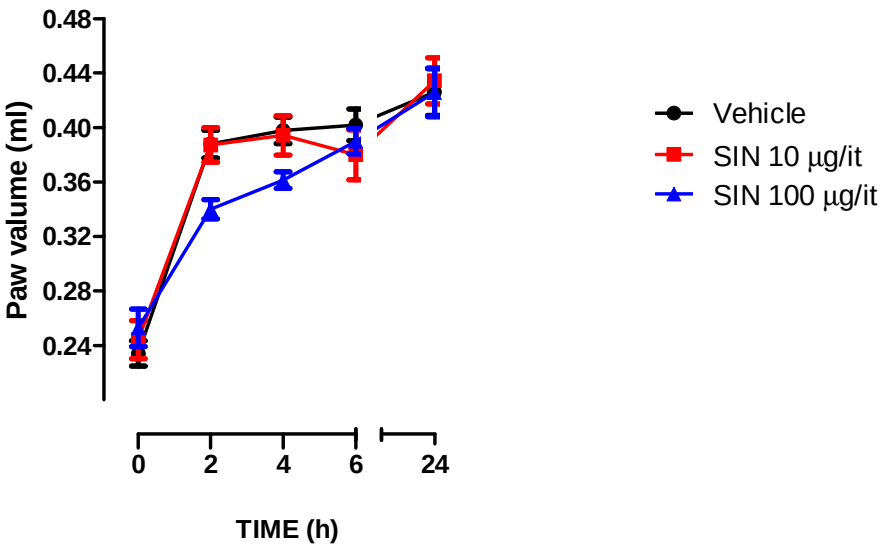


Fig.2 Effect of Sintetico in a model of inflammatory pain in mice, induced by intraplantar injection of carrageenan. Mice received Sintetico (SIN 10-100 µg/it) after carrageenan injection. Paw edema was assessed at 2, 4, 6 and 24h after spinal administration. Data are shown as mean ± SEM of 6 animals per group

For better clarify this weak effect obtained by spinal injection, we studied SIN activity in a postoperative animal pain model. Injury to peripheral tissues may produce prolonged pain, increased sensitivity to painful stimuli (hyperalgesia) and/or pain following innocuous stimulation (allodynia).

As reported, paracetamol can be used alone or in combination with an NSAID to improve postoperative analgesia (Elia N. et al. 2005; Remy C. et al. 2005). Furthermore, Bujalska M. and colleagues (2001) showed that oral administration of paracetamol, increased the nociceptive thresholds for both mechanical (Randall-Selitto test) and chemical (writhing test) stimuli. Previously, Pelissier and colleagues (1996) demonstrated that oral paracetamol at dose of 400 mg/kg produced an antinociceptive effect comparable to 800 mg/kg, suggesting that with 400mg was observed the ceiling effect.

On the basis of these results, we selected a range of oral doses of PARA between 200-500 mg/kg to evaluate its efficacy in a postoperative pain model. As expected, following paw incision, single oral treatment with PARA (200mg/kg) produced a significant antihyperalgesic effect at 2 h, whereas PARA 300 and 500 mg/kg showed a significant antihyperalgesic effect form 2 to 6 h post dose (Fig.3 *$p<0.05$, **$p<0.01$ and ***$p<0.001$).

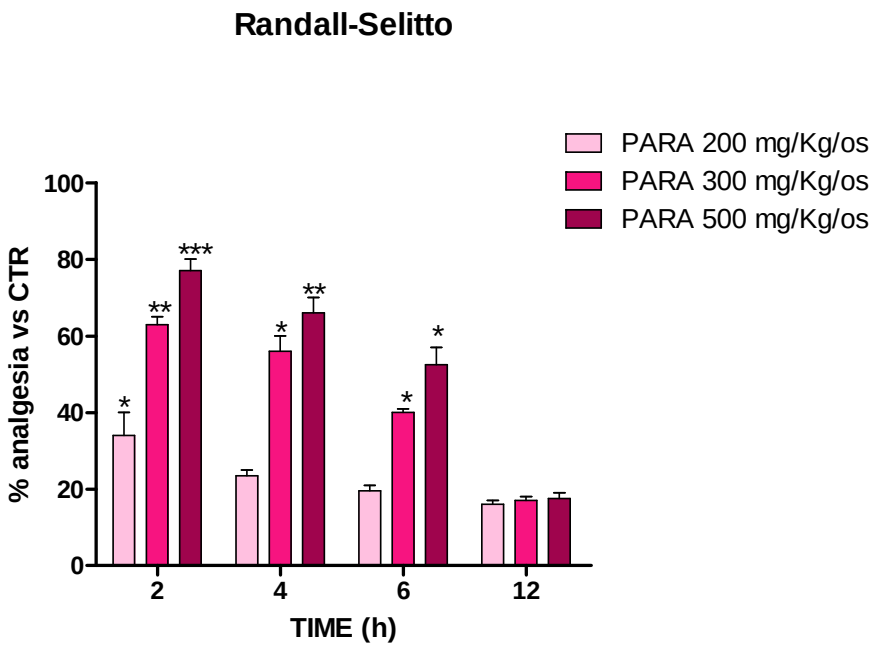


Fig.3: Effect of PARA on incision paw-induced mechanical hyperalgesia. Rats received PARA 200-500 mg/Kg/os 15 min before incision paw. Mechanical hyperalgesia was assessed at 2, 4, 6 and 12 h after oral administration. Data are shown as mean ± SEM of 6 animals per group, and are presented as % analgesia vs CTR. *p<0.05, ** p<0.01 and ***p<0.0001 vs CTR group.

Our data confirm PARA efficacy in reduction of both acute and postoperative pain, underlining the limited activity after oral administration (within 6 h), while shows prolonged analgesia by intrathecal administration. Moreover, our results suggest that to achieve a significant and prolonged analgesia is preferable to administer paracetamol by intrathecal administration, and using this therapeutic approach it is possible to bypass all side effects caused by oral administration.

6.2 Effect of combination of active or inactive doses of oral paracetamol (PARA) and intrathecal Sintetico (SIN) in incisional pain model-induced mechanical hyperalgesia.

Injury to peripheral tissues may produce prolonged pain, increased sensitivity to painful stimuli (hyperalgesia) and/or pain following innocuous stimulation (allodynia) (Woolf CJ. et al. 1983). These changes are usually accompanied by enlargement of the peripheral receptive field and increased excitability of spinal nociceptive cells to peripheral stimulation (Hylden JL. et. al. 1989). A current hypothesis states that excitatory amino acids activating NMDA receptors in the spinal cord produce excessive cell depolarization that contributes to increased pain sensation (Dubner R. et al. 1991). According to this hypothesis, the amplification of pain long after the initial stimulus may be avoided if the treatment of pain is introduced before its initiation (Woolf CJ et. al. 1994). Some studies have reported the efficacy of such "preemptive analgesia" in laboratory animals following pre-surgical administration of opioids (Woolf CJ. et al. 1986). Gaspar AF.et al. (2007) showed that pre or postoperative injection of MK886 (an inhibitor of 5-lipoxygenase-activating protein), combined with indomethacin significantly reduced the mechanical allodynia. However, the combination was

significantly more effective when used before than after surgery, thus fulfilling the criteria for preemptive analgesia.

For this reason, during our study we investigated the efficacy and the activity of pretreatment with oral paracetamol, following up by spinal administration of SIN in postoperative pain. For this purpose we used inactive and active doses of oral PARA (200 and 500 mg/kg, respectively) in combination with inactive and active doses of inthratecal SIN (100 and 500 µg/it/rat, respectively). Firstly, we investigated whether the analgesic effect obtained with an active dose is modified when administered an active dose for the other route of administration.

As expected, oral treatments with a paracetamol high dose (300 mg/Kg) increased pain threshold from 2 h to 6 h after administration; at same way, intrathecal SIN injection (300 µg/it) produced a significant analgesic effect from 2 h to 48 h after administration (Fig. 4; fuchsia and blue columns). This antihyperalgesic effect was also obtained using the combination of these drugs and was comparable to single treatment (Fig.4 *$p<0.05$,**$p<0.01$ ***$p<0.001$ vs CTR).

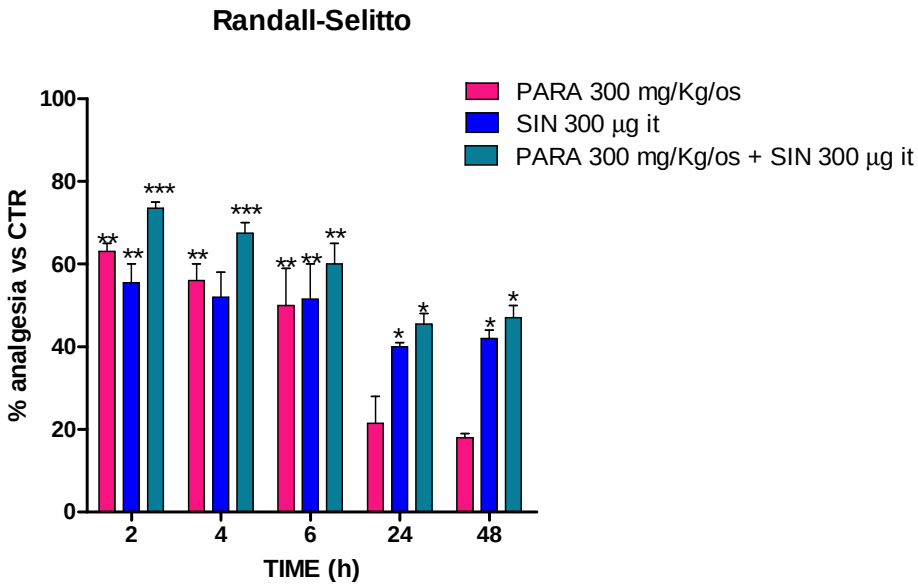


Fig. 4: Effect of single or combination of paracetamol and Sintetico on incision paw-induced mechanical hyperalgesia. Rats received Paracetamol (PARA 300 mg/Kg), Sintetico (SIN 300 µg/it) and Paracetamol +Sintetico (PARA 300 mg/Kg +SIN 300 µg/it). Mechanical hyperalgesia was assessed at 2, 4, 6, 24 and 48 h after administration. Data are shown as mean ± SEM of 6 animals per group (*p<0.05, **p< 0.01, and *** P<0.001 vs CTR group).

Other aim was to investigate the efficacy of combination of active or inactive oral doses of paracetamol with active or inactive intrathecal doses of Sintetico. Oral treatment with a inactive dose of Paracetamol (PARA 200 mg/Kg) showed a weak analgesic effect only 2h after administration; while the intrathecal administration of active dose of Sintetico (SIN 300 µg/it) showed antihyperalgesic effect at all experimental time. The co-administration of these doses showed an antihyperalgesic activity from 2 h to 48 h after administration (Fig. 5A., *p<0.05 and **p<0.01 vs CTR). These data suggested that combination of inactive oral dose of paracetamol and an active intrathecal dose of Sintetico did not produced an significant increased of pain threshold, if

comparator at the effect of IT SIN injection (Fig. 5A). The same results were obtained using active oral doses of paracetamol and an inactive dose intrathecal of Sintetico. In fact, oral treatment with high dose of Paracetamol (PARA 300 mg/Kg) produced a significant analgesic effect from 2 to 6 h after oral administration; no significant activity was obtained following intrathecal administration of Sintetico (SIN 100 µg/it). Combination of these two doses showed a significant antihyperalgesic effect until 24 h (Fig. 5B, *p<0.05 and **p<0.01 vs CTR).

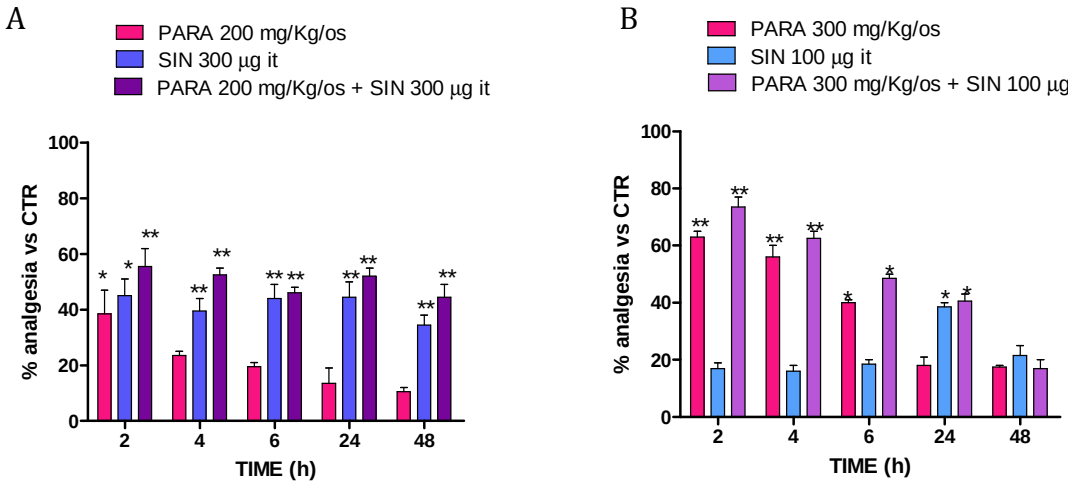


Fig. 5A: Effect of single or combnation of paracetamol and Sintetico on incision paw-induced mechanical hyperalgesia. Rats received paracetamol (PARA 200 mg/Kg/os), Sintetico (SIN 300 µg/it) and Paracetamol +Sintetico (PARA 200 mg/Kg +SIN 300 µg/it). Mechanical hyperalgesia was assessed at 2, 4, 6, 24 and 48 h after administration. Data are shown as mean ± SEM of 6 animals per group (*p<0.05 and **p< 0.01 vs CTR group).

Fig. 5B: Effect of single or combination of paracetamol and Sintetico on incision paw-induced mechanical hyperalgesia. Rats received paracetamol (PARA 300 mg/Kg), Sintetico (SIN 100 µg/it) and paracetamol +Sintetico (PARA 300 mg/Kg +SIN 100 µg/it). Mechanical hyperalgesia was assessed at 2, 4, 6 and 24 h after administration. Data are shown as mean ± SEM of 6 animals per group (*p<0.05 and **p< 0.01, vs CTR group).

Finally, we investigated the activity of the combination of two inactive doses. Results showed that oral paracetamol (PARA 200 mg/Kg) had a weak analgesic effect only 2 h after administration, while single intrathecal injection of Sintetico (SIN 100 µg/it) did not produce analgesic effect in all experimental time. Surprisingly, oral and intrathecal combination of Paracetamol and Sintetico produced a prolonged analgesic effect from 2 up to 24 h after administration (Fig. 6, *p<0.05). The analgesic effect obtained after combination of two inactive doses of drug, has produced an synergic effect (Fig. 6).

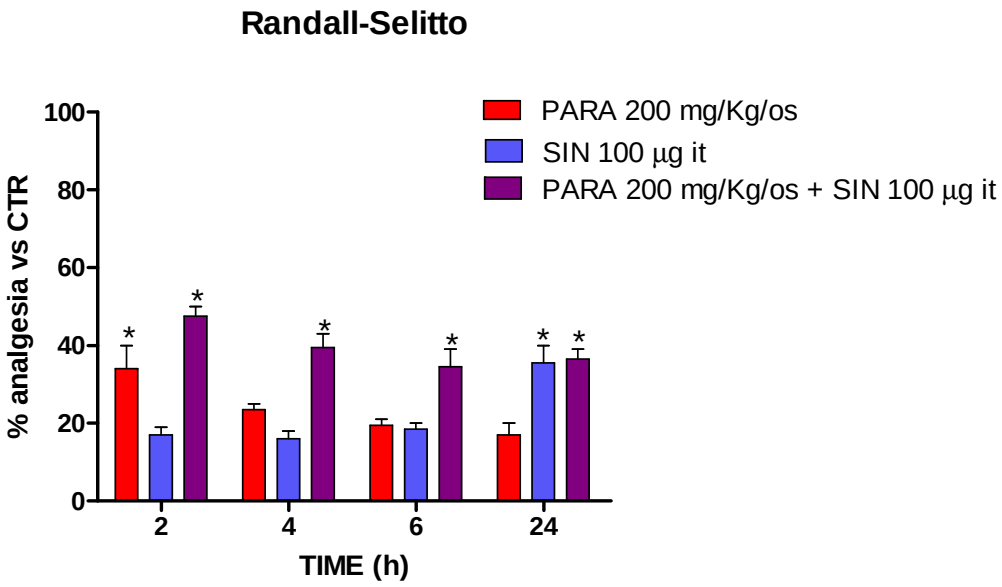


Fig. 6: Effect of single or co-administration of paracetamol and Sintetico on incision paw-induced mechanical allodynia. Rats received oral paracetamol (PARA 200 mg/kg), intrathecal Sintetico (SIN 100 µg/it), paracetamol+Sintetico (PARA 200 mg/Kg+SIN 100 µg/it); mechanical allodynia was assessed at 2, 4, 6 and 24 h after administration. Data are shown as mean ± SEM of 6 animals per group (*p<0.05 vs CTR group).

6.3 Role of cannabinergic, opioidergic and serotoninergic systems after SIN IT administration.

After well more than a century of clinical use, and in spite of being one of the most prescribed and consumed drugs in the world, paracetamol's mechanism of action has remained a mystery. Several data suggest the possibility that the site of action of its antinociceptive effect may be in the CNS. Moreover, endogenous cannabinoids (anandamide and 2-arachidonylglycerol) seem to be tonically released and to control basal nociceptive threshold (Meng et al. 1998). Cannabinoids produce antinociceptive effects by descending spinal inhibition, and cannabinoid CB_1 receptors are almost exclusively involved.

Ottani A. et al. 2006 have demonstrated that analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors in rats. Moreover acethaminophen dose-dependently decreased mechanical allodynia and lowered nociceptive scores associated with hyperalgesia testing. These effects were inhibited by the administration of cannabinoid CB_1 (AM251) and CB_2 (AM630) receptor antagonists (Dani M. et al. 2007). On the basis of these evidences, we have investigated whether the analgesic pathways observed following PARA oral administration, could be involved after SIN IT administration.

In our experiments we evaluated the role of cannabinergic, opioidergic and serotoninergic receptors: specific anatgonist were IT administrated 5 min before SIN spinal injection in both non operated rats (naïve) and in operated rats (incisional paw).

In mechanical hyperalgesia experiment, single spinal treatment with Sintetico (SIN 300μg) incresed pain threshold from 2 to 4 h in naive rats; using the same dose in operated rats, SIN produced a significant antihyperalgesic effect lasted until 6 h post dose.

In naive rats, analgesic effect of SIN was reverted using a CB_1 antagonist, AM281 (10 μg/it), while a CB_2 antagonist, AM630 (10 μg/it), did not produce any effect (Fig. 7A).

In operated rats, results showed that both receptors CB_1 and CB_2 are involved. In fact, AM281 (10 μg/it) reduced SIN analgesic effect from 2 until 6 h after spinal administration, while AM630 (10 μg/it) reverted SIN effect up to 4 h (Fig.7B; *$p<0.05$,**$p<0.01$ and ***$p<0.001$ vs CTR; ## $p<0.01$ and ### $p<0.001$ vs SIN 300 μg). Our data suggested the involvement of cannabinergic system in pain modulation; in particular, CB_1 receptors are involved in pain modulation both in naïve and operated rats, while CB_2 receptors modulated analgesic effect only in operated rats.

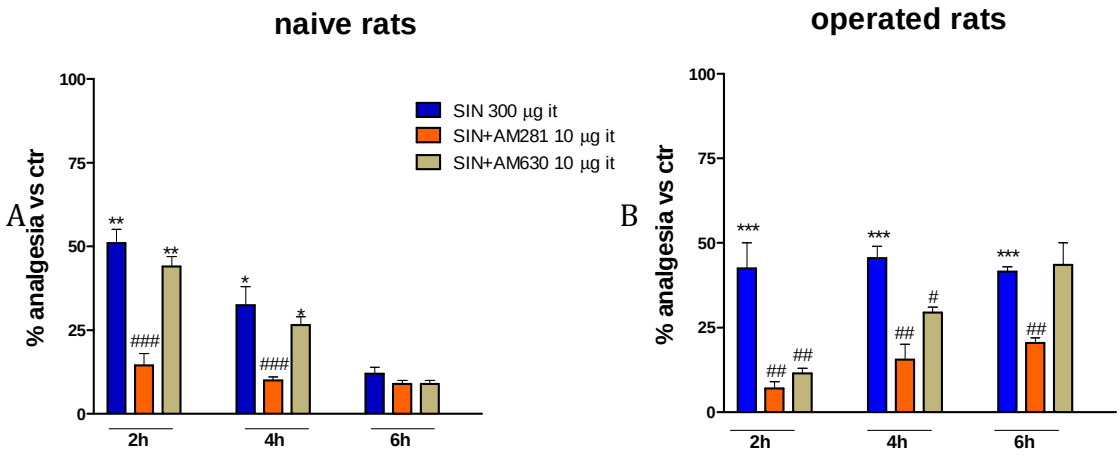


Fig. 7: Effect of Sintetico and AM281/AM630 on naive (A) and operated rats (B) in Randall Selitto test. Rats received Sintetico (SIN 300µg/it), AM281 10µg/it (SIN+AM281) and AM6330 10 µg/it (SIN+AM630). Mechanical hyperalgesia was assessed at 2, 4 and 6 h after spinal administration. Data are shown as mean ± SEM of 6 animals per group. *p<0.05, **p< 0.01, and *** P<0.001 vs CTR group; ## p<0.01 and ### p<0.001 vs SIN 300µg/it.

Moreover it has been reported that the antinociceptive action of oral PARA high- (400 mg/kg) and low dose (100 mg/kg) is antagonized by naloxone, a nonselective opioid receptor antagonist, and that the antinociceptive action caused by morphine is enhanced by PARA low-dose and this effect is due to an interaction with opioidergic systems (Bujalska M. et al. 2004;Sandrini M. et al. 1999).

Furthermore, there is evidence that the antinociceptive effects of opiaces are potentiated by some NSAIDs (Poggioli et al., 1980; Maves et al., 1994) and by paracetamol (Pircio A.W. et al., 1978), whereas naloxone is able to revert

antinociception induced by diclofenac in rats (Björkman R. et al., 1990). Thus studying paracetamol effect on serotonergic and opioidergic systems, might throw some light on the complex antinociceptive activity of this widely used drug.

For this purpose, our experiment was conducted to gain insight into the mechanism of the analgesic action of spinal paracetamol and the influences of opiod system, using a specific antagonists (naloxone and nor-Binaltorphimine) in Randall-Selitto test.

As reported in Fig. 8A, in mechanical hyperalgesia, spinal dose of SIN (SIN 300μg) incresed pain threshold up to 4 h after administration in naive rats; while, in operated rats, the same dose used before the incisional paw, has prolonged analgesic effect to 6 h after administration (Fig. 8B).

Analgesic effect of Sintetico was reverted by Naloxone (10 μg/it), and Nor-Binaltorphimine administration (10 μg/it), in both groups and in all experimental time (Fig. 8 A and B *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs CTR; # $p<0.05$ and ### $p<0.001$ vs SIN 300 μg). Therefore we have hypothesized that opioidergic system is involved in paracetamol mechanism of action.

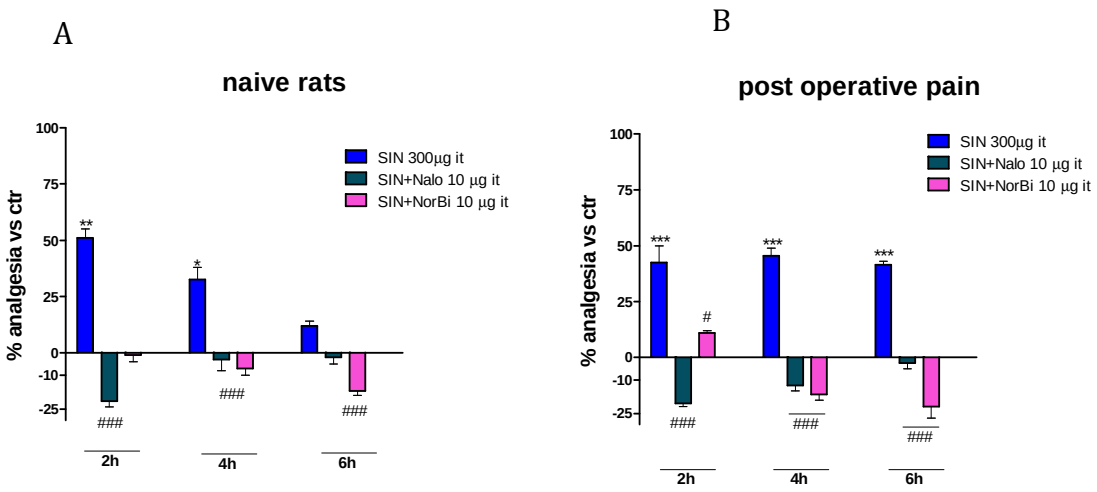


Fig. 8: Effect of administration of Sintetico and Naloxone/Nor-Binaltorphimine (Nalo/NorBi) on naïve (A) and operated rat (B)-induced mechanical hyperalgesia. Rats received Sintetico (SIN300µg/it), Naloxone 10µg/it (SIN+Nalo) and Nor-Binaltorphimine 10 µg/it (SIN+NorBi). Mechanical hyperalgesia was assessed at 2, 4 and 6 h after spinal administration. Data are shown as mean ± SEM of 6 animals per group. *p<0.05, **p< 0.01, and *** P<0.001 vs CTR group; # p<0.05 and ### p<0.001 vs SIN 300µg/it.

Multiple serotonin receptor subtypes have been identified in CNS: 5-HT_1, 5-HT_2 and 5-HT_3 seem to be involved in the 5-HT-mediated antinociceptive mechanism (Sufka KJ. et al. 1992). There are conflicting findings concerning the relationship between the antinociceptive effects of 5-HT to specific subtypes of 5-HT receptors. Recently it has been suggested that 5-HT_2 and 5-HT_3 receptors mediate antinociception to chemical *stimuli* in the spinal cord (Sasaki M. et al. 2001).

In rat paw pressure test, Courade JP. and co-workers showed that the antinociceptive action of paracetamol intravenously was inhibited by intrathecally injection of 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} antagonists and by

Tropisetron, known as a 5-HT_3 specific antagonist (Alloui A. et al. 2002; Alloui A. et al. 1996; Pelissier T. et al 1996).

Our data reveal that spinal administration of Sintetico (SIN 300µg/it) had a significant analgesic effect from 2 up to 4 h after administration in naive rats, while in operated animals this effect resulted more evident until 6 h. Also in this case, Tropisetron (10 µg/it) reduced the analgesic effect of Sintetico in both naive and operated rats (Fig.3 A and B *p<0.05,**p<0.01 and ***p<0.001 vs CTR, # p<0.05, ## p<0.01 and ### p<0.001 vs SIN 300µg).

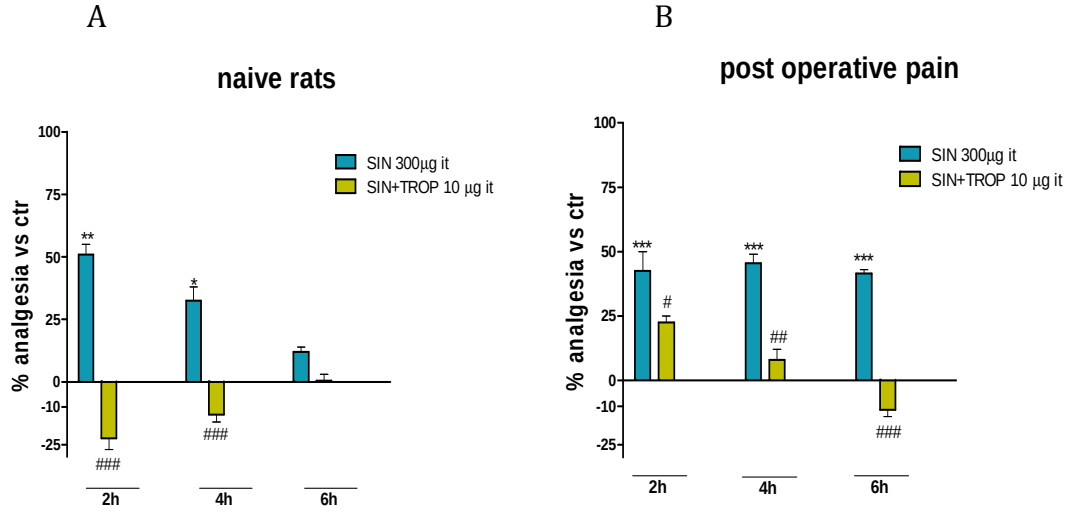


Fig. 9: Effect of single administration of Sintetico and Tropisetron (TROP) on naïve (A) and operated rat (B) induced mechanical hyperalgesia. Rats received Sintetico 300 µg (SIN300 µg/it), Tropisetron 10µg/it (SIN+TROP). Mechanical hyperalgesia was assessed at 2, 4 and 6 h after spinal administration. Data are shown as mean ± SEM of 6 animals per group. *p<0.05, **p< 0.01, and *** P<0.001 vs CTR group; # p<0.05,, ## p<0.01 and ### p<0.001 vs SIN 300µg.

Therefore, also our results suggested an involvement of serotoninergic system in pain modulation.

6.4 Spinal cord and liver toxicity after SIN IT administration

Paracetamol toxicity is not due to drug *per se*, but to one of its metabolites, NAPQ1. Paracetamol biotransformation involves conjugation with glucoronide and sulphate. A small amount of paracetamol is metabolized by mixed function oxidase enzymes to form highly reactive compound NAPQ1, which is immediately conjugated with glutathione (GSH) and subsequently excreted as cysteine and mercapturic conjugates. In overdoses, large amounts of paracetamol are metabolized by oxidation because of saturation of the sulphate conjugation pathway (Benjamin N. et al. 2002;Pajoumand A. et al 2003), but once the protective intracellular glutathione stores are depleted, hepatic and renal damage may ensue. Hepatotoxicity is the most remarkable feature of paracetamol overdose (Rumack BH. et al 1975). Paracetamol acute overdoses can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure. Renal effects of paracetamol overdose are less commonly seen than hepatic effects.

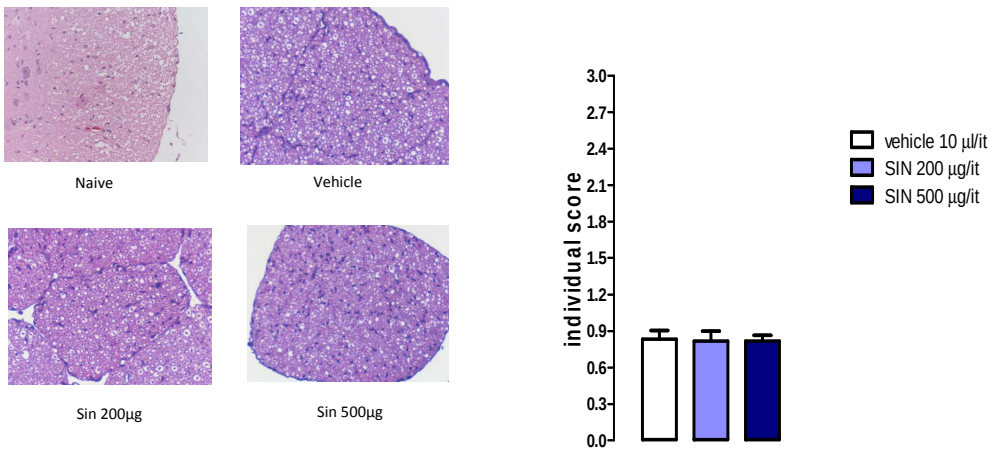
Venkatesan P.S. and colleagues (2014) suggest that in Sprague Dowley rats of either sex paracetamol oral administration up to 500 mg/kg did not show any impact on feeding, body weight gain, behaviour, physiological and biochemical parameters; moreover the suspected target organ, liver and kidney, were found to be normal on histopathological analysis. These results indicated that paracetamol NOAEL in rats following oral administration is found to be 500 mg/kg. Furthermore, El-Kott AF. (2015) showed that oral single-dose administration of paracetamol (800mg/kg) was hepatotoxic in rats as shown by the significant increases in plasma ALT and AST activities as well as ALP concentration. Abnormal levels of hepatic enzymes in plasma are believed to be an indicator of hepatocyte injury (Ozer J. et al. 2008).

According to these data, our aim has been to studied if SIN administration produced side effects in spinal cord and in liver after single and repeated injection.

After single, three and ten SIN administrations at higher dose (500 μg/it), histopathological results of rat spinal cord region indicated a low toxicity degree of SIN within 24 h. In particular, single injection did not produce histopathological alteration while, both three and ten IT administrations produce a weak cell infiltration (not significant) in submeningeal and/or perifascicular

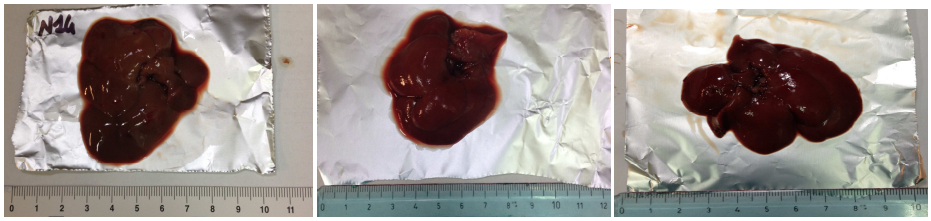
region and/or in spinal cord. These data were considered a sequel to the technical procedures of administration, so no-treatment-related effects were observed in vehicle animals (data not shown).

Finally, repeated SIN (200 and 500 µg/it) administrations for 7 days showed a mild toxicity degree with little or no degeneration of nerve fibers; there was no difference between SIN-treated and vehicle-treated rat (Fig. 10).



FFig.10 Hematoxylin-eosin stained spinal cord sections from different animal groups . Naive is animal group without catheter, Vehicle is animal group with catheter and that recevied only salina for 7 days. SIN 200µg and SIN 500µg are animal groups that received for 7 day Sintetico by spinal catheter.

Furthermore, in this last experiment, we also observed macroscopically whether SIN administrations for 7 days produced liver changes, in terms of margins and sizes. As shown in Fig. 11, no significant alterations were observed between vehicle- and SIN-treated rats.



Vehicle SIN 200µg SIN 500µg

Fig.11 Liver photos of Vehicle, SIN 200 µg and SIN 500 µg after chronic administration (7days).

7.0 CONCLUSION

Acute pain after surgery and trauma represent two of the biggest concern of hospital in patients and the management of pain is of utmost importance (Macintyre PE. et al. 2010). Acute and chronic pain states account for a large proportion of presentations to general practitioner and the emergency department. The past ten years have witnessed a far greater focus upon the management of acute, cancer and chronic pain; these efforts have culminated an international pain summit leading to the declaration of Montreal that access to pain management is a fundamental human right (International Pain Summit Of The International Association For The Study Of P. Declaration of Montreal 2011).

Despite massive progress in the understanding of the physiology and pharmacology of pain there is only a limited number of new compounds used into clinical practice. In an ideal world the management of pain should be associated to one medication that produces little to no side effects, and capable to treat multiple types of pain. To date, pain management is hard to reach, because of the complex nature of pain physiology and the associated social, psychological and economical components.

Therefore, pharmacological pain treatment is centered on a multimodal approach with old medications that have new uses and indications. Combined with the increasing understanding of pain perception, and an appreciation of the multifactorial nature of pain, this could lead to future personalization of analgesic therapy.

During our study, the attention was focused on treatment of postoperative pain. Postoperative pain is an individual multifactorial experience influenced by patient culture, psychology, genetics, previous pain events, beliefs, mood and ability to cope, as well as the type of procedure performed. Inadequate treatment of postoperative pain continues to occur, despite advances in analgesic techniques, placing patients at risk and significant disability.

Optimal pain results from proper management in the preoperative, intraoperative, and postoperative periods, requires appropriate education of physicians, nurses, other health care providers, and patients. An understanding of the pathophysiology of postoperative pain and the various options available for analgesia often results in a procedure-specific, multimodal approach, optimizing pain relief, decreasing adverse effects, and creating a better patient experience.

Many new analgesic medications and techniques have been developed to reduce acute postoperative pain. These include preoperative use of NSAIDs, anxiolytics,

and anticonvulsants; intraoperative use of neuraxial analgesia, continuous local anesthetic wound infusion, epidural morphine, intravenous paracetamol, intravenous ketamine; and postoperative use of intravenous ibuprofen, new opioids (eg, tapentadol) or opioid formulations (morphine- oxycodone).

Many of these drugs have demonstrated analgesic superiority to placebo and a comparable activity to traditional therapy, coupled with a reduction in adverse events. Several of the newer medications and techniques improve analgesia and minimize the risk of adverse events, although additional research is needed to establish their efficacy and safety profile. New approaches to acute postoperative pain management may provide safer and more effective analgesia than traditional therapy such as postoperative spinal analgesics. The development of chronic pain syndromes following surgery is not rare and may be unappreciated by clinicians.

The risk factors for developing chronic pain after surgery are several: preoperative pain, repeat surgery, prolonged surgery, younger age, severe postoperative pain, surgical approaches with a higher risk of nerve damage, chemotherapy or radiation, and some psychological or depressive symptom (Kehlet H. et al. 2006).

To help prevent these, it is important to inform the patient about the management of postoperative pain and patients should be also educated about analgesic

agents, their risks and benefits, and encouraged to ask questions. It is usual to find that many patients underestimate or overestimate the potential risks of opioid analgesics.

Moreover, a written protocol for best practices for postoperative pain should be developed. This should encompass certain established benchmarks: patients should be educated about the risk, benefits, and dosing instructions of their medicines and prescribed break-through pain medication (where appropriate) and antiemetic agents, if required. The protocol should also allow for adjustments to the *regimen* in patients who are at risk for cardiorespiratory morbidity. Shortfalls, gaps, or failures of the analgesic protocol should be promptly detected and rectifed, in no more than 2 hours. Further, the protocol set forth by a hospital should be subject to periodic audit. It has previously show that postoperative epidural analgesia decreased 30-day postoperative mortality, pneumonia, and deep vein thrombosis and shortened intensive care unit and hospital length of stay, nevertheless epidural analgesia should be evaluated for postoperative analgesia only in highly selected cases and for patients who are otherwise at high risk for other analgesic regimens (Rawal N. et al.. 2012; Low JA. et al. 2008).

Today the most widely drug used for treatment of acute and postoperative pain is paracetamol. This drug place on the WHO analgesic ladder, which precisely

defines the rules for application of analgesic drugs, is impressive. It is a recommended oral analgesic of a first choice to be used for a long in time, e.g., in symptomatic treatment of slight and moderate pain occurring in osteoarthritis as well as in muscle or tendon pains. Moreover, it is a drug of choice in patients in whom application of non- steroidal anti-inflammatory drugs (NSAIDs) are contraindicated, e.g., in the case of gastric ulcers, hypersensitivity to aspirin, impairments in blood coagulation, in pregnant women, nursing mothers and children with fever accompanying a disease (Leung L.; 2012).

Although paracetamol was discovered several years ago, its mechanism of action has not been elucidated until now (Smith HS. et al. 2009; Graham GG. et al. 2005; Raffa RB. et al. 2004; Roca-Vinardell A. et al. 2003; Bujalska M. 2004; Ottani A. et al. 2006). The mechanism of action is complex and includes the effects of both the peripheral (COX inhibition), and central (COX, serotonergic descending neuronal pathway, L-arginine/NO pathway, cannabinoid system) antinociception processes and redox mechanism. These evidences underline the possibility of paracertamol to interact with several systems: cyclooxygenase, opioidergic, cannabinergic and serotoninergic. Although higher doses are not associated with hepatotoxicity, the recommended dose at present is 1 g in a 15 min infusion every 6 hours. However, administration of paracetamol by means of

methods alternative (as spinal administration) to traditional methods (oral or intravenous) is still yet to be explored extensively, and essentially no specific applications have been found in the field of analgesic therapy.

In present study we used a new supersaturated aqueous solution of paracetamol (SIN) to verify the effect of this solution in a postoperative pain model in rat after spinal administration. This solution is highly stable, has an increased concentration of acetaminophen in the solvent, and can be mixed with other drugs in order to obtain a solution with a total volume that is compatible with the volume injectable by a single spinal administration. As above reported, the administration of injectable solutions presents physical limitations that could be overcome by this supersaturated solution.

Our data suggested that SIN spinal administration before paw incision, produced an significant, marked and prolonged analgesic effect, that was dose- and time-dependent. Our data support the hypothesis that an alternative administration route, as the spinal one, could be used in both acute and postoperative pain. Moreover, it was observed that in order to obtain an adequate analgesic postoperative effect is necessary to administer high doses of analgesics or opioid (Kodali BS. et al. 2014;American Society of Anesthesiologists Task Force on Acute Pain Management 2012). Both of these therapeutic approaches not only

expose patients to many side effects but in most cases, do not provide an adequate analgesic response. In this regard, the analgesic activity was assessed using combinations of paracetamol of inactive and active oral doses (100 and 500 mg/kg, respectively) with inactive and active IT doses (100 and 500μg/it, respectively). The most important data of this set of experiments was obtained using the combination of oral and spinal inactive doses. In fact, results showed that this combination produced a synergic and significant antihyperalgesic effect.

The possible mechanism of action underlying the prolonged analgesic activity of paracetamol was also investigated, deepening the involvement of cannabinergic (CB_1 and CB_2), opioidergic (μ and κ receptors) and serotonin ($5HT_3$) systems, in naive (not operated) and in operated rats.

The recent discovery that paracetamol acts as a prodrug (a donor of a moiety of an endogenous cannabinomimetic) by triggering CB_1-mediated effects, provided explanation of the peculiar effects of this drug.

Ottani et co-workers suggest a so far unforeseen mechanism for the analgesic effect of paracetamol; i.e the activation of cannabinoid system, or at least of the components of such system that are involved in the modulation of nociception and whose signal trasduction requires the availability of CB_1 receptors.

Our results showed that analgesic effect with single spinal treatment with Sintetico 300 μg was reverted using AM281 (CB_1 antagonist) in naive rats; while, in operated rats both receptors CB_1 and CB_2, are involved.

Many of the documented analgesic effects of cannabinoids are based on the interaction of these compounds with CB_1 receptors on spinal cord interneurons in the superficial levels of the dorsal horn, known for its role in nociceptive processing. In particular, CB_1-receptors are heavily expressed in layers 1 and 2 of spinal dorsal horn and in lamina 10. These localizations of CB_1 receptors are responsible for analgesic and antihyperalgesic effects observed in naive and operated animals.

CB_2 receptors are manly localized on the mast cells, known to facilitate the inflammatory response, and are not expressed on nociceptive sensory neurons; these underline the key role of this receptor only in pathological condition such as inflammation due to surgery.

It has been reported that the antinociceptive action of oral high dose of paracetamol is antagonized by naloxone, which is a non selective opioid receptor antagonist (Bujalska M. et al. 2004, Bujalska M. et al. 2004;Godfrey L, et al. 2005). Our results are in agreement with these data, in fact, IT administration of naloxone reduced SIN analgesic effect, both in naive and operated animals.

Same results were obtained using k-opioid antagonist (Nor-Binaltorphimine). These data clearly indicated the role of opioids receptors in SIN-induced analgesia. Finally, as reported by Sandrini M. et al. (1999) the antinociceptive action obtained by morphine is enhanced by paracetamol low-dose and this effect is dependent on the cross-talk between opioidergic and serotoninergic systems. Moreover, the involvement of serotonergic system in analgesia induced by non-opioid analgesics has been demonstrated (Björkman R. et al. 1995), but the detailed mechanism by which serotonin acts, together with the exact nature of the receptor subtypes involved, has not yet been elucidated (Richardson BP. et al. 1990; Courade JP. et al. 2001).

Paracetamol antinociceptive effect may be mediated by different serotonin receptor subtypes at spinal and supraspinal levels. This is suggested by results obtained by some authors, indicating that paracetamol activity is prevented by 5-HT_3 receptor antagonist Tropisetron IT injected (Pellissier T. et al. 1995).

Many studies support the hypothesis that 5-HT participates in the central antinociceptive effect of paracetamol. 5-HT and NA are the two main neurotransmitters involved in the endogenous descending pain inhibitory pathway, known as the “analgesic system”, which originates at the level of the midbrain in the periaqueductal gray and in the magnus raphe nucleus that lies

within the medulla. In rat brain, the antinociceptive action of paracetamol is associated with changes in the serotoninergic system. A significant down-regulation of $5HT_{2A}$ binding sites in the frontal cortex in response to 5-HT release was demonstrated in rats after the administration of paracetamol; this indicates that the serotoninergic system plays a major role in the mechanism underlying analgesia produced by this drug. Moreover, the potent 5-HT_3 receptor antagonist Tropisetron has been reported to reverse the antinociceptive effect of paracetamol in the paw pressure test in rats. However, IT injection of other 5-HT_3 receptor antagonists, such as Ondansetron and Granisetron, were unable to block its activity. This suggested that a specific spinal Tropisetron-sensitive receptor could be involved in the antinociceptive mechanism of action of paracetamol (Courade JP. et al. 2001) In agreement with this, our data showed that spinal administration of Tropisedron reverted analgesic effect of SIN both in naive and operated rats, underlying the involvement of this receptor.

During our studies we also investigated the possible spinal cord toxicity, following acute or repetitive spinal administration of SIN. As we know, liver is the largest complex organ in the body, which plays an important role in the internal environment maintenance by its multiple functions. It plays a central role in the metabolic pathways of carbohydrates, lipids and proteins. It is also

involved in the detoxification and excretion of many endogenous and exogenous compounds by its xenobiotic metabolism. The liver is the organ that is most affected by acute paracetamol toxicity. This potential hepatotoxicity could still represent a perceived limitation to its use among some physicians.

This problem could be overcome, using an alternative route of administration, such as the spinal one. In fact, our results showed that single and repetitive treatment with SIN, using low and high doses, showed no signs of spinal and liver toxicity.

These results open a new scenario for treatment of postoperative pain, because this new formulation and administration route allow to obtain a prolonged analgesic effect using low doses of paracetamol. Considering these evidences and lower toxicity, this new therapeutic approach to postoperative pain could be a great benefit for public health; more studies and researches are needed for developing more information guidelines and education activities to fight pain. When patients receive effective postoperative analgesia, it can reduce postoperative morbidity, enhance and accelerate recovery, shorten the hospital stay, and improve patient satisfaction (Kehlet H. et al. 1994). Considering the relatively low cost of analgesic agents, this type of treatment has a very favorable cost-to-benefit ratio.

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