The effects of iv. lidocaine on inflammatory factors and circulation in burn injured rats

Sif Ólafsdóttir

Thesis for the degree of Master of Science
University of Iceland
Faculty of Medicine
School of Health Sciences

HÁSKÓLI ÍSLANDS

Effect of iv. lidocaine on inflammatory factors and circulation in burn injured rats

Sif Ólafsdóttir

Thesis for the degree of Master of Science

Supervisor: Jón Ólafur Skarphéðinsson

Masters committee: Guðmundur Björnsson and Jóna Freysdóttir

Faculty of Medicine

School of Health Sciences

June 2015

Áhrif lídókaíns í æð á bólguþætti og blóðrás í brunasköðuðum rottum

Sif Ólafsdóttir

Ritgerð til meistaragráðu í líf- og læknavísindum

Umsjónarkennari: Jón Ólafur Skarphéðinsson

Meistaranámsnefnd: Guðmundur Björnsson og Jóna Freysdóttir

Læknadeild

Heilbrigðisvísindasvið Háskóla Íslands

June 2015

Prentun: Háskólaprent ehf.

Reykjavík, Ísland 2015

Ágrip

Sársauki af völdum bruna getur verið óbærileg upplifun, sem fer þá aðallega eftir stærð og dýpt brunasársins. Stórt annars stigs brunasár er mjög alvarlegur áverki sem er ýmsum vandkvæðum bundinn. Þörf er á að gefa stóra skammta af morfíní í æð til að lina sársaukann, sem hefur sínar aukaverkanir, m.a. öndurnarbælandi áhrif. Tilfelli hafa sýnt að lídókaín í æð getur haft kröftug sársaukadeyfandi áhrif í sjúklingum með alvarlegan annars stigs bruna og minnkaða þörf á morfíni. Ekki er vitað hvernig þessi verkjastillandi áhrif lídókaíns er framkölluð, en sýnt hefur verið fram á að lídókaín getur haft víðtæk bólgueyðandi áhrif með því að draga úr myndun og áhrifum bólgumiðlara. Mikil losun verður á cytókínum í brunaskaða en cytókín eru þekkt fyrir að taka þátt í stjórnun ónæmissvarsins og geta haft bein eða óbein áhrif á sársaukaskyn. Því er áhugavert að skoða áhrif lídókaíns á bólguþætti í annars stigs brunatilfelli. Þá eru einnig vísbendingar um að sársaukaminnkandi áhrif lídókaíns gætu verið í miðtaugakerfinu, en ekki verður farið í það nánar hér.

Blóðsýni voru tekin úr svæfðum brunasköðuðum rottum sem fengu lídókaín- eða saltlausn. Annars stigs brunaskaði var framkallaður með því að dýfa aftari limum í 80°C heitt vatn í 10 sek. Styrkur bólguhvetjandi miðlara (IL-1β, IL-6, TNF-α, IL-8, and rCRAMP) sem taka þátt í upphafs bólgusvari, aðrir bólguhvetjandi miðlarar (IL-2, IL-5, IFN-γ) og bólguhamlandi miðlarar (IL-4, IL-10, IL-13) voru mældir í plasma. Auk þess var fylgst með áhrifum lídókaíns á hjartsláttartíðni og meðalslagæðaþrýsting.

Helstu niðurstöður sýndu aukningu í upphafs bólguþáttum (TNF-α, IL-1β, and IL-6) 2 klst eftir brunaskaðann ($p=0,007$; $p=0,007$; $p<0,001$). Hinsvegar tókst ekki að sýna fram á marktæk áhrif af lídókaíni í þeim styrk sem prófaður var, hvorki á cytókínum, rCRAMP/LL-37, hjartsláttartíðni eða meðalslagæðaþrýstingi.

Aukning í upphafs bólguþáttum við brunaskaðann gefur til kynna að brunamódelið henti til að skoða áhrif lídókaíns í æð í annars stigs bruna í rottum. Hinsvegar hafði lídókaín skammturinn 2.0 mg kg^{-1} bólus og 1 mg kg^{-1} $klst^{-1}$ innflæði ekki marktæk áhrif á bólguþætti. Ekki er útilokað að skammtur lídókaíns hafi verið of lágur og frekari rannsóknir þarf til að útiloka þann möguleika.

Abstract

Burn injury is one of the most severe form of trauma and can cause an unbearable pain sensation, which is difficult to manage and has generally been treated with large doses of opioids that can cause severe side effects, such as respiratory depression. Lidocaine given intravenously (iv.) has been shown to have analgesic effect and reduce pain sensation in burn injury patients and reduced opioid requirement. The analgesic effect of iv. lidocaine is poorly understood, but lidocaine has shown extensive anti-inflammatory effects by e.g. diminishing the formation and effects of inflammatory mediators. Burn injury causes the release of a large amount of cytokines which are known to be the main regulators of the immune response and can stimulate nociception directly and indirectly. Thus, it is interesting to study the effects of lidocaine on pro- and anti-inflammatory cytokines in second degree burn injury. That might give insight into some of its analgesic properties. In addition, there is evidence that the effects might be centrally mediated as well, but that is beyond the scope of this study.

In this study, blood samples were collected from anaesthetized second degree burn injured rats, receiving randomized treatment of either iv. lidocaine or saline infusion. The injury was induced by immersing the hind limbs into 80°C hot water for 10 sec. Concentration of acute pro-inflammatory (IL-1β, IL-6, TNF-α, IL-8, and rCRAMP/LL-37), other pro-inflammatory (IL-2, IL-5, IFN-γ), and anti-inflammatory (IL-4, IL-10, IL-13) mediators were measured in plasma. In addition, heart rate (HR) and mean arterial pressure (MAP) were recorded during the experiment.

The results show a significant increase in the levels of TNF-α, IL-1β, and IL-6 ($p=0.007$; $p=0.007$; $p<0.001$, respectively) in plasma, 2 hours after the burn injury. However, there were no significant effects of i.v. lidocaine on cytokine levels, rCRAMP/LL-37 level, HR or MAP.

Increase in the levels of acute inflammatory cytokines suggests that the burn model was effective, and can be used to study the effect of lidocaine (iv.) in second-degree burn injury in rats. However, the iv. lidocaine dose, 2.0 mg kg^{-1} bolus followed by 1 mg kg^{-1} h^{-1} infusion for 60 min, failed to show any significant effects on inflammatory factors or circulation. There is a possibility that the dose might be too low to induce a detectible effect, and therefore further studies are required.

Acknowledgements

This study was supported by the University of Iceland Research Fund. The study was performed at the Department of Physiology, Faculty of Medicine, School of Health Science, University of Iceland, Reykjavik, Iceland, Department of Life and Environmental Sciences, School of Engineering and Natural Sciences, University of Iceland, Reykjavik, Iceland and Department of Aanesthesiology and Intensive Care, Sahlgrenska University Hospital, Möldal, Sweden.

I would like to extend my gratitude to all those who helped me in one way or another in accomplishing this study. First and most of all I would like to recognize Professor Jón Ólafur Skarphéðinsson, my supervisor, thank you for giving me the opportunity, your guidance and inspiration from day one. As well a grateful appreciation to the co-authors Dr. Jean Cassuto and Professor Guðmundur Hrafn Guðmundsson, thank you for your feedback, direction and assistance, as well as access to your time and laboratory. Grateful appreciation is extended to Guðmundur Björnsson and Jóna Freysdóttir, thank you for the feedback and editing skills during the completion of this thesis and for being part of my masters committee. My appreciation is extended to Mr. Nikhil Kulkarni and Hans Guttormsson for their guidance and all the help they provided with the western blot process. Furthermore, I would like to thank Lilja Guðrún Steinsdóttir for her help and assistance during the research. I would like to thank as well all the members of the physiology department and the students and staff at Læknagarður for being my second family through this study. Finally, I would like to thank my family and friends for being patient and for their support, and a special gratitude goes to my boyfriend Anton Sigurðsson for being my mental and financial support, as well as my best friend through all the sweet and sour that this journey offered.

Table of Contents

List of figures

List of tables

List of tables in appendix

List of abbreviations

CAMP	Cathelicidin antimicrobial peptide
COX	Cyclooxygenase
HR	Heart rate
IFN-γ	Interferon gamma
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
ip.	Intraperitoneal
iv.	Intravenous
KC/GRO	Keratinocyte chemoattractant/growth-related oncogene
LA	Local anesthetic
LPS	Lipopolysaccharide
LT	Leukotrienes
MAP	Mean arterial pressure
NO	Nitric oxide
PBST	Phosphate buffer saline Tween
PG	Prostaglandin
PGI	Prostacyclin
rCRAMP	rat cathelicidin-related antimicrobial peptide
SEM	Standard error of the mean
TX	Thromboxane
TNF-α	Tumor necrosis factor alpha

1 Introduction

Acute burn injury pain can be a source of immense suffering (1). Several analgesic drugs are available, depending on the severity of pain being experienced. Non-steroidal anti-inflammatory drugs like ibuprofen and paracetamol are used for pain management in minor burns or as adjuncts to opioids in major burns (2). For centuries, opioids have been used successfully for the treatment of severe pain following trauma but high doses may lead to severe complications, such as respiratory depression and in some cases addiction (3). Case studies have shown that intravenous (iv.) lidocaine can relieve the severe pain in major burn injury patients (4). In 1998, following a major fire in a discotheque in Gothenburg, an 18 year old boy suffering from a severe burn injury was treated with iv. lidocaine for 48 hours postburn. This was done because of the shortage of respirators due to the number of victims from the fire catastrophe. The patient reported no pain and did not require additional analgesics during the period of the lidocaine treatment. After he was transferred to another hospital, lidocaine infusion was discontinued and a morphine treatment initiated. During the next 10 days his analgesic requirements ranged from 200-600 mg morphine per day, initiating the need of a respirator (5). This and other case studies (4-6) thus indicate that iv. lidocaine can be effective in treating burn injury pain, but fear of toxicity by using systemic lidocaine seems to have limited its use (2, 7). The mechanism behind the analgesic effects of iv. lidocaine infusion is not known and it is not likely that the well-known cation-channel blocking properties of the local anesthetic are important in such systemic injection (8-10).

To some extent, pain is induced as a result of the enhanced production and release of inflammatory mediators (11). Cytokines are known to be the main regulators of the immune system and numerous experiments provide evidence that pro-inflammatory cytokines induce or facilitate inflammatory as well as neuropathic pain and hyperalgesia (12). Therefore, it is interesting to see if changes in plasma cytokine levels could give insight into the effects of systemic administration of lidocaine, although central mechanisms may be important as well (10). The aims of this thesis are primarily to develop an effective burn model by inducing second degree burn on the hind limbs of anesthetized rats. Furthermore, to study the effect of lidocaine on selected cytokines, cathelicidin, and circulatory parameters (heart rate (HR) and mean arterial pressure (MAP)) in the anesthetized burn injured rat.

1.1 Innate immune response to burn

Burn injury is initiated when heat stimuli destroys the physical barrier of the skin, providing microorganisms with an easy entry. The increased temperature (higher than 44°C) causes protein denaturation, disruption of cellular components (membrane, protein, organelles), and eventually cell death (13-15). The damaged tissue and invasion of bacteria trigger an immediate acute inflammatory reaction, which is regulated by inflammatory mediators. It is a cascade of events involving vasodilation, increased vascular permeability, as well as transmigration, activation, and chemotaxis of leukocytes (16) (Figure 1). Leukocytes are derived from hematopoietic stem cells that are produced in the bone marrow, they are constantly being produced but their production is increased in inflammation and infection. The inflammatory reaction is regulated by inflammatory mediators (e.g. cytokines, histamine, serotonin, bradykinin, nitric oxide (NO), eicosanoids, complement factors and substance P), which are produced and released by various types of cells, both immune and tissue cells, located at the injured and infected area (17).

Symptoms of inflammation have been described as redness (*rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*), and altered function (*functio laesa*). Symptoms such as redness and heat appear with increased blood supply to the damaged area by vasodilation and increased vascular permeability. The symptoms are induced by vasoactive mediators (e.g. histamine, bradykinin, NO, and substance P). Increased vascular permeability allows fluid and larger molecules than usual to cross the capillaries into the injured area and generate swelling. Leukocytes such as neutrophils and monocytes are attracted to the site of infection and transmigrate out of the capillaries into the damaged tissue by chemotaxis and activation of adhesion molecules. There they can start phagocytosis (18).

The first leukocytes to appear and transmigrate to the damaged tissues are neutrophils. Neutrophils have granules in their cytoplasm, where they store several inflammatory proteins, including large amount of cathelicidin. Cathelicidins are known to contain antimicrobial peptides and are widely distributed in tissues as they are expressed in epithelial cells and monocytes/macrophages (19). Its main function is to fight invaders but it also takes part in several other functions, such as chemotaxis (20, 21), induce release of histamines (22), participate in wound healing (23), induction of angiogenesis (24), induce release of proinflammatory cytokine (25), as well as modulation of the ATP receptor function (26) which has been implicated in inflammatory and neuropathic pain (27).

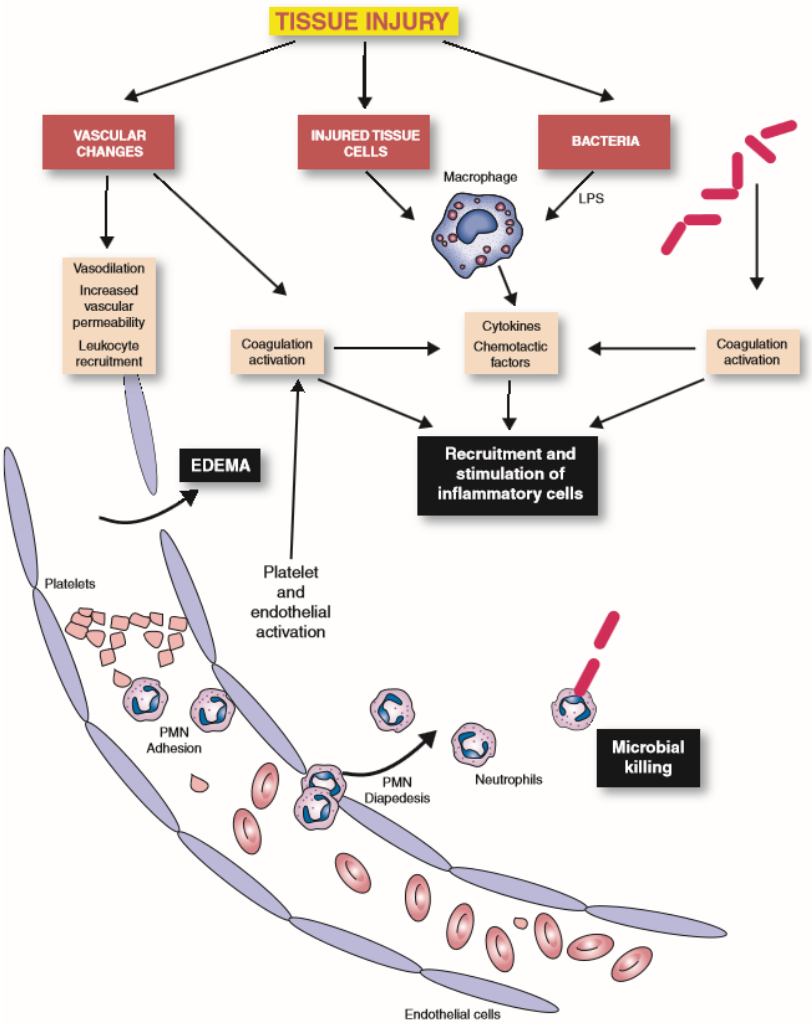

Figure 1. Tissue damage activates the innate immune response by different pathways. They induce the release of inflammatory mediators that act on the endothelial cells and result in vasodilation and increased permeability. Activation of adhesion molecules, priming of neutrophils, and chemotaxis lead to recruitment of neutrophils by crossing the post-capillary venules, ready to start phagocytosis. LPS, lipopolysaccharide; PMN, polymorphonuclear leukocytes (28).

Cytokines are a family of over hundred different proteins (29) produced by local and migrating cells in the inflammatory response. They are the main communicators of the immune system, and act by binding to specific high-affinity cell surface receptors on the target cells. In some cases only a few receptors on a cell need to be activated to elicit an effect. The communicators activate a secondary signal cascade that regulates transcription of number of cellular genes. Cytokines are both multifunctional and redundant and generally act at a short range, either paracrine or autocrine, with some entering the circulation via lymph or plasma (endocrine). The cytokine network is very complex (Figure 2). Various cytokines can be synthesized by the same cell, various cells can synthesize the same cytokine, different cytokines can have similar function, and one cell can have receptors for several cytokines. They can also stimulate expression and release of more cytokines (30), and in some cases they can overlap or have diverse function, depending on their concentration, the target cell and the presence of other cytokines and mediators. Therefore, their activities are tightly regulated,

by controlling the secretion and the expression of receptors. Thus, the information which a cell is exposed to depends on the pattern of cytokines bound to its cytokine receptors, but not by a single cytokine (31).

The central role of cytokines is to regulate, amplify and control the duration of the immune response, as well as controlling the remodeling of tissues, e.g. after inflammation, infection, and wounding. Cytokines regulate the inflammatory response with pro-inflammatory and anti-inflammatory agents. The main functions of pro-inflammatory cytokines in acute response are activating leukocytes, up-regulating adhesion molecule expression, amplifying the release of reactive oxygen intermediates, NO, vasoactive amines, neuropeptides, as well as activating kinins and eicosanoids, which then regulate cytokine release (32). There are several cytokines known to play key roles in mediating acute inflammatory reactions, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α (32). Inflammatory cytokines have been shown to work in sequence, where IL-1 is first to appear in systemic circulation in the inflammatory response after e.g. thermal injury, followed by IL-6 and TNF-α (33).

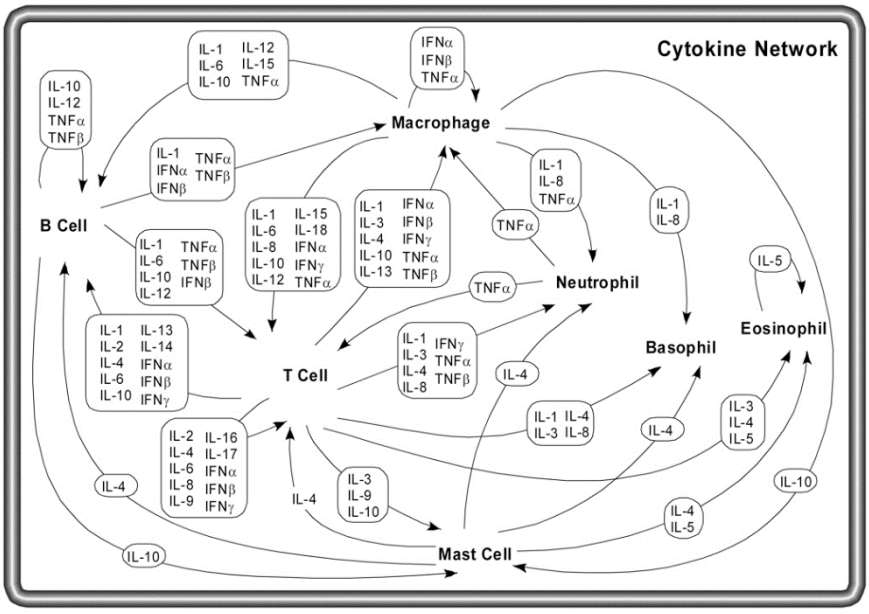

Figure 2. Cytokines are synthesized and secreted by many different types of cells of the immune system, including e.g. B cells, T cells, macrophages, mast cells, neutrophils, basophils and eosinophils, where every cell type has a distinct role in the immune response, and communicates with other immune cells using secreted cytokines. Cytokines boost up and degrade the immune response (30). IL, interleukin; TNF, tumor necrosis factor; IFN, interferon.

One class of cytokines, the chemokines, exhibit a specialized function in inflammation and repair. They have a major role in leukocytes trafficking, recruitment and activation (34). One such chemokine is IL-8 (also called CXCL8) plays a crucial role in acute inflammation by recruiting and activating neutrophils (35). Furthermore, IL-8 increases the production of reactive oxygen intermediates as well as participates in the release of granule enzymes of neutrophils (36, 37), and induces permeability of

endothelial cells (38, 39). Lipopolysaccharides (LPS) have been shown to induce massive elevation of IL-8 plasma levels, reaching maximal levels in 2 h following injection (40). Intradermal injection of IL-8 in rats or rabbits induced accumulation of neutrophils at 2-3 h after injection (41). Activated neutrophils have been observed in the circulation of burn patient shortly after burn injury (42). Plasma IL-8 has been seen to increase in concentration in relation to burn size (42). Of a particular interest are the cytokines IL-1, IL-6, IL-8, and TNF-α, for their involvement in the acute phase inflammatory response (32, 40).

1.2 Pain

Nociceptors are specific receptors on peripheral sensory neurons that are in normal condition only stimulated by pressures and temperatures extreme enough to be potentially harmful. These primary sensory neurons are mainly pseudo-unipolar, with their cell bodies located in the dorsal root ganglions, and a central process extending into the dorsal horn synapsing on an ascending neuron. The peripheral (dendritic) axon branches into the skin, muscles, blood vessels and internal organs. Axon terminals detect noxious stimuli and convey signals to higher brain center, where pain feeling is sensed (43). Nociceptive fibers can be activated by inflammatory acidic environment, heat, pressure, inflammatory mediators, and other chemical factors. Environmental stimuli, such as extreme heat, cold, and mechanical deformation, can only be detected by the peripheral terminals, but both terminals can be affected by endogenous molecules that enhance nociceptor sensitivity, such as by pH, prostaglandin, and neurotransmitters peptides (44). At the injured and infected area, inflammatory mediators, such as protons, ATP, bradykinin, prostaglandin, and cytokines, are released and cause enhanced response of nociceptors by lowering the action potential threshold and thereby enhance pain sensation (44, 45). In addition, neuropeptides such as substance P and calcitonin gene related peptide that are released via axon reflexes following tissue injury can sensitize or stimulate nociceptors (46, 47).

There are two major classes of nociceptive fibers: Aδ and C fiber, classified by its morphology and function. Aδ fibers have a medium diameter myelinated afferents that mediate acute, well-localized fast pain. Most nociceptors are C fibers, which have unmyelinated, small diameter axons and convey slow pain (43, 44). These fibers can be subdivided further depending on their reaction to stimuli. Nociceptors can also be divided by expression of channels; sensitive to heat, (the transient receptor potential cation channel subfamily V member 1 (TRPV1) also known as the capsaicin receptor), cold (transient receptor potential cation channel subfamily M member 8 (TRPM8) also known as the menthol receptor), acidic (acid-sensing Ion channels (ASICs)), and chemical irritants (transient receptor potential cation channel, subfamily A member 1 (TRPA1)).

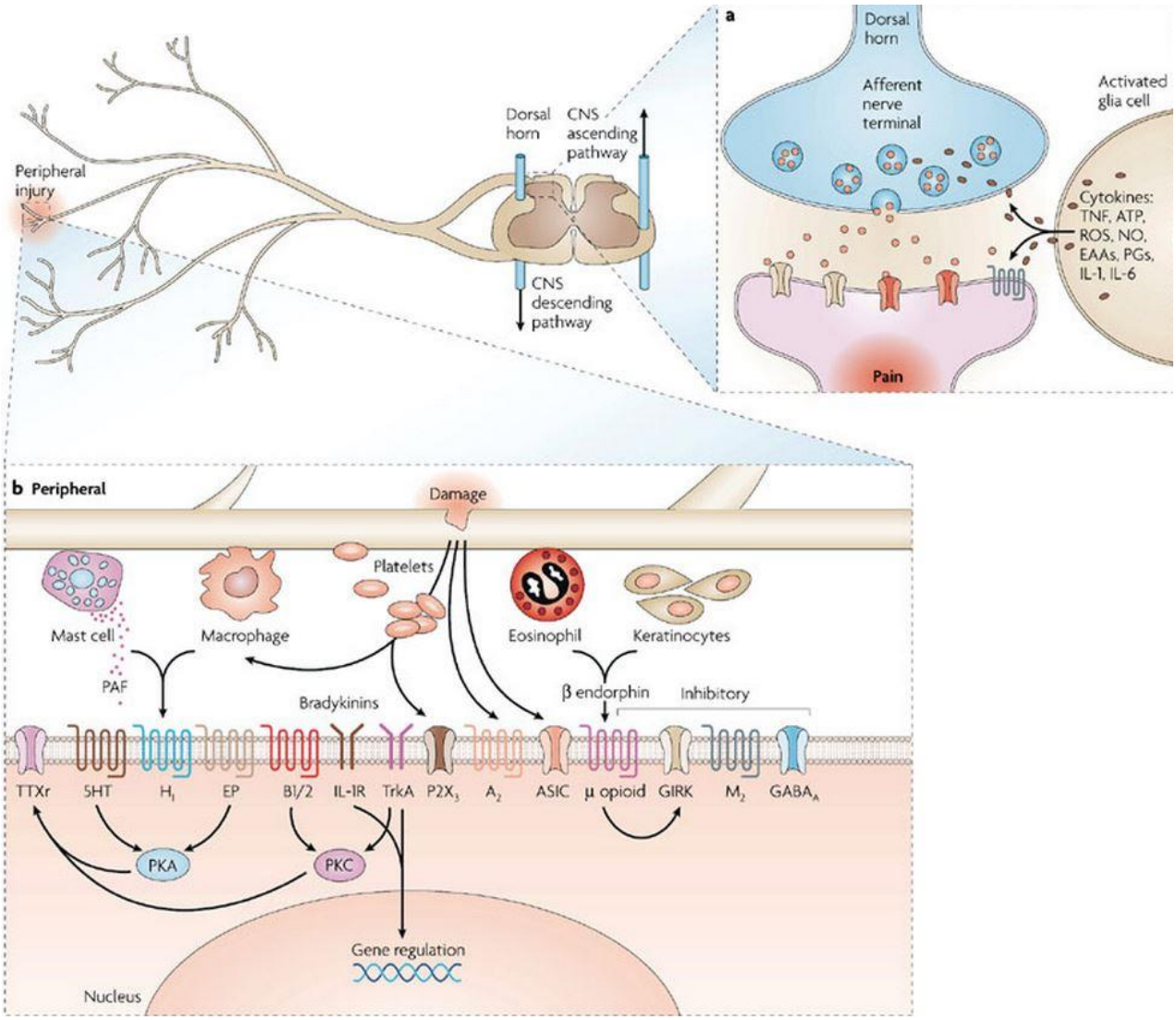

Figure 3. Environmental stimuli or inflammatory agents trigger action potentials by activating various receptors on nociceptive fibers. The signal travels to the dorsal horn of the spinal cord, where interneurons GABAergic/glycinergic- and enkephalinergic neurons can suppress, and astrocytes and microglia can enhance the signal, before it travels to higher centers of the brain, where pain is felt. a) Showing examples of receptors and ion channels in the dorsal horn that are activated by excitatory mediators released by activated glial cell following nerve injury. b) Pain transduction after tissue injury. Inflammation leads to release of numerous chemicals from various cells e.g. mast cells, macrophages, and injured cells that act directly or indirectly to sensitize receptors and ion channels on peripheral nerve terminals. These receptors release secondary messengers such as protein kinase A (PKA) and PKC that can activate other membrane bound receptors and gene transcription. A_2, adenosine A_2 receptor; ASIC, acid-sensing channels; B1/2, bradykinin receptors 1 and 2; CNS, central nervous system; EAAs, excitatory amino acids; EP, prostaglandin E receptor; GABA, γ-aminobutyric acid; GIRK, G-protein-coupled inwardly rectifying K^+; H_1, histamine H_1 receptor; 5-HT, 5-hydroxytryptamine; IL, interleukin; IL-1R, interleukin 1 receptor; M_2, muscarinic M_2 receptor; NO, nitric oxide; $P2X_3$, purinergic receptor X_3; PAF, platelet-activating factor; PGs, prostaglandins; ROS, reactive oxygen species; TNF, tumor necrosis factor; TTXr, tetrodotoxin receptor; TrkA, tyrosine receptor kinase A (48).

Over the last decades evidence has accumulated that the release of several classic hyperalgesic mediators during acute and chronic inflammation is mediated by a cascade of cytokines produced by local or migrating cells (49, 50). Cytokines and their effects on pain have been intensely investigated. Several *in vivo* studies report that cytokines activate and sensitize pain fibers (51, 52). They have been shown to modulate receptors playing a key role in pain sensation on afferent nerve fibers, by up-regulating inflammatory mediators, such as prostaglandins, nerve growth factor, bradykinin, and extracellular protons (12, 44). Furthermore, the direct effect of cytokines on nociceptors has also been described (51). However, little is yet known about the role of cytokines in burn injury pain (53). Other trauma injuries have been studied more deeply in relation to cytokine involvement in pain, such as surgery (54), where one study reports that reduction of pro-inflammatory cytokines by cytokine inhibitor in the pre-operative period resulted in less severe pain in the post-operative period (55). The main pro-inflammatory cytokines participating in the process of pathological pain are IL-1β, IL-6, and TNF-α, whereas the anti-inflammatory cytokines IL-4, IL-10 and IL-13 have been shown to have analgesic properties, by preventing or diminishing the effects of the pro-inflammatory cytokines (12, 30, 56).

Cunha and Ferreira (56) concluded in their review that cascade of inflammatory cytokines is induced by e.g. injury of local cells, and release of local inflammatory mediators, such as eicosanoids, amines, peptides and cytokines themselves. The release of these mediators induces hyperalgesia by sensitizing primary sensory neurons. The cytokine cascade can be initiated by the release of TNF-α from local cells, which in turn acts upon local or migrating cells which release IL-1β (through release of IL-6) and IL-8. These cytokines then induce eicosanoids formation and release of amines, respectively. Low dose of the endotoxin LPS indirectly releases TNF-α by activation of kinin system, while higher doses directly release TNF-α. IL-β can in some nociceptive models stimulate the release of nerve growth factor from mast cells, which indirectly causes hyperalgesia through leukotriene B4 (LTB_4). Analgesic cytokines or anti-inflammatory cytokines can reduce inflammatory hyperalgesia, as well as interleukin-1 receptor antagonist (IL-1RA) that are released later or together with the acute inflammatory response (49).

1.3 Lidocaine

Lidocaine is a well-known local anesthetic, originally derived from cocaine which was the first local anesthetic (LA) agent to be widely used. Its anesthetic effects were recognized by the lack of feeling and taste when placed on the tongue. In 1884, Carl Koller reported that cocaine caused insensitivity to pain of the corneas. The same year, William Halsted used it as a regional anesthesia in dentistry. Several harmful effects were observed with the use of cocaine, leading to development of other LAs, both ester and amide-based (57). Lidocaine is an amid-based agent derived in 1943 by a Swedish chemist, Nils Löfgren (58). Besides from being a known local anesthetic, it has also been used to treat certain ventricular arrhythmias (59, 60).

The local anesthetic properties of lidocaine prevent action potential by blocking fast sodium channels and thereby prevent action potentials in sensory neurons. Lidocaine has an amphipathic structure, where one end of the molecule has a lipophilic aromatic ring structure and the other end a

hydrophilic amino group (Figure 4). It has an intermediate linkage that provides a separation between the lipophilic and hydrophilic end. It has a sufficient water solubility to avoid precipitation when injected into interstitial fluid, and is able to penetrate the hydrophobic nerve sheath and axolemma with satisfactory lipid solubility. Free base and charged acidic forms can block sodium channels (61).

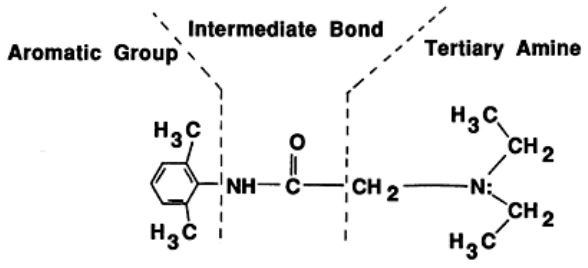

Figure 4. Lidocaine structure, one end of the molecule has a lipophilic aromatic ring and the other end a hydrophilic amino group.

Intravenous lidocaine has been proposed to relieve different types of severe pain (62), such as neuropathic pain (63), postoperative pain (64-67), hyperalgesic pain (68), and chronic pain (69). Furthermore, lidocaine has been reported to shorten hospital stay (70, 71), promoting analgesia in surgical procedures (acute pain) (72), decreased symptoms of chronic inflammatory conditions, such as interstitial cystitis (73) and ulcerative proctitis (74). Thus, analgesic effect of lidocaine in these inflammatory diseases might therefore indicate that its pain relieving effect might be due to the anti-inflammatory properties of lidocaine (6). However, these properties have not been well described, and since effects of lidocaine on sodium channels can in many cases be ruled out, researchers have pondered on the possible effects. Several mechanisms of LAs action on inflammatory cells have been proposed. It includes lidocaine interfering with cyclic adenosine monophosphate, G protein-coupled receptors, nicotinamide adenine dinucleotide phosphate, Na+-H+ exchanger, and protein kinase C (75). Furthermore, anti-inflammatory effect of lidocaine has been linked with inhibition of signaling pathways, such as mitogen activated protein kinase and nuclear factor kappa B (76-80).

Lidocaine has gained interest as a potential drug for pain relieving in burn injury patients. Thus, systemic lidocaine has been reported to be effective in treating burn injury in numerous case studies and experimental studies where it improved the analgesic effect and diminished the requirement of opioid administration in burn injuries (5, 7, 10, 81). However, another study has failed to show its analgesic effect (82), which might be explained by the dose of the lidocaine.

When lidocaine is given iv. the patient needs an effective dosage that rapidly produces therapeutic blood levels without causing toxicity. That usually involves a bolus dose followed by a steady infusion to maintain a certain plasma concentration. Safe and effective plasma level is considered approximately 2-6 µg ml^{-1}, with a plasma elimination half-life of 1-2 h (59, 83-85), as lidocaine is metabolized rapidly by the liver (61, 86). Lidocaine can depress the central nervous system in a dose-dependent manner. Low serum concentration is used clinically for suppressing cardiac dysrhythmias and status seizures, but higher concentration induces seizure activity. Therapeutic antiarrhythmic

effects in humans are normally within plasma concentration of 5 μg ml^{-1}. However, lidocaine can generate seizure, coma, and death at higher levels, approximately 15-50 μg ml^{-1} (87). A state of chronic pain responded to iv. lidocaine at plasma concentration of 2-7 μg ml^{-1} (88, 89), and more recently chronic cancer pain has been treated for periods of several months with infusion of lidocaine, achieving plasma concentration of 3-15 μg ml^{-1} (90).

In the last two decades lidocaine has been shown to interfere with various inflammatory processes (7, 91). Several studies both *in vitro* and *in vivo* have shown that lidocaine attenuated immune cell function, such as leukocytes mobility (92), adhesion and migration of leukocytes (7, 93), and activation and function of neutrophils (94, 95), as well as reduce granulocyte phagocytosis (96, 97). Studies have shown that lidocaine inhibits the release of eicosanoids, prostaglandin E_1 (PGE_1), PGE_2, $PGF_{2\alpha}$, thromboxane A_2 (TXA_2), TXB_2, PGI_2, 6-keto-$PGF_{1\alpha}$ and LTB_4 (see review (7)). Furthermore, iv. lidocaine has been shown to inhibit expression of mucosal cyclooxygenase 2 (COX-2) enzyme in horses with ischemia-injured jejunum (98), but expressions of COX-2 enzymes is dramatically increased in inflamed tissues. Additionally, nonsteroidal anti-inflammatory drugs like aspirin are known to act through inhibition of COX enzymes (99). Furthermore, iv. lidocaine inhibits the release of histamine from mast cells (100), and affect the free radical production by decreasing NO synthase expression through suppressing the activation of its transcriptional factor (101). Therefore, it can be said that lidocaine has an antioxidant effect. Moreover, in *in vitro* studies, lidocaine has been found to inhibit the release of cytokines, such as IL-1, from human leukocytes activated by LPS and zymosan (93), inhibit IL-8 and IL-1β secretion, both in non-stimulated and TNF-α-stimulated epithelial cell lines, and stimulate the release of the anti-inflammatory agent IL-1RA (102). Moreover, lidocaine decreased cell injury induced by cytokines (IL-1β TNF-α, and INF-γ), both in vascular smooth muscle cells and human microvascular endothelial cells, and increased cell survival in both cell types (103). *In vivo* studies on lidocaine also reported inhibition of cytokine release, such as IL-1β and TNF-α, after hyperoxia-induced acute lung injury in rabbits (104). In addition, lidocaine up-regulated the antimicrobial peptide CRAMP (cathelicidin) in mice with zymosan-induced peritonitis (105).

When taken together, in the view of the complex functions of cytokines and their role in inflammatory responses and nociception, as well as the documented effect of lidocaine on inflammatory processes, it is possible that the observed analgesic effect of iv. lidocaine in second-degree burn injury may be related to changes in pro-inflammatory and anti-inflammatory agents.

2 Aims of the study

In view of the apparent pain-relieving effect of iv. lidocaine after severe burn injury in humans and its documented effects on inflammatory responses, we ran our experiments to evaluate the effects of lidocaine on plasma cytokines in a second degree burn model in anesthetized rats. Thus, we were interested in:

1. developing a burn model to study the effect of lidocaine on different factors that might explain its analgesic effect.
2. determining whether iv. lidocaine has an effect on changes in plasma levels of pro- and anti-inflammatory cytokines in the model that could give insight in the proposed analgesic effect of the drug in burn injury.
3. determining whether lidocaine (iv.) has an effect on HR and MAP in the model.

3 Materials and methods

All animal procedures were approved by the Experimental Animal Committee, Ministry for the Environment in Iceland (Licence number: 0312-1501).

3.1 Experimental animals

Male Wistar Han® rats (Taconic Europe, Ejby, Denmark) weighing 300-450 g were used throughout the study. Upon arrival rats were acclimatized for at least 7 days before used in the experiments. They were housed three to five per cage in a controlled environment; temperature 22-26°C, humidity 45-65% and a reversed light/dark cycle 12/12 hours. All rats had free access to dry food (RMI (E) rat food from SDS (Special Diets Services), Essex, England) and drinking water, until anesthetized.

3.2 Anesthesia and basal surgical preparation

Anesthesia was induced with methohexital sodium (Brevimytal®, Hikma *Farmacêutica S.a.,* Estrada do Rio da Mó, Terrugem, Portugal), 70 mg kg^{-1}, intraperitoneal injection (ip.), and maintained with thiobarbital (Inactin®, Sigma Aldrich Co., St. Louis, MO, USA) 166 mg kg^{-1}, ip. injection. Rectal temperature was continuously monitored and maintained at 38°C by a heating plate. The animals were prepared with catheters in the tail artery (PE25), connected to pressure sensor for measuring blood pressure and heart rate, recorded in LabChart (AD Instruments Ltd, Oxford, UK), and a constant infusion of 0.9% saline (1 mL h^{-1} of 9.7 g NaCl L^{-1} solution). The animals were tracheotomized (PE240), for easy breathing, and *vena jugularis externa* was catheterized (PE25) for blood sampling and iv. injections.

The choice of the anesthetic agent, Inactin, was based on its extended and stable anesthesia, with a minimal change in basal mean arterial pressure, heart rate, and renal sympathetic nerve activity (106, 107).

3.3 Experimental procedure

Following preparation, at least one hour recover period was allowed. At the end of that period control recordings were made for 15 min. Then, to induce a secondary degree burn and inflammatory response, the animals were scalded by immersing the hind paws into 80°C hot water for 10 sec. Just before scalding, anesthesia was enhanced with 0.05 mL 1.5% w/v Brevimytal bolus iv. Following scalding, 60 minutes were allowed before the first blood sample. The animals were first injected with iv. heparin (Heparin, Leo Pharma A/S, Ballerup, Denmark) 100 AE/300 g. Then, 1 mL of blood was collected from the venous catheter and replaced with an equal amount of 0.9% saline solution. Following this, the animals received a random treatment, either lidocaine as a bolus (2 mg kg^{-1}) followed by an infusion (1 mg kg^{-1} h^{-1}) or 0.9% saline solution, all iv. After 60 min infusion the second blood sample was collected (2-4 mL).

After each experiment, the animal was decapitated in a guillotine, head and right hind limb were immediately frozen (N_2) and stored at -80°C for further research. Blood samples were kept on ice, centrifuged (5000 rpm for 10 min) and plasma samples were frozen and kept at -80°C until analyzed.

3.4 Analysis

3.4.1 Multiplex cytokine assay

Plasma samples were transferred to Gothenburg, Sweden and stored there at -80°C until analyzed. MSD® Multi-spot assay system was used to measure pro- and anti-inflammatory cytokines (IFN-γ, IL-1β, IL-10, IL-13, IL-2, IL-4, IL-5, IL-6, KC/GRO (keratinocyte chemoattractant/growth-related oncogene, the rat homologue for IL-8) (108, 109) and TNF-α) according to the manufacturer's instructions (Meso Scale Discovery (MSD®), Rockville, MD, USA).

MSD MULTI-SPOT cytokine assay with 96 wells and 10-spot plate was used. Each plate contained 10 spots and each spot had a capture antibody for a particular cytokine assay. The assays are independent of one another and each is optimized for maximum performance in detecting the particular cytokine.

Reagents were prepared according to the manual instructions (Pro-inflammatory Panel 1 (rat) Kits, MSD® Multi-Spot Assay system). The plate was pre-coated with capture antibodies and needed no additional preparation. Blocker was added (150 µL/ well) and the plate sealed and incubated at room temperature with shaking for an hour. Blocking prevents nonspecific binding and thereby reduces background. Next step was washing the plates 3 times with at least 150 µL/well of wash buffer and adding the prepared diluted samples, calibrator, and control (50 µL/well). The plate was then sealed and incubated at room temperature with shaking for 2 hours. Again the plate was washed 3 times as before. Detection antibody solution was added (25 µL/well), and the plate sealed and incubated at room temperature with shaking for 2 hours. The plate was washed 3 times as before before adding 150 µL of 2X Read Buffer to each well. Finally, the plate was analysed in the MSD instrument.

3.4.2 Western blot

Western blot analysis was used for measuring rCRAMP plasma concentration. The protein concentration was determined by measuring optical density in a photo-spectrometer, with Bradford protein assay (110). Reagents were prepared according to NuPAGE® Bis-Tris Mini Gel electrophoresis protocol (Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples and the rCRAMP positive control were electrophoresed on 4-12% gradient SDS-PAGE gels for 60 min at 120 V. Proteins were blotted onto a nitrocellulose membrane (with 0.2 µm pore size for low molecular weight proteins) for 70 min at a 25 V. After transfer, the membrane was washed with phosphate buffer saline containing 0.05% Tween (PBST) and incubated in a blocking buffer (PBST, 5% non-fat dry milk) for 1 hour with shaking at room temperature. Following blocking, membrane was washed with PBST before incubating with primary antibody (1:3500 rCRAMP, diluted in 2.5% milk (PBST, 2.5% non-fat dry milk)) overnight with shaking at 4°C.

Next day the membrane was washed five times with PBST, before incubattion with horseradish peroxidase (HRP)-conjugated secondary antibody, diluted in 2.5% milk, for 1 hour with shaking at room temperature. The membrane was washed three times with PBST and once with water before adding the detection reagent. The detection reagent activated the HRP-conjugated secondary

antibody, which generated light that was captured with a digital image of a chemiluminescence (ImageQuant™ LAS 4000, GE Healthcare Bio-Sciences AB, Björkgatan, Uppsala, Sweden).

Nitrocellulose membrane was stained with Ponceau S dye for loading control. Neither creatine nor albumin could be used as a loading control, since lidocaine affects their concentration (111, 112). Densitometric analysis of the blots was done by using Image Quant TL software (GE Healthcare Bio-Sciences AB, Björkgatan, Uppsala, Sweden).

3.4.3 Blood pressure and heart rate

Effects of treatments on MAP and HR were analyzed. Mean values for each parameter were calculated from 30s periods in the Lab Chart records, just before blood samples were collected.

3.4.4 Statistical evaluation

Data are presented as mean $\pm$ standard error of the mean (SEM) for each treatment group. Two-way ANOVA for repeated measurements with Bonferroni post hoc test, was used to analyze factor effects within and between groups, as well as the interaction between factors, for cytokines, blood pressure and heart rate. An independent samples t-test was used to compare the levels of rCRAMP in plasma of burn injured rats between treatment groups. Effects were considered significant if $p<0.05$. All statistical tests were performed in SPSS.

4 Results

4.1 Plasma levels of cytokines and rCRAMP

The results of the cytokine measurements are presented in Tables 1, 2 and 3. Calculated changes of cytokines in the groups are presented in Tables A1, A2 and A3 (see appendix) and for rCRAMP in Fig. 6. A great variation was observed in the plasma levels of both cytokines and rCRAMP. Statistical analysis showed a significant main time effect for TNF-α, IL-1β, and IL-6 ($p=0.007$, $p=0.007$, $p<0.001$, respectively; see Fig 5). Changes in other cytokines and rCramp (Fig 6) were not significant. The interaction between the time and group factors was not significant for any parameter and thus only main effects were analysed.

Table 1. Plasma concentration of cytokines before and after saline treatment.

Inflammatory mediators (ng/mL)	Before	SEM	After	SEM
IFN-γ	97.4	4.8	90.7	2.5
IL-10	441.5	13.8	444.5	14.5
IL-13	95.0	2.3	95.2	1.7
IL-1β	365.8	37.9	396.1	23.5
IL-2	1491.7	47.8	1439.9	29.2
IL-4	17.7	0.7	16.4	0.3
IL-5	189.6	15.8	178.8	6.3
IL-6	765.7	79.7	1695.2	462.1
KC/GRO	6776.1	1393.6	7069.9	1315.5
TNF-α	83.0	19.6	152.4	54.0

The data are shown as mean ± SEM with n=16.

Table 2. Plasma concentration of cytokines before and after lidocaine treatment.

Inflammatory mediators (ng/mL)	Before	SEM	After	SEM
IFN-γ	85.0	4.0	84.3	4.9
IL-10	424.2	10.5	417.7	10.6
IL-13	90.7	4.6	89.6	4.2
IL-1β	293.9	20.8	397.4	28.9
IL-2	1447.9	41.7	1497.2	28.8
IL-4	16.3	0.5	16.4	0.5
IL-5	180.0	8.9	191.4	11.2
IL-6	774.9	88.0	1823.8	332.9
KC/GRO	8540.3	2267.8	10429.4	2223.8
TNF-α	60.4	10.7	144.5	38.8

The data are shown as mean ± SEM with n=14.

Table 3. Change in plasma concentrations of cytokines for saline and lidocaine treatment before and after treatment (delta Δ).

Inflammatory mediators (ng/mL)	Saline Mean (n=16)	SEM	Lidocaine Mean (n=14)	SEM
ΔIFN-γ	-6.7	4.8	-0.7	2.9
ΔIL-10	3.0	19.5	-6.5	7.4
ΔIL-13	0.2	2.8	-1.1	2.1
ΔIL-1β	30.3	37.6	103.5	18.1
ΔIL-2	-51.8	44.4	49.3	49.5
ΔIL-4	-1.3	0.7	0.1	0.4
ΔIL-5	-10.8	16.3	11.5	12.1
ΔIL-6	929.5	372.3	1048.9	267.9
ΔKC/GRO	293.8	728.6	1889.1	1515.8
ΔTNF-α	69.4	34.3	84.1	38.3

Data are expressed as mean difference in concentration (after-pre) ± SEM, with n=16 for saline group and n=14 for lidocaine group.

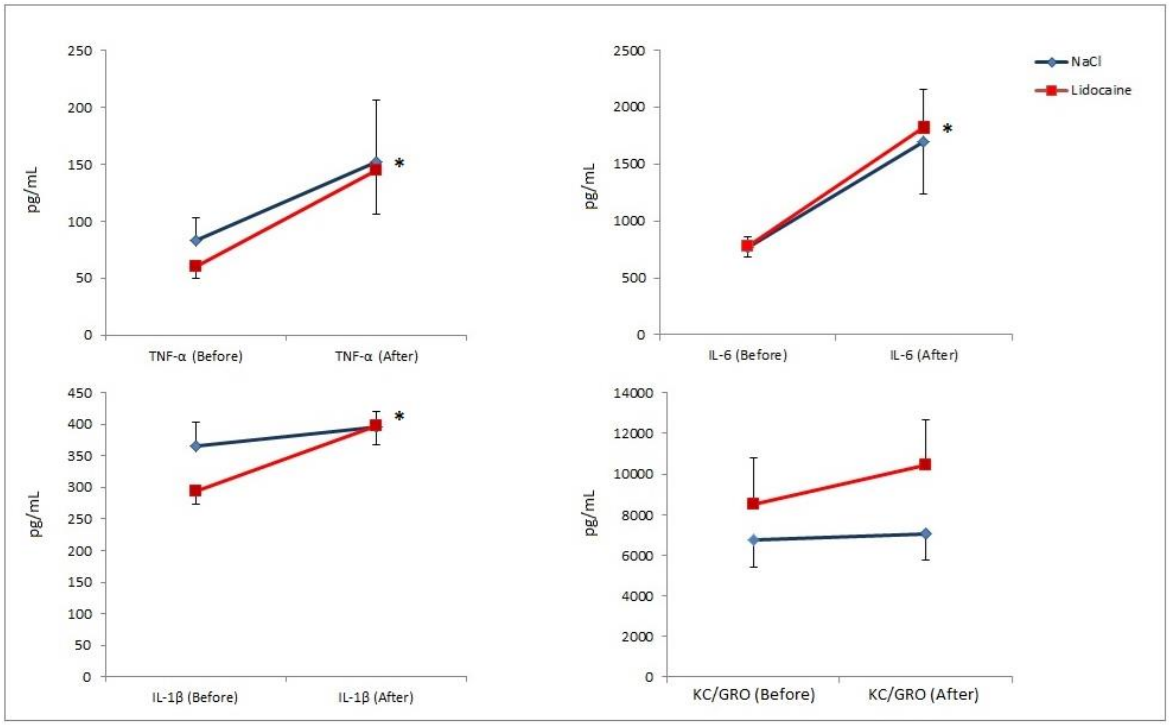

Figure 5. Plasma concentration for selected cytokines in burn injured rats treated with either iv. saline or lidocaine. The main time effect for the increase in TNF-α, IL-1β, and IL-6 was significant (*p=0.007, *p=0.007, *p<0.001, respectively). The data are shown as mean ± SEM, n=16 and 14 in the saline and lidocaine groups, respectively.

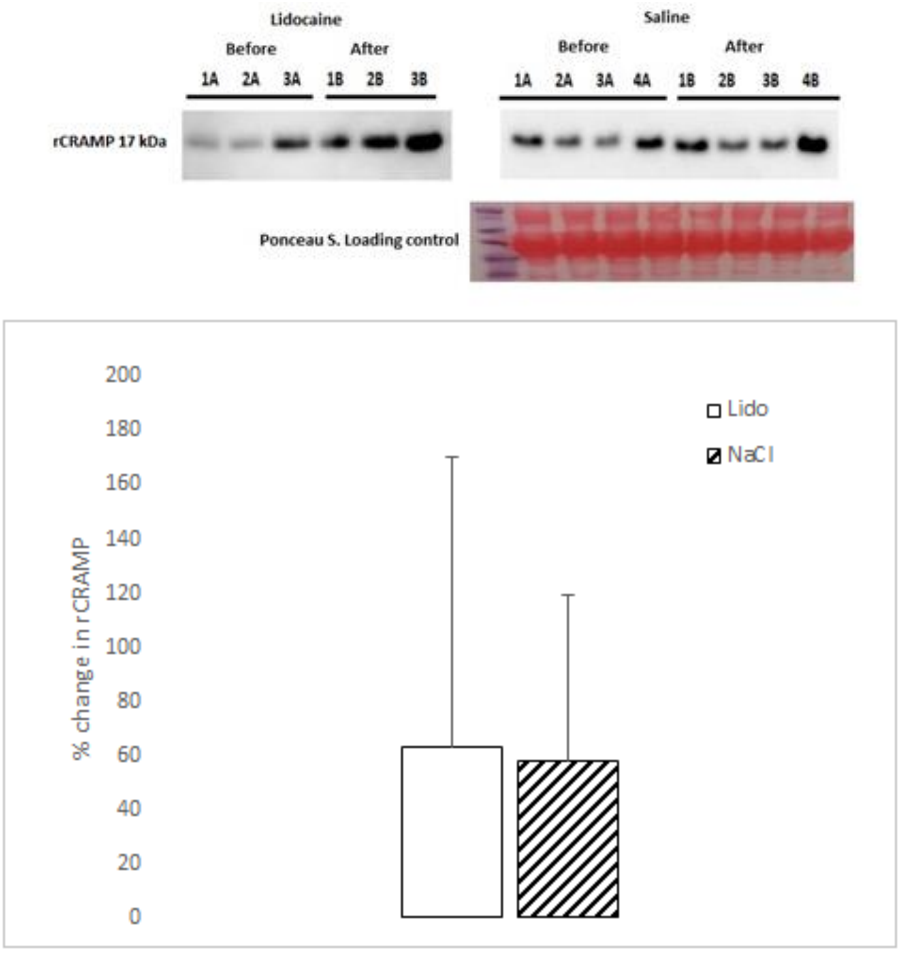

Figure 6. Western blot analysis shows the level of rCRAMP (17 kDa) measured in plasma in burn injured rats treated with either iv. saline or lidocaine. An illustrated example of the blots and loading control (Ponceau S.) are shown. Bars represent percentage change between before and after treatment, in saline treated rats (n=15) and lidocaine treated rats (n=10). Data are expressed as mean ± SEM. Densitometric analysis of the blots were done by using Image Quant TL software.

4.2 Changes in blood pressure and heart rate

Figure 7 shows HR and MAP just before blood samples were taken in the two treatment groups. There was a significant main group effect in HR (p=0.03) indicating lower HR in lidocaine treated animals. For MAP there was a significant main time effect (p<0.001) indicating lower MAP when the second blood sample was collected. However, the group*time interaction was not significant for either parameter indicating similar change in both groups (Table A3, see appendix).

Table 4. HR and MAP before and after 60 min drug treatments.

Mean	Before infusion 1	SEM	After infusion 2	SEM
NaCl HR (bpm)	404.1	9.7	403.2	7.0
Lido HR (bpm)	384.6	6.5	371.0	13.1
NaCl MAP (mmHg)	105.0	4.5	95.3	3.2
Lido MAP (mmHg)	100.5	3.5	94.4	2.2

Data are expressed as mean ± SEM with n=16 for saline group and n=12 for lidocaine group.

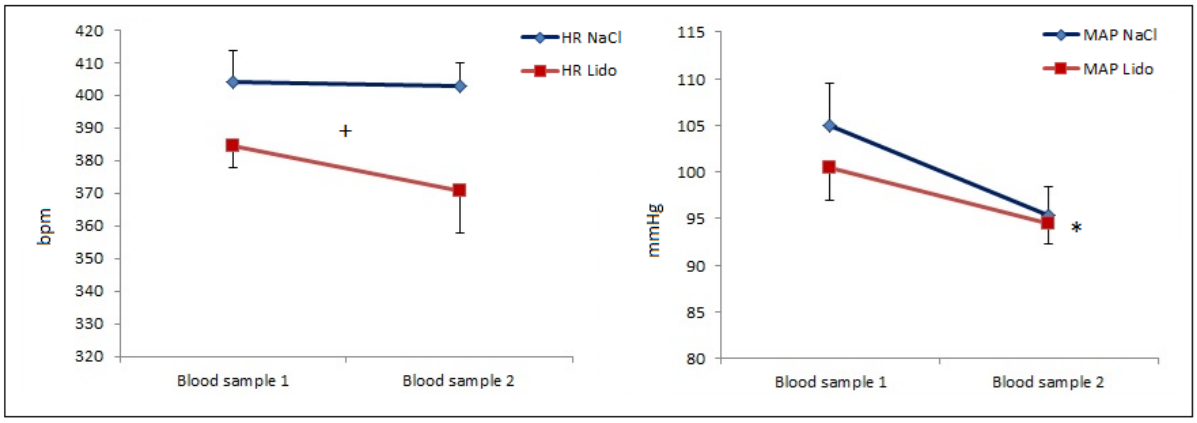

Figure 7. Heart rate (HR) and mean arterial pressure (MAP) measured at two time points, just before blood samples were collected. The first blood sample was taken 60 min after burn injury and the second sample 60 min later. Data are expressed as mean ± SEM, (NaCl n=16, Lido n=12), + p=0.03 main group effect. * p<0.001 main time effect.

5 Discussion

The primary goal of this study was to develop a second-degree burn injury model in rats, which could be used to study the effects of iv. lidocaine on the inflammatory response. The results presented in this thesis indicate that the burn injury model was effective. This could be seen by an increase in plasma levels of the cytokines IL-1β, IL-6, and TNF-α, known to participate in early inflammatory response.

The second goal was to examine changes in cytokine- and cathelicidin levels in plasma due to systemic administration of lidocaine (bolus dose of 2.0 mg kg^{-1} followed by an infusion of 1 mg kg^{-1} h^{-1} for 60 min). No significant difference in changes in cytokines or rCRAMP concentration between saline and lidocaine treated rats were observed.

The third goal was to examine possible effects of lidocaine treatment on HR and MAP. MAP showed a significant decrease in both treatment groups ($p<0.001$, time main effect), but no significant difference were observed between treatments. HR showed no significant changes in time, but was generally lower in the lidocaine treated animals ($p=0.03$, group main effect) without any significant interaction between the treatment factors (time*drug). Thus, it can be concluded that iv. lidocaine treatment did not have significant effect on HR and MAP in the anaesthetized burn injured rats.

5.1 Validation of the burn model

The extent of burn damage depends on both the temperature and the duration of its exposure. The first animal burn model for research was the boiling water burn model described by Mason and Walker over 40 years ago (113), where they reported that 10 sec exposure produced a full-thickness burn, and 3 sec a partial-thickness burn. This model has since been effectively used with some modulations, e.g. in studies on burn infection (114). One study used 90°C hot water for 6 sec to induce second-degree burn injury on the back of the rats, first shaving the area that was burned (115). Another study used similar method by scalding the back in 70°C hot water for 12 sec to induce deep dermal burn (116). Another study induced second-degree injury by immersing one hind-paw into 70°C hot water for 8 sec (117). Studies done by Wang S. et al. (118), found that minimum time for hind-paw immersion in rats, that is effective to produce a first, second, and third-degree burn injury in 85°C water were 4, 7, and 12 sec, respectively. In the present study, duration and temperature to obtain second degree burns were based on these results. Thus, it was assumed that by immersing both hind-legs of a rat into 80°C hot water for 10 sec, would be effective to obtain second-degree burn injury of the extent that changes in inflammatory agents could be detected in plasma.

Second-degree burn injury induces a vigorous innate immune response, resulting in great increase in inflammatory mediators, such as cytokines. The cytokines are released by immune cells, such as macrophages, as well as by a variety of other cell types, such as endothelial cells located at site of injury. Extensive tissue injury may lead to the spillover of such mediators into the bloodstream (16). Early pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, are detectable in plasma after thermal injury in rats (33). Our results show a significant increase of these cytokines suggesting that our burn model probably is effective and can be used to study the effect of lidocaine (iv.) on

inflammatory mediators in second-degree burn injury in rats. However, this has to be further tested specifically.

There were no significant changes in IL-8 concentration in this study although activated neutrophils have been observed in the circulation of burn patient shortly after burn injury (42). It is possible that it can be explained by the time factor or the extent of the burn injury.

5.2 Effect of lidocaine on inflammatory cytokines

Hyperalgesia induced by mechanical stimulation at site of injury, is the major source of severe pain after a burn injury (1). Burn causes tissue damage, which triggers an inflammatory cascade. Excessive release of inflammatory mediators, such as cytokines, can induce an activation of Aδ- and C- nociceptive fibers as well as maintenance of peripheral and central hyperalgesia (119). Lately, iv. lidocaine has been used to treat hyperalgesia and pain, such as acute and chronic neuropathic- and inflammatory pain (6, 63, 65) as well as pain from burn injury (4, 5). The mechanisms of the analgesic ability of iv. lidocaine are poorly understood, but both peripheral- and central nervous system effects have been proposed (90). Thus, lidocaine may depress conduction of Aδ- and C-fibers by interacting with receptors and ion channels, modulate inflammatory processes, as well as inhibit dorsal horn neuronal transmission, and/or modify the cerebral perception of pain (7, 62, 75, 91, 120, 121). In postoperative pain, analgesic effect of lidocaine may partly be due to blockade of fast voltage gated sodium channels on nociceptor nerve endings, which are sensitive to low doses of lidocaine, both in the dorsal horn of the spinal cord and in the dorsal root ganglion (122). Also, lidocaine blocks voltage-gated and voltage-independent potassium channels (123) as well as calcium channels (124) both directly and indirectly (125). Lidocaine interacts with a G protein-coupled receptor, that modulates potassium and calcium channel function. However, a much greater concentration of lidocaine is necessary to block potassium and calcium channels than is needed for sodium channel blockade (123, 124).

Cytokines have a major effect on the inflammatory processes and the release of various mediators in the inflammatory soup that can stimulate nociceptors and cause pain (30). Thus, cytokines can sensitize nociceptors directly as well as indirectly by triggering the release of more mediators sensitizing the nociceptors (126). As several studies, both *in vitro* and *in vivo*, have shown that lidocaine reduces the release of acute pro-inflammatory cytokines (93, 102, 104), as well as many inflammatory processes (7), our hypothesis is that systemic administration of lidocaine may decrease pro-inflammatory cytokines plasma levels in the acute inflammatory response, which could explain to some extent its apparent analgesic effect in severe burn injury. However, contrary to our hypothesis, iv. lidocaine in our burn model failed to show any significant effects on cytokine levels. This might be explained by the size of the dose which might not have been large enough to induce a detectible effect.

It is well known that high systemic doses of lidocaine are toxic. Widespread sodium channel blockade in the body is obviously very dangerous and can induce seizures and cardiac arrest (87). Souza et al. (69) concluded from their studies of the analgesic effect of iv. lidocaine in chronic pain that it is not possible to uniformly specify the most effective and safe dose of iv. lidocaine for the treatment of neuropathic or musculoskeletal pain (69) and the same seems to be with burn injury pain. At least there is no information on the specific therapeutic concentration effective for treating severe

burn injury pain. The lidocaine doses used in studies differ widely, depending on the method of administration (75) as well as the type of the study. It has been shown that in order to achieve systemic antiarrhythmic effects after iv. administration of lidocaine in humans, its plasma level needs to be at least 0.5–5.0 μg mL^{-1}, while concentration of lidocaine in plasma above 10 μg mL^{-1} can cause adverse effects (75). *In vivo* study in rabbits showed that iv. lidocaine with a bolus dose of 2 mg kg^{-1} followed by 2 mg kg^{-1} h^{-1} infusion suppressed the elevation of IL-6 in plasma in hydrochloride acid induced acute lung injury (127). Another study in pigs showed that iv. lidocaine infusion of 1.5 mg kg^{-1} h^{-1} during lung resection surgery attenuated TNF-α both locally and systemically (128). Cassuto et al. (129) reported that systemic administration of lidocaine in a bolus dose of 2 mg kg^{-1} followed by infusion of 10 μg kg^{-1} min^{-1} (0.6 mg kg^{-1} h^{-1}) reduced albumin extravasation in burn injury rats. However, they failed to detect any effect at a lower dose of lidocaine with a bolus dose 2 mg kg^{-1} followed by infusion of 5 μg kg^{-1} min^{-1} (0.3 mg kg^{-1} h^{-1}). A case study by Cassuto et al. (5) in 18 year old male reported a complete relief of severe burn injury pain when receiving iv. lidocaine bolus dose of 75 mg (1.1 mg kg^{-1}) followed by infusion of 3.4 mg kg^{-1} h^{-1}. Another case study by Cassuto et al. (4) in 7 patients (4 female and 3 males) reported a significantly reduced pain score following 1 mg kg^{-1} bolus dose and 40 μg kg^{-1} min^{-1} (2.4 mg kg^{-1} h^{-1}) infusion. Furthermore, there is evidence that the duration of the exposure to lidocaine could be more important than the dose itself (130). Based on the relatively scarce information the therapeutic doses of lidocaine, we decided to begin our studies by testing the effects of iv. lidocaine in a dose of 2 mg kg^{-1} bolus followed by infusion of 1 mg kg^{-1} h^{-1} for 60 min, which may have turned out to be too low.

5.3 Effect of lidocaine on cathelicidin

Cathelicidin is an antimicrobial peptide and has been shown to participate in several pro-inflammatory processes, such as the release of histamine (22) and being chemotactic factor for neutrophils, monocytes, mast cells and T cells (20). A study by Nan Chiang et al. (105) showed that lidocaine given intraperitoneally upregulated CRAMP in zymosan challenged mice. However, in the present study, systemic administration of lidocaine failed to show any changes on plasma levels of rCRAMP, thus indicating that iv. lidocaine may not have an effect on the release of cathelicidin in rats.

5.4 Effect of lidocaine on HR and MAP

Lidocaine is a class I cardiac antiarrhythmic drug, for the treatment of specific ventricular arrhythmias. It is administered systematically, achieving concentration levels of 1.4 – 6.0 μg mL^{-1} whole blood in humans (59). Lidocaine (iv.) has been shown to alter ion fluxes, reduce action potential duration and prolong the effective refractory period in both purkinje fibers and ventricular muscle cells and thus suppress the automaticity of cardiac cells (59, 131). In addition, lidocaine has been shown to increase acetylcholine release in rats (120), which can reduce HR. The use of lidocaine to treat ventricular arrhythmias is well documented and has been given to a large number of patients during the past decades (132), where it appears that iv. lidocaine causes no, or minimal, decrease in HR and MAP (59). One study (131) in rabbits, showed a decrease in HR but no changes in MAP, with plasma concentration of 2.3 μg mL^{-1} while another study in rats (133) with a mean plasma concentration of 3.6 μg mL^{-1}, did not change MAP or HR. A pilot study by our group showed that iv. infusion of lidocaine in

unburned anesthetized rats, neither affected MAP nor HR (134). In the present study, we confirm that systemic infusion of moderate dose of lidocaine does not affect MAP or HR in anesthetized burn injured rats.

5.5 Limitations of the study

The rise in plasma levels of important cytokines in our model suggests that it may be valuable to study inflammatory mechanisms in burn injury. However, this has to be confirmed with a compatible study without inducing a burn injury.

The dose of lidocaine tested in our study is comparable with those that have been reported successful in humans but since the basal metabolic rate in rats is much higher than in humans (135) there is a possibility that the dose tested may not have been high enough to induce detectible effects. Therefore, it would be interesting to test a higher dose of lidocaine in our model.

Furthermore, to measure cytokines in plasma is a rather crude method and might not give a correct picture of the dynamics of local changes in damaged tissue. Therefore a possible extension of the present experiments would have been to analyze cytokines in a burn damaged tissue.

6 Conclusions

Immersing of the hind limbs of anesthetized rats in 80°C hot water for 10 seconds appears to be a useful method to induce a second degree burn injury. The use of this model could give valuable information on the anti-inflammatory effects of iv. lidocaine in burn injury and thus, possibly, an insight into its analgesic effects after systemic administration. Moreover, this model could as well be used to study other factors and drugs in acute second degree burn injury in anesthetized rats.

References

1. Summer GJ, Puntillo KA, Miaskowski C, Green PG, Levine JD. Burn injury pain: the continuing challenge. J Pain. 2007;8(7):533-48.
2. Pal SK, Cortiella J, Herndon D. Adjunctive methods of pain control in burns. Burns. 1997;23(5):404-12.
3. Fields HL. The doctor's dilemma: opiate analgesics and chronic pain. Neuron. 2011;69(4):591-4.
4. Jonsson A, Cassuto J, Hanson B. Inhibition of burn pain by intravenous lignocaine infusion. Lancet. 1991;338(8760):151-2.
5. Cassuto J, Tarnow P. Potent inhibition of burn pain without use of opiates. Burns. 2003;29(2):163-6.
6. Edwards AD. The role of systemic lidocaine in neuropathic pain management. J Intraven Nurs. 1999;22(5):273-9.
7. Cassuto J, Sinclair R, Bonderovic M. Anti-inflammatory properties of local anesthetics and their present and potential clinical implications. Acta Anaesthesiol Scand. 2006;50(3):265-82.
8. Scholz A. Mechanisms of (local) anaesthetics on voltage-gated sodium and other ion channels. Br J Anaesth. 2002;89(1):52-61.
9. Kawamata M, Takahashi T, Kozuka Y, Nawa Y, Nishikawa K, Narimatsu E, Watanabe H, Namiki A. Experimental incision-induced pain in human skin: effects of systemic lidocaine on flare formation and hyperalgesia. Pain. 2002;100(1–2):77-89.
10. Holthusen H, Irsfeld S, Lipfert P. Effect of pre- or post-traumatically applied i.v. lidocaine on primary and secondary hyperalgesia after experimental heat trauma in humans. Pain. 2000;88(3):295-302.
11. Dray A. Inflammatory mediators of pain. Br J Anaesth. 1995;75(2):125-31.
12. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neuroscience Letters. 2004;361(1–3):184-7.
13. Despa F, Orgill DP, Neuwalder J, Lee RC. The relative thermal stability of tissue macromolecules and cellular structure in burn injury. Burns. 2005;31(5):568-77.
14. Orgill DP, Porter SA, Taylor HO. Heat injury to cells in perfused systems. Ann N Y Acad Sci. 2005;1066:106-18.
15. Moritz AR, Henriques FC. Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns. Am J Pathol. 1947;23(5):695-720.
16. Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. Injury. 2007;38(12):1336-45.
17. Baldridge MT, King KY, Goodell MA. Inflammatory signals regulate hematopoietic stem cells. Trends Immunol. 2011;32(2):57-65.
18. Medzhitov R. Inflammation 2010: New Adventures of an Old Flame. Cell. 2010;140(6):771-6.
19. Dürr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2006;1758(9):1408-25.
20. Kurosaka K, Chen Q, Yarovinsky F, Oppenheim JJ, Yang D. Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. J Immunol. 2005;174(10):6257-65.
21. De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim JJ, Chertov O. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med. 2000;192(7):1069-74.

22. Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I. Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. Eur J Immunol. 2001;31(4):1066-75.

23. Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, Stahle-Backdahl M. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol. 2003;120(3):379-89.

24. Koczulla R, von Degenfeld G, Kupatt C, Krotz F, Zahler S, Gloe T, Issbrucker K, Unterberger P, Zaiou M, Lebherz C, Karl A, Raake P, Pfosser A, Boekstegers P, Welsch U, Hiemstra PS, Vogelmeier C, Gallo RL, Clauss M, Bals R. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest. 2003;111(11):1665-72.

25. Elssner A, Duncan M, Gavrilin M, Wewers MD. A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release. J Immunol. 2004;172(8):4987-94.

26. Tomasinsig L, Pizzirani C, Skerlavaj B, Pellegatti P, Gulinelli S, Tossi A, Di Virgilio F, Zanetti M. The human cathelicidin LL-37 modulates the activities of the P2X7 receptor in a structure-dependent manner. J Biol Chem. 2008;283(45):30471-81.

27. Bourinet E, Altier C, Hildebrand ME, Trang T, Salter MW, Zamponi GW. Calcium-permeable ion channels in pain signaling. Physiol Rev. 2014;94(1):81-140.

28. Rubin E, Reisner HM. Essentials of Rubin's pathology. 6th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2014. xii, 826 p. p.

29. Dinarello CA. Historical insights into cytokines. European Journal of Immunology. 2007;37(S1):S34-S45.

30. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27-37.

31. Curfs JH, Meis JF, Hoogkamp-Korstanje JA. A primer on cytokines: sources, receptors, effects, and inducers. Clin Microbiol Rev. 1997;10(4):742-80.

32. Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. Front Biosci. 1997;2:d12-26.

33. Kataranovski M, Magic Z, Pejnovic N. Early inflammatory cytokine and acute phase protein response under the stress of thermal injury in rats. Physiol Res. 1999;48(6):473-82.

34. Coondoo A. Cytokines in dermatology - a basic overview. Indian J Dermatol. 2011;56(4):368-74.

35. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K. Essential involvement of interleukin-8 (IL-8) in acute inflammation. J Leukoc Biol. 1994;56(5):559-64.

36. Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. FEBS Lett. 1992;307(1):97-101.

37. Jones SA, Wolf M, Qin S, Mackay CR, Baggiolini M. Different functions for the interleukin 8 receptors (IL-8R) of human neutrophil leukocytes: NADPH oxidase and phospholipase D are activated through IL-8R1 but not IL-8R2. Proc Natl Acad Sci U S A. 1996;93(13):6682-6.

38. Biffl WL, Moore EE, Moore FA, Carl VS, Franciose RJ, Banerjee A. Interleukin-8 Increases Endothelial Permeability Independent of Neutrophils. Journal of Trauma and Acute Care Surgery. 1995;39(1):98-103.

39. Petreaca ML, Yao M, Liu Y, Defea K, Martins-Green M. Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability. Mol Biol Cell. 2007;18(12):5014-23.

40. Martich GD, Danner RL, Ceska M, Suffredini AF. Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. J Exp Med. 1991;173(4):1021-4.

41. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology. 1989;68(1):31-6.

42. Vindenes H, Ulvestad E, Bjerknes R. Increased levels of circulating interleukin-8 in patients with large burns: relation to burn size and sepsis. J Trauma. 1995;39(4):635-40.

43. Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. J Clin Invest. 2010;120(11):3760-72.

44. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009;139(2):267-84.

45. Brady ST, Siegel GJ, Albers RW, Price DL, ScienceDirect (Online service). Basic neurochemistry principles of molecular, cellular, and medical neurobiology. Amsterdam ; Boston: Elsevier Academic Press,; 2012.

46. Sann H, Pierau FK. Efferent functions of C-fiber nociceptors. Z Rheumatol. 1998;57 Suppl 2:8-13.

47. Meyer RA, Raja SN, Campbell JN. Coupling of action potential activity between unmyelinated fibers in the peripheral nerve of monkey. Science. 1985;227(4683):184-7.

48. Woodcock J, Witter J, Dionne RA. Stimulating the development of mechanism-based, individualized pain therapies. Nat Rev Drug Discov. 2007;6(9):703-10.

49. Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Br J Pharmacol. 1992;107(3):660-4.

50. Ferreira SH, Lorenzetti BB, Cunha FQ, Poole S. Bradykinin release of TNF-alpha plays a key role in the development of inflammatory hyperalgesia. Agents Actions. 1993;38 Spec No:C7-9.

51. Verri Jr WA, Cunha TM, Parada CA, Poole S, Cunha FQ, Ferreira SH. Hypernociceptive role of cytokines and chemokines: Targets for analgesic drug development? Pharmacology & Therapeutics. 2006;112(1):116-38.

52. Poole S, Queiroz Cunha F, Ferreira S. Hyperalgesia from subcutaneous cytokines. In: Watkins L, Maier S, editors. Cytokines and Pain: Birkhäuser Basel; 1999. p. 59-87.

53. Summer GJ, Romero-Sandoval EA, Bogen O, Dina OA, Khasar SG, Levine JD. Proinflammatory cytokines mediating burn-injury pain. Pain. 2008;135(1-2):98-107.

54. Shavit Y, Fridel K, Beilin B. Postoperative pain management and proinflammatory cytokines: animal and human studies. J Neuroimmune Pharmacol. 2006;1(4):443-51.

55. Wordliczek J, Szczepanik AM, Banach M, Turchan J, Zembala M, Siedlar M, Przewlocki R, Serednicki W, Przewlocka B. The effect of pentoxifiline on post-injury hyperalgesia in rats and postoperative pain in patients. Life Sci. 2000;66(12):1155-64.

56. Cunha FQ, Ferreira SH. Peripheral hyperalgesic cytokines. Adv Exp Med Biol. 2003;521:22-39.

57. Budenz AW. Local anesthetics in dentistry: then and now. J Calif Dent Assoc. 2003;31(5):388-96.

58. Singh P. An emphasis on the wide usage and important role of local anesthesia in dentistry: A strategic review2012.

59. Collinsworth KA, Kalman SM, Harrison DC. The clinical pharmacology of lidocaine as an antiarrhythymic drug. Circulation. 1974;50(6):1217-30.

60. Southworth JL, Mc KV, Pierce EC, 2nd, Rawson FL, Jr. Ventricular fibrillation precipitated by cardiac catheterization; complete recovery of the patient after 45 minutes. J Am Med Assoc. 1950;143(8):717-20.

61. Yagiela JA. Local anesthetics. Anesth Prog. 1991;38(4-5):128-41.

62. Doherty TJ, Seddighi MR. Local anesthetics as pain therapy in horses. Vet Clin North Am Equine Pract. 2010;26(3):533-49.

63. Kosharskyy B, Almonte W, Shaparin N, Pappagallo M, Smith H. Intravenous infusions in chronic pain management. Pain Physician. 2013;16(3):231-49.

64. Tikuisis R, Miliauskas P, Samalavicius NE, Zurauskas A, Samalavicius R, Zabulis V. Intravenous lidocaine for post-operative pain relief after hand-assisted laparoscopic colon surgery: a randomized, placebo-controlled clinical trial. Tech Coloproctol. 2014;18(4):373-80.

65. Koppert W, Weigand M, Neumann F, Sittl R, Schuettler J, Schmelz M, Hering W. Perioperative intravenous lidocaine has preventive effects on postoperative pain and morphine consumption after major abdominal surgery. Anesth Analg. 2004;98(4):1050-5, table of contents.

66. Koppert W, Ostermeier N, Sittl R, Weidner C, Schmelz M. Low-dose lidocaine reduces secondary hyperalgesia by a central mode of action. Pain. 2000;85(1-2):217-24.

67. Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. The effect of systemic lidocaine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Anesth Analg. 2000;91(4):967-72.

68. Koppert W, Zeck S, Sittl R, Likar R, Knoll R, Schmelz M. Low-dose lidocaine suppresses experimentally induced hyperalgesia in humans. Anesthesiology. 1998;89(6):1345-53.

69. de Souza MF, Kraychete DC. The analgesic effect of intravenous lidocaine in the treatment of chronic pain: a literature review. Revista Brasileira de Reumatologia (English Edition). 2014;54(5):386-92.

70. Kaba A, Laurent SR, Detroz BJ, Sessler DI, Durieux ME, Lamy ML, Joris JL. Intravenous lidocaine infusion facilitates acute rehabilitation after laparoscopic colectomy. Anesthesiology. 2007;106(1):11-8; discussion 5-6.

71. El-Deeb A, El-Morsy GZ, Ghanem AAA, Elsharkawy AA, Elmetwally AS. The effects of intravenous lidocaine infusion on hospital stay after major abdominal pediatric surgery. A randomized double-blinded study. Egyptian Journal of Anaesthesia. 2013;29(3):225-30.

72. de Oliveira CM, Issy AM, Sakata RK. Intraoperative intravenous lidocaine. Rev Bras Anestesiol. 2010;60(3):325-33.

73. Asklin B, Cassuto J. Intravesical lidocaine in severe interstitial cystitis. Case report. Scand J Urol Nephrol. 1989;23(4):311-2.

74. Bjorck S, Dahlstrom A, Ahlman H. Topical treatment of ulcerative proctitis with lidocaine. Scand J Gastroenterol. 1989;24(9):1061-72.

75. Hollmann MW, Durieux ME. Local Anesthetics and the Inflammatory Response: A New Therapeutic Indication? Anesthesiology. 2000;93(3):858-75.

76. Yuan T, Li Z, Li X, Yu G, Wang N, Yang X. Lidocaine Attenuates LPS-Induced Inflammatory Responses in Microglia. Journal of Surgical Research. (0).

77. Su D, Gu Y, Wang Z, Wang X. Lidocaine Attenuates Proinflammatory Cytokine Production Induced by Extracellular Adenosine Triphosphate in Cultured Rat Microglia. Anesthesia & Analgesia. 2010;111(3):768-74 10.1213/ANE.0b013e3181e9e897.

78. Wang H-L, Zhang W-H, Lei W-F, Zhou C-Q, Ye T. The Inhibitory Effect of Lidocaine on the Release of High Mobility Group Box 1 in Lipopolysaccharide-Stimulated Macrophages. Anesthesia & Analgesia. 2011;112(4):839-44 10.1213/ANE.0b013e31820dca9f.

79. Lee P-Y, Tsai P-S, Huang Y-H, Huang C-J. INHIBITION OF TOLL-LIKE RECEPTOR-4, NUCLEAR FACTOR-κB AND MITOGEN-ACTIVATED PROTEIN KINASE BY LIGNOCAINE MAY INVOLVE VOLTAGE-SENSITIVE SODIUM CHANNELS. Clinical and Experimental Pharmacology and Physiology. 2008;35(9):1052-8.

80. Feng G, Liu S, Wang GL, Liu GJ. Lidocaine attenuates lipopolysaccharide-induced acute lung injury through inhibiting NF-kappaB activation. Pharmacology. 2008;81(1):32-40.

81. Cassuto J, Hanson B, Jonsson A. Inhibition of burn pain by intravenous lignocaine infusion. The Lancet. 1991;338(8760):151-2.

82. Wasiak J, Spinks A, Costello V, Ferraro F, Paul E, Konstantatos A, Cleland H. Adjuvant use of intravenous lidocaine for procedural burn pain relief: a randomized double-blind, placebo-controlled, cross-over trial. Burns. 2011;37(6):951-7.

83. Campbell TJ, Williams KM. Therapeutic drug monitoring: antiarrhythmic drugs. Br J Clin Pharmacol. 2001;52 Suppl 1:21s-34s.

84. Ikeda Y, Oda Y, Nakamura T, Takahashi R, Miyake W, Hase I, Asada A. Pharmacokinetics of lidocaine, bupivacaine, and levobupivacaine in plasma and brain in awake rats. Anesthesiology. 2010;112(6):1396-403.

85. Hornchen U, Lauven PM, Schuttler J, Dorer A, Stoeckel H. [The pharmacokinetics of lidocaine in resuscitation conditions. Results of experimental studies on swine]. Anaesthesist. 1990;39(2):107-12.

86. Huang YS, Lee SD, Deng JF, Wu JC, Lu RH, Lin YF, Wang YJ, Lo KJ. Measuring lidocaine metabolite--monoethylglycinexylidide as a quantitative index of hepatic function in adults with chronic hepatitis and cirrhosis. J Hepatol. 1993;19(1):140-7.

87. Covino BG. Toxicity of local anesthetic agents. Acta Anaesthesiol Belg. 1988;39(3 Suppl 2):159-64.

88. Boas RA, Covino BG, Shahnarian A. Analgesic responses to i.v. lignocaine. Br J Anaesth. 1982;54(5):501-5.

89. Bach FW, Jensen TS, Kastrup J, Stigsby B, Dejgard A. The effect of intravenous lidocaine on nociceptive processing in diabetic neuropathy. Pain. 1990;40(1):29-34.

90. Tanelian DL, MacIver MB. Analgesic concentrations of lidocaine suppress tonic A-delta and C fiber discharges produced by acute injury. Anesthesiology. 1991;74(5):934-6.

91. Wasiak J, Mahar P, McGuinness SK, Spinks A, Danilla S, Cleland H. Intravenous lidocaine for the treatment of background or procedural burn pain. Cochrane Database Syst Rev. 2012;6:Cd005622.

92. Moudgil GC, Allan RB, Russell RJ, Wilkinson PC. Inhibition, by anaesthetic agents, of human leucocyte locomotion towards chemical attractants. Br J Anaesth. 1977;49(2):97-105.

93. Eriksson AS, Sinclair R, Cassuto J, Thomsen P. Influence of Lidocaine on Leukocyte Function in the Surgical Wound. Anesthesiology. 1992;77(1):74-8.

94. Picardi S, Cartellieri S, Groves D, Hahnenkamp K, Gerner P, Durieux ME, Stevens MF, Lirk P, Hollmann MW. Local anesthetic-induced inhibition of human neutrophil priming: the influence of structure, lipophilicity, and charge. Reg Anesth Pain Med. 2013;38(1):9-15.

95. Hollmann MW, Gross A, Jelacin N, Durieux ME. Local anesthetic effects on priming and activation of human neutrophils. Anesthesiology. 2001;95(1):113-22.

96. Hu WS, Muscoplat CC. Lidocaine: effect on phagocytosis and purification of monocytes in bovine peripheral blood. Am J Vet Res. 1980;41(3):447-9.

97. Cullen BF, Haschke RH. Local anesthetic inhibition of phagocytosis and metabolism of human leukocytes. Anesthesiology. 1974;40(2):142-6.

98. Cook VL, Jones Shults J, McDowell MR, Campbell NB, Davis JL, Marshall JF, Blikslager AT. Anti-inflammatory effects of intravenously administered lidocaine hydrochloride on ischemia-injured jejunum in horses. Am J Vet Res. 2009;70(10):1259-68.

99. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31(5):986-1000.

100. Kazimierczak W, Peret M, Maśliński C. The action of local anaesthetics on histamine release. Biochemical Pharmacology. 1976;25(15):1747-50.

101. Huang YH, Tsai PS, Kai YF, Yang CH, Huang CJ. Lidocaine inhibition of inducible nitric oxide synthase and cationic amino acid transporter-2 transcription in activated murine macrophages may involve voltage-sensitive Na+ channel. Anesth Analg. 2006;102(6):1739-44.

102. Lahav M, Levite M, Bassani L, Lang A, Fidder H, Tal R, Bar-Meir S, Mayer L, Chowers Y. Lidocaine inhibits secretion of IL-8 and IL-1beta and stimulates secretion of IL-1 receptor antagonist by epithelial cells. Clin Exp Immunol. 2002;127(2):226-33.

103. de Klaver MJ, Buckingham MG, Rich GF. Lidocaine attenuates cytokine-induced cell injury in endothelial and vascular smooth muscle cells. Anesth Analg. 2003;97(2):465-70, table of contents.

104. Takao Y, Mikawa K, Nishina K, Maekawa N, Obara H. Lidocaine attenuates hyperoxic lung injury in rabbits. Acta Anaesthesiol Scand. 1996;40(3):318-25.

105. Chiang N, Schwab JM, Fredman G, Kasuga K, Gelman S, Serhan CN. Anesthetics impact the resolution of inflammation. PLoS One. 2008;3(4):e1879.

106. Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract. Dias ACR, Vitela M, Colombari E, Mifflin SW, editors2005 2005-01-01 00:00:00. H256-H62 p.

107. Fish R, Danneman PJ, Brown M, Karas A. Anesthesia and analgesia in laboratory animals: Academic Press; 2011.

108. Shiratori Y, Hikiba Y, Mawet E, Niwa Y, Matsumura M, Kato N, Shiina S, Tada M, Komatsu Y, Kawabe T, Yoshida H, Okano K, Omata M. Modulation of KC/gro Protein (Interleukin-8 Related Protein in Rodents) Release from Hepatocytes by Biologically-Active Mediators. Biochemical and Biophysical Research Communications. 1994;203(3):1398-403.

109. Himi T, Yoshioka I, Kataura A. Production and gene expression of IL-8-like cytokine GRO/CINC-1 in rat nasal mucosa. Acta Otolaryngol. 1997;117(1):123-7.

110. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

111. Sena SF, Syed D, Romeo R, Krzymowski GA, McComb RB. Lidocaine metabolite and creatinine measurements in the Ektachem 700: steps to minimize its impact on patient care. Clin Chem. 1988;34(10):2144-8.

112. Hein KL, Kragh-Hansen U, Morth JP, Jeppesen MD, Otzen D, Møller JV, Nissen P. Crystallographic analysis reveals a unique lidocaine binding site on human serum albumin. Journal of Structural Biology. 2010;171(3):353-60.

113. Walker HL, Mason AD, Jr. A standard animal burn. J Trauma. 1968;8(6):1049-51.

114. Dai T, Kharkwal GB, Tanaka M, Huang YY, Bil de Arce VJ, Hamblin MR. Animal models of external traumatic wound infections. Virulence. 2011;2(4):296-315.

115. Khorasani G, Hosseinimehr SJ, Zamani P, Ghasemi M, Ahmadi A. The effect of saffron (Crocus sativus) extract for healing of second-degree burn wounds in rats. Keio J Med. 2008;57(4):190-5.

116. Santos Heredero F, Hamann C, Obispo Martin J, Rodriguez Arias C, Coca Menchero S. Experimental burn models. Ann Burns Fire Disaster. 1996;9:96.

117. Gao Y, Xu C, Yu K, Li G, Wan F, Liu S, Lin J, Liu H, Zhang J, Li X, Liang S. Effect of tetramethylpyrazine on DRG neuron P2X3 receptor involved in transmitting pain after burn. Burns. 2010;36(1):127-34.

118. Wang S, Lim G, Yang L, Zeng Q, Sung B, Jeevendra Martyn JA, Mao J. A rat model of unilateral hindpaw burn injury: Slowly developing rightwards shift of the morphine dose–response curve. Pain. 2005;116(1–2):87-95.

119. Pedersen JL. Inflammatory pain in experimental burns in man. Dan Med Bull. 2000;47(3):168-95.

120. Abelson KS, Hoglund AU. Intravenously administered lidocaine in therapeutic doses increases the intraspinal release of acetylcholine in rats. Neurosci Lett. 2002;317(2):93-6.

121. Attal N, Gaude V, Brasseur L, Dupuy M, Guirimand F, Parker F, Bouhassira D. Intravenous lidocaine in central pain: a double-blind, placebo-controlled, psychophysical study. Neurology. 2000;54(3):564-74.

122. Osawa Y, Oda A, Iida H, Tanahashi S, Dohi S. The effects of class Ic antiarrhythmics on tetrodotoxin-resistant Na+ currents in rat sensory neurons. Anesth Analg. 2004;99(2):464-71, table of contents.

123. Brau ME, Vogel W, Hempelmann G. Fundamental properties of local anesthetics: half-maximal blocking concentrations for tonic block of Na+ and K+ channels in peripheral nerve. Anesth Analg. 1998;87(4):885-9.

124. Xiong Z, Strichartz GR. Inhibition by local anesthetics of Ca2+ channels in rat anterior pituitary cells. European Journal of Pharmacology. 1998;363(1):81-90.

125. Hollmann MW, Wieczorek KS, Berger A, Durieux ME. Local anesthetic inhibition of G protein-coupled receptor signaling by interference with Galpha(q) protein function. Mol Pharmacol. 2001;59(2):294-301.

126. Verri WA, Jr., Cunha TM, Parada CA, Poole S, Cunha FQ, Ferreira SH. Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? Pharmacol Ther. 2006;112(1):116-38.

127. Nishina K, Mikawa K, Takao Y, Shiga M, Maekawa N, Obara H. Intravenous lidocaine attenuates acute lung injury induced by hydrochloric acid aspiration in rabbits. Anesthesiology. 1998;88(5):1300-9.

128. Garutti I, Rancan L, Simon C, Cusati G, Sanchez-Pedrosa G, Moraga F, Olmedilla L, Lopez-Gil MT, Vara E. Intravenous lidocaine decreases tumor necrosis factor alpha expression both locally and systemically in pigs undergoing lung resection surgery. Anesth Analg. 2014;119(4):815-28.

129. Cassuto J, Nellgard P, Stage L, Jonsson A. Amide local anesthetics reduce albumin extravasation in burn injuries. Anesthesiology. 1990;72(2):302-7.

130. Baranowski AP, De Courcey J, Bonello E. A trial of intravenous lidocaine on the pain and allodynia of postherpetic neuralgia. J Pain Symptom Manage. 1999;17(6):429-33.

131. Beltrame J, Aylward PE, McRitchie RJ, Chalmers JP. Comparative haemodynamic effects of lidocaine, mexiletine, and disopyramide. J Cardiovasc Pharmacol. 1984;6(3):483-90.

132. Chow MS. Advanced cardiac life support controversy: where do antiarrhythmic agents fit in? Pharmacotherapy. 1997;17(2 Pt 2):84S-8S; discussion 9S-91S.

133. Johns RA, DiFazio CA, Longnecker DE. Lidocaine constricts or dilates rat arterioles in a dose-dependent manner. Anesthesiology. 1985;62(2):141-4.

134. Bjarkardóttir AV. Áhrif lidocaines í æð á hjarta, blóðrás og bólguþætti hjá heilbrigðum rottum [BSc. research]. Skemman: Háskóli Íslands; 2010.

135. White CR, Seymour RS. Mammalian basal metabolic rate is proportional to body mass2/3. Proc Natl Acad Sci U S A. 2003;100(7):4046-9.

Appendix

Table A 1. The results of the statistical analysis of the plasma concentrations of cytokines following burn at the two time points (before and after treatment), between groups (lidocaine and saline group) and the interaction between treatments.

cytokine	factors	F value	df	p value	partial eta squared	Observed power
IFN-γ	Group	3.5	(1. 28)	0.071	0.112	0.443
	time	1.5	(1. 28)	0.237	0.050	0.215
	time*group	1.0	(1. 28)	0.334	0.033	0.158
IL-10	Group	2.5	(1. 28)	0.123	0.083	0.337
	time	0.0	(1. 28)	0.880	0.001	0.052
	time*group	0.2	(1. 28)	0.687	0.006	0.068
IL-13	Group	1.4	(1. 28)	0.250	0.047	0.206
	time	0.1	(1. 28)	0.817	0.002	0.056
	time*group	0.1	(1. 28)	0.722	0.005	0.064
IL-1β	Group	1.1	(1. 28)	0.311	0.037	0.169
	time	8.6	(1. 28)	0.007*	0.236	0.809
	time*group	2.6	(1. 28)	0.119	0.084	0.342
IL-2	Group	0.0	(1. 28)	0.874	0.001	0.053
	time	0.0	(1. 28)	0.970	0.000	0.050
	time*group	2.2	(1. 28)	0.147	0.074	0.302
IL-4	Group	1.3	(1. 28)	0.263	0.045	0.197
	time	2.1	(1. 28)	0.162	0.069	0.284
	time*group	2.8	(1. 28)	0.103	0.092	0.370
IL-5	Group	0.0	(1. 28)	0.902	0.001	0.052
	time	0.0	(1. 28)	0.974	0.000	0.050
	time*group	1.1	(1. 28)	0.308	0.037	0.171
IL-6	Group	0.0	(1. 28)	0.842	0.001	0.054
	time	16.3	(1. 28)	0.001*	0.368	0.974
	time*group	0.1	(1. 28)	0.809	0.002	0.056
KC/GRO	Group	1.1	(1. 28)	0.298	0.039	0.176
	time	1.8	(1. 28)	0.194	0.060	0.251
	time*group	0.9	(1. 28)	0.339	0.033	0.156
TNF-α	Group	0.1	(1. 28)	0.729	0.004	0.063
	time	8.4	(1. 28)	0.007*	0.231	0.800
	time*group	0.1	(1. 28)	0.783	0.003	0.058

Data are expressed as statistical measurements, *p<0.05, two way ANOVA for repeated measurements, n=30.

Table A 2. Results of the ANOVA analysis of pro-inflammatory cytokines (IFN-γ, IL-1β, IL-2, IL-5, IL-6, KC/GRO, and TNF-α) and anti-inflammatory cytokines (IL-4, IL-10, and IL-13), respectively.

cytokines	factors	F value	df	p value	partial eta squared	Observed power
Pro-inflammatory	**group**	2.45	(1. 28)	0.129	0.08	0.327
	time	6.81	(1. 28)	0.014*	0.196	0.712
	time*group	1.29	(1. 28)	0.266	0.044	0.195
Anti-inflammatory	**group**	0.94	(1. 28)	0.34	0.033	0.155
	time	0.04	(1. 28)	0.835	0.002	0.055
	time*group	0.12	(1. 28)	0.729	0.004	0.063

*p<0.05, two way ANOVA for repeated measurements, n=30.

Table A 3. Statistical analysis of heart rate and mean arterial pressure following burn injury. The effects within groups (before and after 60 min drug treatments), between groups (lidocaine and saline group), and interaction between time and group factors were analyzed.

	factors	F value	df	p value	partial eta squared	Observed power
HR	**Group**	5.26	(1.26)	0.03+	0.168	0.598
	time	1.10	(1.26)	0.304	0.041	0.172
	time*Group	0.83	(1.26)	0.371	0.031	0.142
MAP	**Group**	0.31	(1.26)	0.582	0.012	0.084
	time	17.82	(1.26)	0.001*	0.407	0.982
	time*Group	0.96	(1.26)	0.336	0.036	0.157

Data are expressed as statistical measurements, *P<0.05 main time effect, +P<0.05 main group effect, two-way ANOVA for repeated measurements, n=28.