The Role of Meloxicam in Proteinuria in Spinal Cord Injury

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Master's thesis / Diplomski rad

2024

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Rijeka / Sveučilište u Rijeci**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:193:841296

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Download date / Datum preuzimanja: **2025-02-19**

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Mentor: doc. dr. sc. Željka Minić

SVEUČILIŠTE U RIJECI
FAKULTET BIOTEHNOLOGIJE I RAZVOJA LIJEKOVA
Diplomski sveučilišni studij
"Biotehnologija u medicini"

Eva Mihelec

Uloga meloksikama u proteinuriji kod ozljede kralježnične moždine

Diplomski rad

Rijeka, 2024.

Mentor rada: doc. dr. sc. Željka Minić

Diplomski rad obranjen je dana _____ pred povjerenstvom:

1. _____
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Rad ima 34 stranica, 12 slika, 0 tablica i 50 literaturnih navoda.

Abstract

BACKGROUND: Spinal cord injury (SCI) resulting from trauma to spinal column leads to changes in organ function above and below the level of the injury. There are many preclinical animal models of SCI which recapitulate different forms and degrees of injury occurring in human population. SCI procedure carried out in preclinical animal models usually involves some surgical manipulation of spinal cord or vertebral column and therefore requires analgesic treatment in order to ensure optimal recovery. Non-steroid anti-inflammatory (NSAID) drugs such as meloxicam or carprofen are generally used to alleviate surgical pain in animal models of SCI. Since SCI leads to a multitude of changes in organs which handle metabolism and excretion of drugs, one needs to choose appropriate analgesic agent with minimal side effect profile. Previously we have observed increased mortality in animals which received meloxicam as part of the analgesic regiment following SCI surgery. The mortality was likely due to the suboptimal effects of the drug on the bladder function which is compromised by SCI. Therefore, the purpose of this study was to: (i) investigate the mechanism via which administration of meloxicam contributes towards the adverse outcomes following induction of experimental SCI in rats, and to (ii) determine the gender differences in response to SCI and in the response to the drug administration.

MATERIALS AND METHODS: 29 12 weeks old Wistar rats were used in the present study. All animals were subjected to T3 SCI and received either meloxicam (1mg/kg) or vehicle control administered subcutaneously once a day for three days following the surgery. Urine was collected once a day for three days and protein concentration was determined using Bradford Assay. Animals were euthanized 72 hours after the injury. Organs, tissues and blood were collected for future analyses.

RESULTS: Both female and male animals exhibited hematuria following SCI. However, the proportion of female animals which exhibited hematuria following SCI was significantly less than male animals.

Furthermore, male animals exhibited proteinuria while female animals were less affected. Finally, meloxicam administration tended to increase protein concentration in urine 72 hours following spinal cord injury.

CONCLUSION: These data suggest that there are significant gender differences in response to experimental SCI in Wistar rats. Furthermore, due to the effect of SCI on renal and urinary physiology, utility of meloxicam for alleviating pain following SCI in male Wistar rats may be suboptimal and other NSAIDs should be used.

Keywords: bladder; kidney; meloxicam; proteinuria; spinal cord injury

Sažetak

POZADINA: Ozljeda kralježnične moždine (OKM) uzrokovana traumom dovodi do promjena u funkciji organa iznad i ispod područja ozljede. Pretklinički modeli životinja OKM koji prikazuju različite oblike i stupnjeve ozljede do kojih dolazi u ljudima su brojni. Postupak OKM u pretkliničkim životinjskim modelima obično uključuje kiruršku manipulaciju kralježnične moždine ili kralježnice i stoga zahtjeva tretman analgetikom kako bi se osigurao najpovoljniji oporavak. Obično se koriste nesteroidni protuupalni lijekovi (NSAID) kao što su meloksikam ili karprofen za ublažiti bol nakon kirurškog zahvata u OKM modelima životinja. OKM dovodi do mnogih promjena u organima koji sudjeluju u metabolizmu i izlučivanju lijekova i zato je potrebno izabrati prikladan analgetski agens sa minimalnim mogućim nuspojavama. Prethodno smo uvidjeli povećanu smrtnost u životinjama koje su primale meloksikam kao analgetik nakon što su podvrgnute operaciji OKM. Stoga, cilj ovog istraživanja je bio: (i) istražiti mehanizam kojim administracija meloksikama pridonosi nepovoljnim ishodima uslijed indukcije eksperimentalne OKM u štakorima i (ii) odrediti razlike između spolova prema odgovoru na OKM i odgovoru na administraciju lijeka.

MATERIJALI I METODE: U ovome je istraživanju korišteno 29 Wistar štakora stara 12 tjedana. Sve su životinje bile podvrgnute operaciji ozljede T3 kralješka te su primile meloksikam (1mg/kg) ili kontrolu koji su potkožno administrirani jednom dnevno tri dana uslijed operacije. Mokraća je prikupljana jednom dnevno tri uzastopna dana, a koncentracija proteina je određena pomoću Bradford eseja. Životinje su eutanazirane 72 sata nakon ozljede. Organi, tkiva i krv su prikupljeni za daljnja istraživanja.

REZULTATI: Obje ženske i muške jedinke su doživjele hematuriju uslijed OKM. Međutim, omjer ženskih životinja koje su doživjele hematuriju uslijed OKM je značajno manji u odnosu na muške životinje. Nadalje, koncentracija proteina u mokraći je značajno manja u ženkama u odnosu

na mužjake. Administracijom meloksikama se povećala koncentracija proteina u mokraći 72 sata nakon operacije OKM.

ZAKLJUČAK: Ovi podatci nalažu da postoje značajne razlike u odgovoru na eksperimentalnu OKM između muških i ženskih Wistar štakora. Nadalje, radi utjecaja OKM na renalnu i urinarnu fiziologiju, upotreba meloksikama za ublažavanje boli uslijed OKM u muškim Wistar štakorima nije povoljna i drugi bi se NSAID-ovi trebali upotrijebiti.

Klučne riječi: bubreg; meloksikam; mokraćni mjehur; ozljeda kralježnične moždine; proteinuria

Contents

1. Introduction

Spinal cord injury (SCI) refers to damage to the spinal cord that causes temporary or permanent changes in its function. SCI can be of traumatic or non-traumatic origin. The resulting disabilities are of great concern to the medical community, the affected person, their family, and society in general. It is a traumatic and life-altering event. Traumatic SCI occurs when the spinal cord sustains acute physical damage from an outside force (such as from a car accident, a fall, a sports injury, or violence), whereas non-traumatic SCI occurs when the spinal cord sustains acute or chronic disease damage (such as from a tumor, infection, or degenerative disease) (1).

1.1. Incidence and classification

According to the WHO, between 250 000 and 500 000 people suffer a spinal cord injury annually with most cases being due to preventable causes such as motor vehicle accidents, falls or violence. A study from 2014 aimed to summarize the literature on the incidence and prevalence of SCI. The countries with the highest reported incidences were the United States (40.1 per million), Estonia (35.4 per million and 39.7 per million), Japan (39.4 per million), and New Zealand (49.1 per million) and lowest in the Rhone-Alpes region, France (250 per million) and Helsinki, Finland (280 per million) (2). Studies also found that males were more susceptible to SCI in cases of both traumatic and non-traumatic SCI. However, males suffer from traumatic SCI at a higher rate than females, more so than in SCI in general. (3). The primary causes of traumatic SCI are traffic accidents, falls and sports related injuries. In developed countries, the percentage of traumatic SCI caused by traffic accidents is at a stable or declining rate but rising in developing nations. Developed countries generally have safer cars, better road design, higher regulation and stricter licensing rules (4). Around two thirds of traumatic SCI occurs at the cervical spine level, followed by thoracic and lumbosacral (1).

The most widely adapted score for diagnostics and classification has been the American Spinal Injury Association (ASIA) impairment scale developed by International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) (5). The ISNCSCI consists of the ASIA motor score, sensory score and impairment scale grade. The impairment scale measures the grade of SCI, ranging from grade A to grade E. Grade A is the most severe degree of injury with loss of sensory or motor function below the neurological level of injury, along with absence of sacral function. Grade E is the least severe degree where sensorimotor function is normal in all segments (1).

1.2. Physiology of micturition

The process of micturition requires connections between different areas of the brain and extensive tracts in the spinal cord where sympathetic, parasympathetic and somatic systems are involved. Simple on-off switching circuits maintain a reciprocal interaction between the urine bladder and the urethral outlet. Bladder storage reflexes are engaged during bladder filling and are predominantly organized in the spinal cord, while voiding is handled by reflex mechanisms of the brain. During urine storage or bladder filling, distention of the bladder stimulates sympathetic outflow of the hypogastric nerve towards the bladder base and urethra and the pudendal nerve to the external urethral sphincter. These events cause the bladder outlet to contract and inhibit the contraction of the detrusor muscle. This then prevents involuntary bladder emptying. Many neuron populations in the brain regulate the bladder, urethra, and urethral sphincter. Micturition specific neurons include neurons from the pontine micturition centre (PMC) and the periaqueductal grey (PAG), cell groups in the caudal and preoptic hypothalamus, and neurons from several parts of the cerebral cortex, particularly the medial frontal cortex. These areas can project synaptic inputs to spinal interneurons or preganglionic neurons (6).

The registration of bladder filling sensations and manipulation of the firing of the voiding reflex are two crucial features of voluntary control of the bladder and urethra. The PAG plays a critical part in both. It receives and transmits ascending bladder signals to higher brain centers and into the conscious sensation. Functional brain imaging scans have shown activation of the PAG during bladder filling. Pelvic visceral afferent nerves communicate information about bladder distension, leading to proper coordination of autonomic/somatic output to cause retention or voiding of urine. Muscarinic, nicotinic, adrenergic, tachykinin, bradykinin and transient-receptor-potential vanilloid receptors are expressed in the urothelium, which allow it to respond and engage in chemical communication with nerves in the bladder wall. It can release chemical mediators and respond to transmitters that are released from afferent nerves. Micturition is a process mediated by both the spinal cord and the brain, with intricate neural pathways coordinating between these regions to control the storage and release of urine (6).

1.3. Differences in male and female lower urinary tracts

There are significant differences in the anatomy and physiology between male and female lower urinary tracts that affect their form, function and ultimately, recovery after SCI. The urethral striated muscle provides support to the pelvic floor and controls the initiation of micturition (7) and somatic motor nerves that arise from the S2-S4 motor neurons supply the striated muscles of the external urethral sphincter (6). In rats, female striated muscles are thin and circular, while the male striated muscles form a thick layer. Fast twitch muscle fibers are predominant in both sexes, but females contain a higher percentage of slow twitch fibers than males (7). The presence of different muscle fiber types in males and females suggests variations in muscle endurance and contraction speed, potentially affecting the control and support provided during micturition. Acid-sensing ion channels play a role in control of sensitivity of the urothelium to changes in pH. Different forms of those channels are

present in different quantities in the sexes. The male urethra is significantly longer than the female urethra, which makes females more susceptible to infection. Male rats tend to have more severe bladder dysfunction post SCI, including higher incidences of detrusor overactivity and incontinence, while females often demonstrate better control over bladder function and fewer complications. Although the length of the urethra favors males, difficulties in bladder management and increased residual urine volumes make males more susceptible to infection post SCI (8).

1.4. Effect of SCI on micturition

Continence, the storage of urine in the bladder, and micturition, the elimination of urine from the bl
adder, are fundamental functions of the lower urinary tract. Continence relies on the contraction of the external urethral sphincter and relaxation of the bladder, while micturition involves bladder contraction alongside sphincter relaxation. These actions are normally coordinated by the brain and spinal cord. However, SCI disrupts the coordination between bladder and sphincter functions, resulting in both urinary incontinence and difficulties with bladder emptying, which can lead to serious kidney damage. Consequently, individuals with SCI often experience frequent urine leakage and difficulty emptying their bladder, resulting in significant residual urine, frequent bladder infections, and possibly the formation of bladder stones (9).

SCI can impact the process of micturition, leading to neurogenic bladder dysfunction, which disrupts the normal storage and emptying of urine. Decreased bowel and bladder sensation with no voluntary control of evacuation is often result of SCI (10). Injuries above the lumbar level interrupt innervation of the detrusor and sphincter muscles. This leads to urinary incontinence, acontractile bladder and highly increases the risk of infections (1). The acontractile bladder (AcB) is a urodynamic-based diagnosis wherein the bladder is unable to demonstrate any contraction

during a pressure flow rate study. In AcB, the bladder does not exhibit the normal rhythmic contractions needed to expel urine. The underlying causes may include damage of the nerves that control bladder function, as well as myogenic factors (11). Damage to the kidney or urinary tract can result in hematuria and proteinuria. Hematuria is defined as the presence of red blood cells in the urine. It can be classified into two types: gross hematuria and microscopic hematuria. Gross hematuria is visible to the naked eye, while microscopic hematuria requires microscopic examination of the urine sample to be detected (12). Proteinuria is a condition characterized by an abnormal amount of protein in the urine. The primary protein found in blood is albumin. Proteins serve as essential building blocks for various body parts, including muscles, bones, hair, and nails. In the bloodstream, proteins perform several critical functions, such as protecting the body from infections, aiding in blood clotting, and maintaining proper fluid balance throughout the body (13). Injuries above the lumbar spine interrupt innervation of the detrusor muscle and urinary sphincters, which causes dysfunction of the urinary system and increased risk of urinary tract infection (UTI). The gastrointestinal system is also affected where voluntary control of the anal sphincter is interrupted (1). In suprasacral spinal lesions, loss of the micturition reflex occurs, but consciousness of bladder filling can still be present. Contractions of the detrusor and sphincter muscles are not coordinated which results in higher voiding pressure, residual urine volume and incontinence (14). Afferent neurons change morphologically and physiologically in rats following SCI (6).

SCI permanently alters bladder function in humans. They exhibit sustained ulceration and other abnormalities of the uroepithelium which can lead to cystitis, inflammation and bacterial infection. This can result in long term abnormalities of the bladder structure. The uroepithelium is a multi-cellular superficial layer of tissue that lines the distal portion of the urinary tract, including the bladder. It functions as a barrier that can hold large volumes of urine without unregulated exchange of molecules

between the urine and blood supply. In addition, it releases various mediators and neurotransmitters which allows communication with other tissues in the bladder (15). One of the layers is comprised of specialized umbrella cells that align the lumen. Umbrella cells are connected by a network of tight junction proteins which contribute to the layer's permeability (16). Bladders from chronically injured rats demonstrated significant deficits in expression of uroepithelial proteins compared to uninjured, age-matched controls, indicating that the bladder may never fully recover from SCI (16).

1.5. Animal models of SCI

Animal models have improved our understanding of the molecular pathways involved in SCI, predicted clinical outcomes and provided prognostic information. The ideal animal models should be reproducible, accessible, and able to generate various levels of SCI severity and functional outcomes. They should also closely resemble the pathological and anatomical characteristics of human SCI (1)(17). Rats are relatively inexpensive, readily available and have a similar response to SCI injury to the one seen in humans. Thus, they are the most frequently used animal model in preliminary studies. Mice, on the other hand, are advantageous for genetic research (18). Notably, there are differences between the spinal cord pathology and recovery in the two most widely used experimental mammals, rats, and mice. However, no model can exactly mimic every aspect of the injury due to the complexity of SCI in humans. Biological differences such as difference in size, anatomy, molecular signaling and path of recovery have made potential therapies ineffective when translated to humans. In the rat model of SCI, the cells around the injury do not proliferate in a way that keeps the opposing ends of the transected spinal cord in contact, leading to the formation of fluid-filled cysts. This contrasts with the mouse, where such cellular proliferation prevents cyst formation (1). Rats are an important mammalian model that continues to provide insight and understanding of the pathological

basis of SCI. Most types of SCI observed in humans can be replicated on adult rats. With proper care, these animals can be studied for several months. There are several behavioral tests available that test:

- *Locomotor function*: Open-field locomotor test, Digital systems, Ladder, rope or wire grid test, Staircase test
- *Limb strength*: Inclined plane test, Forelimb grip strength
- *Sensory function*: Tail-flick test, Von Frey filaments, The Hargreaves assay (1).

Rats exhibit anatomical and physiological characteristics of the bladder that are comparable to humans and findings can often be translated to humans. Rats have been shown to exhibit similar pathological responses to spinal cord injuries as humans, making them a robust model for studying bladder dysfunction and recovery post-injury (19).

Prior to the use of rodents, cats, dogs, and monkeys were used as spinal cord injury animal models. Early animal models had variable outcomes and researchers have assessed the effects of treatment on functional recovery using a variety of behavioral tests. Some of these early models laid the groundwork for the first clinical trials of spinal cord injury treatments. However, unsatisfactory results led researchers to seek out more adequate rodent models (19). The type and location of SCI are important for the development of clinically relevant and translatable animal models. Furthermore, testing novel therapies on multiple animal models helps in determining safety and effectiveness before initiating clinical trials (1).

1.6. Models of SCI

The different models of traumatic SCI are contusion, compression, transection, distraction, dislocation and chemical models. Compression models inflict prolonged, acute injuries through calibrated clip-compression and forceps. Longer compression aggravates outcome, but decompression improves recovery and reduces secondary pathology (19).

Most compression models require at least a partial laminectomy to access the spinal cord. The main limitations of these methods include the obstruction and removal of spinal processes and lamina, as well as alterations to the spinal cord's fluid dynamics (20). Contusion models typically inflict acute injuries through weight drop, electromagnetic or pneumatic factors. Devices that can produce contusion injury in animal models are weight-drop apparatus, electromagnetic impactor, and the air gun device (18). These forces displace and damage the spinal cord. Transection models involve partial or complete lesions. Since rats develop human-like symptoms, locomotor function, limb strength and sensory function behavioral tests are carried out to measure the impact of SCI. These models are not ideal for investigation of complex SCI pathophysiology because they are not common in clinical settings. Partial transections are more likely to be seen clinically. In distraction models the spinal cord is stretched in a controlled environment to simulate the tension forces. Dislocation models use a device that replicates column dislocation. The advantage of this model is that surgical exposure of the spinal cord is not necessary, and the spinal column remains intact. Chemical models use chemicals to mimic the secondary injury cascade. They are useful for the investigation of molecular mechanisms involved in SCI and effects of therapies. Examples of chemical models include photochemically induced ischemia and demyelination (18).

1.7. Post operative treatment plans following SCI

Spinal injuries and experimental procedures are associated with significant pain in the postoperative period and effective pain management is important as it can positively affect rehabilitation and long-term outcomes. Following induction of experimental SCI, post operative analgesic plan most commonly includes NSAIDs and opioid treatments each of which carry a unique side effect profile. Multiple pharmacologic agents are available to manage pain in research animals. These agents have different mechanisms and duration of action as well as

varying potencies for providing pain relief. A multimodal approach to analgesia is recommended to offer a broad spectrum of pain control, which includes the use of different categories of analgesics. Standard practice also involves continuous monitoring of animals' behavior and physiological parameters, ensuring optimal pain relief (21). The most commonly used analgesic agents are buprenorphine, meloxicam and carprofen. Buprenorphine is an opioid that is less toxic than other opiates, but it has been reported to cause respiratory depression and pica behavior in rats, common effects with the use of opioid analgesics (22).

Opioid-related side effects include postoperative nausea and vomiting, urinary retention, ileus, constipation, sedation, and ventilatory depression, while nonopioid analgesics such as acetaminophen, classic and cyclooxygenase selective nonsteroidal anti-inflammatory drugs (NSAIDs), ketamine and gabapentanoids also have their own unique side-effect profiles (e.g., hepatic and renal toxicity, coagulation, confusion, sedation, and dizziness), which may be exacerbated when they are administered as a part of a multimodal regimen after surgery.

Opioids, which are standard of postoperative analgesia, are not optimal for use following SCI. Adverse effects of opiates are sedation accompanied by reduced food and water intake, depressed respiration, pica behavior and bradycardia, which affects thermoregulation (23).

1.8. Pharmacological mechanism of action of NSAIDs

Most NSAIDs are organic acids that work by inhibition of enzymatic activity of the cyclooxygenase (COX) enzymes. They are usually metabolized in the liver through the microsomal cytochrome P450 and excreted in the bile and urine. COX-1 and COX-2 are enzymes that biosynthesize prostaglandins (PGs) and other bioactive lipids. PGs are important mediators of many physiological and pathophysiological processes in the kidney, they play a vital role in solute balance and renovascular homeostasis. In cases of prolonged renal vasoconstriction, such as in kidney damage caused by SCI, PG mediated arteriole

vasodilation plays a very crucial role in preserving renal blood flow and glomerular filtration rate (24). Use of NSAIDs and blocking the production of PGs can trigger adverse renal effects that involve proteinuria and acute interstitial nephritis.

Meloxicam (Figure 1.) is a long-acting NSAID available by prescription only and used in therapy of chronic arthritis in humans. Meloxicam has been linked to rare instances of acute, clinically apparent liver injury. It is an oxicam derivative and a NSAID with anti-inflammatory, antipyretic and analgesic activities. Unlike traditional nonselective NSAIDs, meloxicam preferentially inhibits the activity of COX-2, resulting in a decreased conversion of arachidonic acid into prostaglandin precursors. The resulting decrease in prostaglandin synthesis is responsible for the therapeutic effects of meloxicam (25).

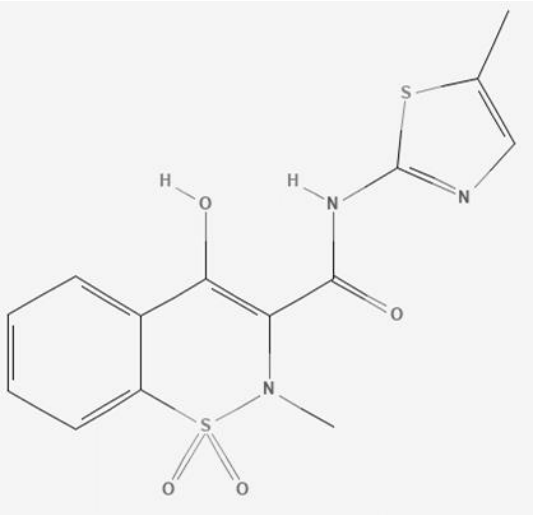

Figure 1. Meloxicam structure

PGs are a type of immunomodulatory lipid called eicosanoids. Eicosanoids are synthesized by the COX, lipoxygenase and cytochrome P450 pathways and exert multiple effects on inflammation and immunity depending on the organ they are produced in. They have roles in the regulation of the vascular, renal, gastrointestinal and reproductive systems, but are also associated with inflammatory disease. PGs, thromboxanes and leukotrienes are three major classes of eicosanoids.

Generally, NSAIDs function by inhibiting COX and/or PG-endoperoxide synthase (PGHS) enzymes.

Arachidonic acid is an unsaturated 20-carbon fatty acid present in almost all cell membranes that acts as a precursor for eicosanoid synthesis. It is not free in the cell, but instead the enzyme phospholipase A2 cleaves membrane phospholipids after activation of Toll-like receptors to release the arachidonic acid. PG production occurs in most tissues, but the kidney is one of the major organs where PG production is abundant. PGE2 is an important renal metabolite that has a role in modulating the effect of vasopressin on osmotic water reabsorption, where it reduces antidiuretic action. It is also involved in tubular water and sodium transport, glomerular filtration and vascular resistance.

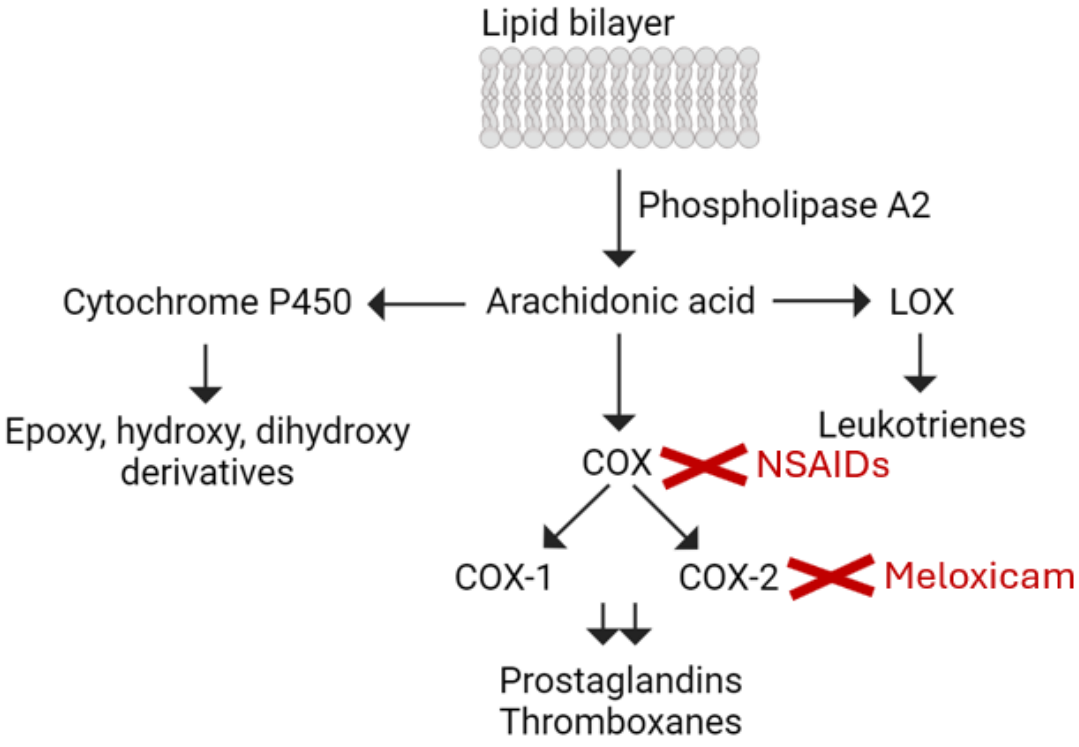

Figure 2. Pathways of eicosanoid synthesis and effect of NSAIDs and meloxicam on the pathway.

The renal arterioles and glomerular capillaries of the kidney are significantly vulnerable to NSAIDs, since the kidney is an organ for drug excretion, receiving almost 25% of the cardiac output (26). In a

histopathological study executed on rats, meloxicam has been linked to glomerular stasis-related hypertrophy and focal interstitial nephritis in the kidneys. Along with nephrotoxicity, meloxicam might cause hepatotoxicity, and gastric metaplasia dependent on the dose and duration. Light microscopic observations of rat kidney histological staining after meloxicam treatment include shrinkage of Bowman's capsule, dilatation in collecting tubules and distal tubules in medulla and vasoconstriction of arterioles all of which can lead to alterations in glomerular filtration (27).

Recent studies have found evidence that the use of meloxicam can alter renal and urinary function in cats. Cats with chronic kidney disease that received a low dose of meloxicam over 6 months had greater proteinuria than cats in the placebo group (28). Another study revealed that repeated administration of meloxicam to cats altered a minimum of 40 metabolic pathways in the renal cortex and medulla (29). Repeated administration of meloxicam also altered plasma and urine lipidome of cats (30). Since SCI negatively impacts renal function, it is likely that administration of meloxicam following induction of experimental SCI will exacerbate renal function even further.

PGHS-derived prostanoid inhibition by NSAIDs is important in the progression of nephrotoxicity. PGHS-1 is synthesized constitutively in the collecting ducts and in diverse cells of the Bowman’s capsule, while PGHS-2 is expressed in the medullary interstitial cells of renal papillae, epithelial cell lining of the ascending loop of Henle and cells of the macula densa. Kidney related complications that may arise with NSAID administration are acute kidney injury, CKD, minimal change disease, papillary necrosis, hyponatremia, hyperkalemia, renal tubular acidosis, interstitial nephritis (26).

2. Materials and methods

The study was approved by the Animal Welfare Ethics Committee of School of Medicine at University of Rijeka and the Ministry of Agriculture of Croatia. A total of 29 animals was used in the study. Upon reaching the appropriate age animals were randomly assigned into the control and meloxicam treated groups. These groups both underwent SCI surgery. In addition to the injured animals, five animals were used as sham animals. Urine was collected each day during the morning manual bladder expression. Three days following the surgery, the animals were euthanized using isoflurane anesthesia.

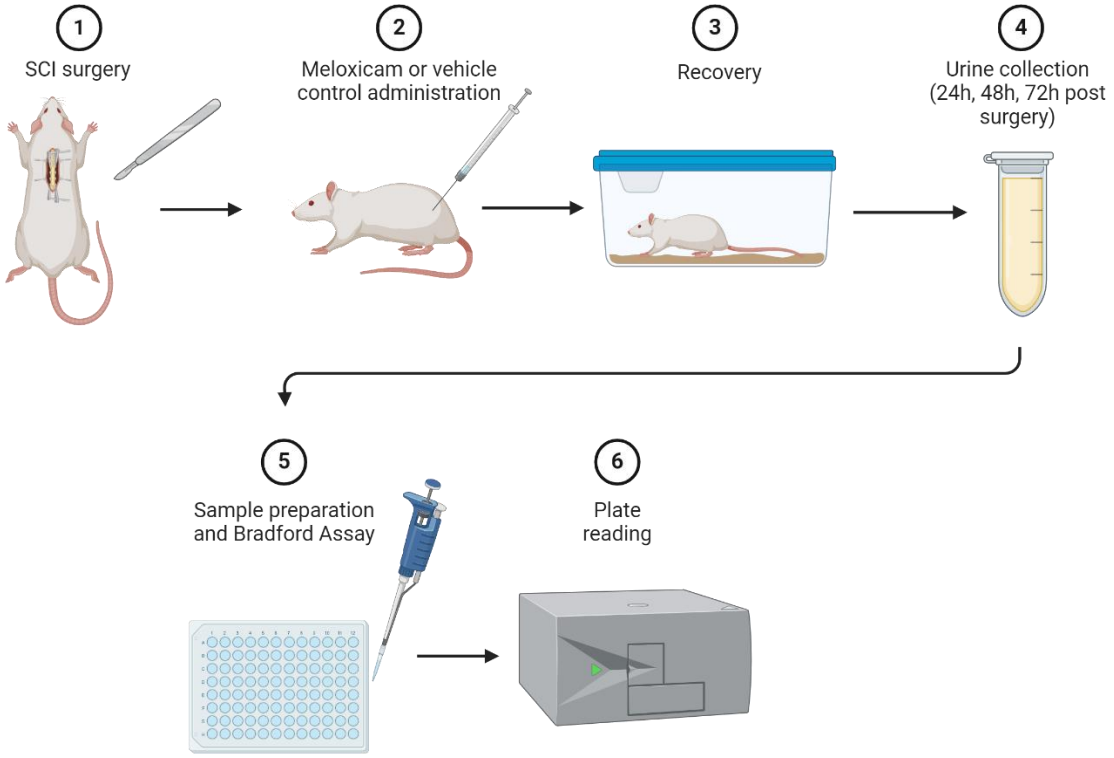

Figure 3. Experimental design. Created with BioRender.com

2.1. Spinal cord injury surgery

29 (12 weeks old) male and female adult Wistar rats were used in the present study. The rats were bred and born in house at University of Rijeka School of Medicine. The rats had access to a standard mice and rat diet (Mucedola Diete Standard) provided ad libitum. Each of the animals underwent a T3 SCI compression operation. The animal was anesthetized

via intraperitoneal injection ketamine and xylazine anesthesia (70mg/kg, Ketamidor, Richter Pharma and 12mg/kg, Alfasam International, respectively). Steps of the operative procedure are presented in figure 5. As soon as the rat went into deep anesthesia and had no palpebral or acoustic reflex, it was placed on a homeothermic heating pad to preserve a constant temperature of 37°C. The animal's fur was shaved about 1cm above and below the scapula and wiped with 10% betadine (Alkaloid) followed by 70% ethanol, repeating the process three times. A sterile ocular lubricant (Puralube Vet Ointment, Dechra) was applied around the eyes to prevent the eyes from drying out. The animal was placed on a 10ml syringe to elevate and expose the underlying vertebrae. Using a scalpel blade, a 3 cm skin incision was made between the shoulder blades and infiltrated with 2% lidocaine (Belupo) to reduce pain from sensory endings in the skin. Skin and muscles were dissected and retracted to expose the T1 to T4 vertebrae. The underlying bone was cleaned off connective tissue and the laminectomy was performed on T2 vertebral segment which corresponds to the T3 spinal segment. Muscle layers were sutured using an absorbable suture (Ethicon Ethibond Excel 4-0) and the skin was sealed with stainless steel staples. Subsequently, the animal was administered an antibiotic, enrofloxacin (10mg/kg, Enroxil, Krka-Farma) and either meloxicam (1mg/kg, Meloxidolor, Dechra) or vehicle control. Sham animals underwent the same surgical procedures, except for the lesioning of the spinal cord.

2.2. Post operative management and urine collection

The animals recovered in a temperature-controlled environment while housed in cages with wood shavings. They were examined at approximate 24-hour intervals for 3 consecutive days. Postoperative care included administration of enrofloxacin and meloxicam and collection of urine once per day for three days following the surgery, the first time being right after surgery. Following spinal cord injury surgery, the animals require assistance to void the bladder until spontaneous micturition returns

approximately 10 to 14 days following the surgery. The manual bladder expression occurs by manually applying external pressure to the bladder to induce voiding. Voided urine was collected in clean Eppendorf tubes and stored in -20°C for further analysis.

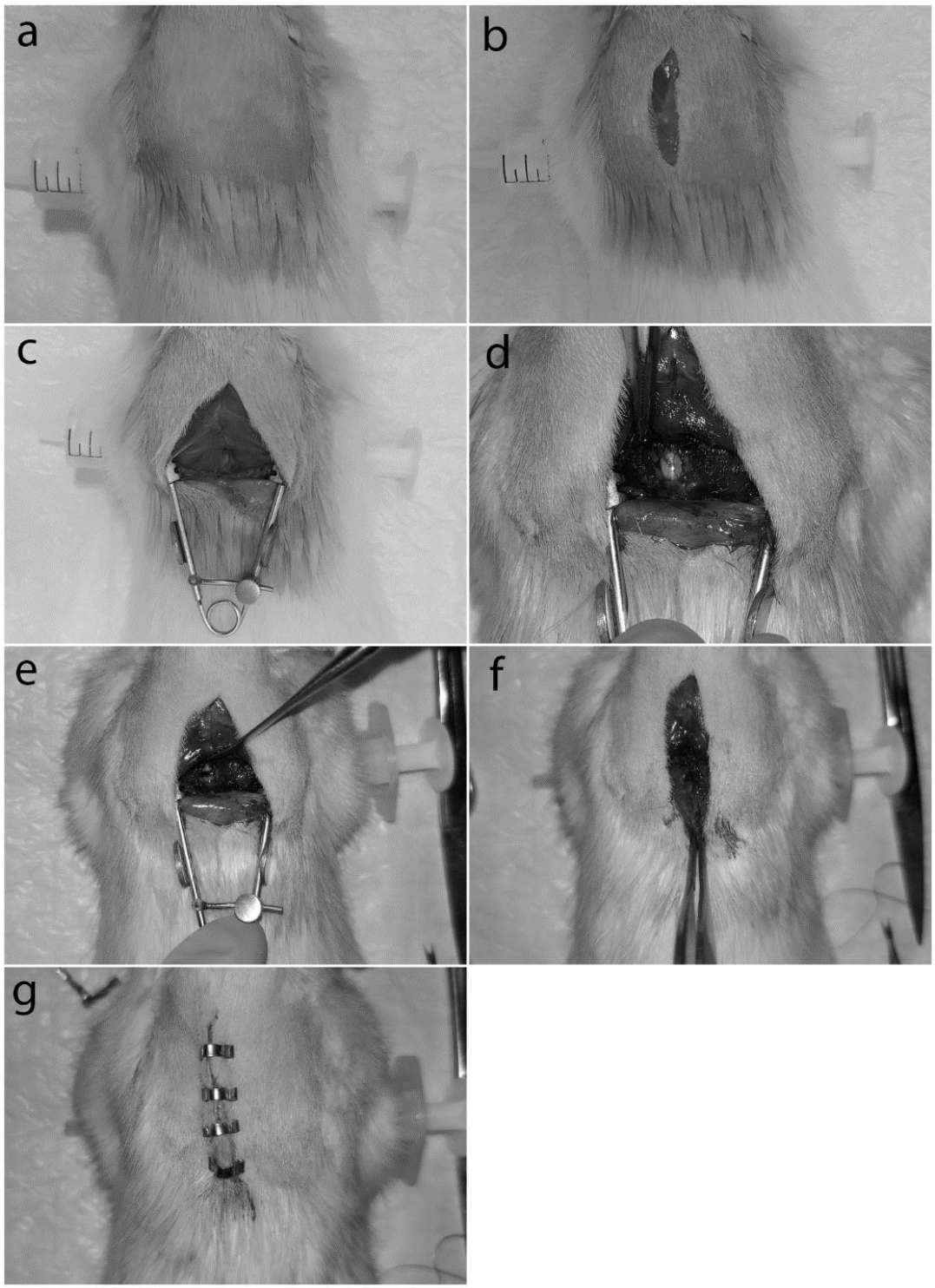

Figure 4. SCI surgery. (a) Rat positioned in surgery unit with sterilized and shaved dorsal region of back. (b) Longitudinal incision of skin with scalpel. (c) Retractors positioned to reveal muscles and brown adipose tissue. (d) Exposed vertebral column. (e) Exposed T1 vertebra. Localization of T1 facilitates T2 recognition. (f) Performed laminectomy. (g) Sutured skin with staples after laminectomy. Surgery completed.

2.3. Assessment of urine protein content

Protein concentrations in each urine sample were determined using a Bradford assay employing bovine serum albumin (BSA) as the standard. The Bradford assay is a colorimetric assay used for protein quantification. It is based on the formation of a complex between the Bradford reagent (acidified Coomassie Brilliant Blue G-250) to proteins in the solution that causes a shift in the absorption from 470nm to 595nm and a change of color in the visible spectrum. The Bradford reagent is of a reddish brown color, but changes color into blue when said complex is formed. The absorbance at a wavelength of 595nm is then read in a spectrophotometer. The protein concentration of the sample is determined by interpolation from a standard curve that is generated using a protein standard at known concentrations. Bovine serum albumin (BSA) is the most commonly used standard. To prepare the samples, they were removed from the freezer and allowed to thaw. Meanwhile, a stock solution of BSA at 1000µg/mL was prepared by dissolving BSA in Milli-Q water to predict the unknown protein concentrations using a calibration curve. The solution was vortexed thoroughly. Following that, a 1:1 dilution series of the stock solution to 1000, 750, 500, 250 and 100µg/mL was performed, with vortex spinning between each subsequent dilution. Pure Milli-Q water was used as a blank (0µg/mL of BSA). 10µl of each of the diluted standards and 10µl of the blank solution were transferred into triplicate wells of a clear bottom 96 well plate.

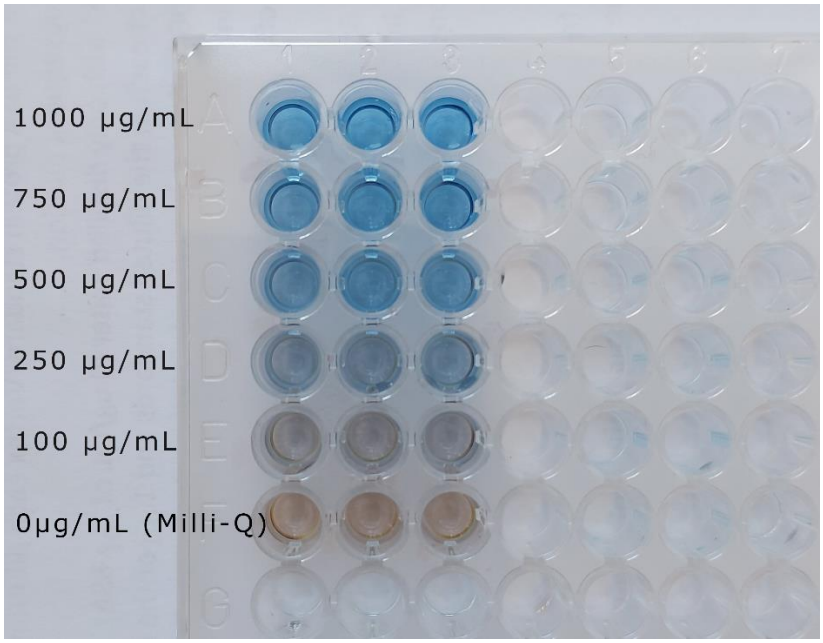

Figure 5. Dilution series of BSA stock solution for calibration curve creation.

As soon as the urine samples thawed, they were centrifuged in spin down mode in a fixed angled rotor centrifuge at 3000rpm at 20°C for 2 minutes and 30 seconds. The supernatant was transferred into new Eppendorf tubes and diluted in a 1:3 ratio by adding 335µl of urine supernatant to 665µl of Milli-Q water. 10µl of each of the diluted urine samples was transferred into triplicate wells of a clear 96 well bottom plate. 100µl of Bradford reagent (Sigma) was added to each well, then shaken gently to mix making sure there are no bubbles as the measured optical density (OD) will not be correct. The plate was incubated at room temperature for 5 minutes for the Bradford reaction to occur. The device used for measuring OD is Tecan Pro Infinite 2000 spectrophotometer with the appropriate software (Tecan i-control 1.11.1.0.). OD was measured at a wavelength of 595nm and bandwidth of 9nm with 25 flashes in "Absorbance" mode which are the default Bradford assay settings in the software. The absorption data was exported to Microsoft Excel. By constructing the calibration curve of the stock solution, we were able to calculate the unknown protein concentrations of our samples by using the determined linear equation.

2.4. Statistical analysis

Statistical analysis was performed using Microsoft Excel and GraphPad Prism 9.4.1 (GraphPad Software, Inc, USA). Microsoft Excel was used to calculate the standard curve and predict unknown concentrations of protein in the samples. GraphPad Prism was used for visualization and statistical tests. Differences between groups were analyzed using two-way ANOVA, mixed effects analysis, Student t-test and contingency table. Results are presented as mean ± standard deviation (SD). Differences are considered significant at $p<0.05$.

3. Results

First, we monitored the weight of the rats and how it is affected by SCI in the acute stage in different genders. Male rats are heavier than female rats. The mean female starting weight was 204.9g and the mean male starting weight was 314.4g. The difference between means is 109.5±5.26. We found differences in average weight change between male and female rats statistically significant. Male rats experienced a significant decrease in weight 48h after SCI, while female rats did not. The differences in mean weight change between males and females 48h after SCI was equal to -8.57g. The mean of female weight change was -1.88g or -0.92% and the mean of male weight change, on the other hand, was -6.19g or 1.97%.

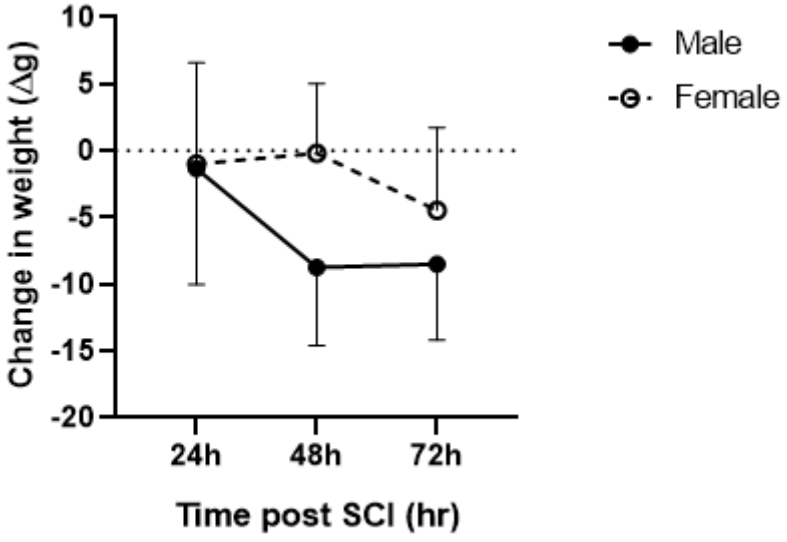

Figure 6. Males experience greater weight loss than females following SCI. (a) Body weight change of animals by gender 0, 24, 48 and 72h after SCI surgery. Statistical analysis performed using 2way ANOVA mixed effects model with Geisser-Greenhouse's correction and Šídák multiple comparisons test. Differences were considered significant at $p>0.05$.

We wished to examine changes and differences between subjects in urine color following spinal injury. The assessment of color is subjective and

may bring different interpretations, so we divided them into clear and colored samples by inspecting the sediment after centrifugation. Colors ranged from brown and red to green. Out of a total of clear samples, female ones were in the majority. On the contrary, there was a greater number of male samples in the group of colored samples (Figure 7.). We compared the effect of meloxicam administration on male and female subjects. Out of 15 samples in the male control group, 60% had an aberrant color. Out of 12 samples in the meloxicam treated male group, 83.3% had an aberrant urine color. In the female control group, there were 17 total urine samples and 23.5% had an aberrant color. In the meloxicam treated female group there were 37.5% colored samples out of a total of 8 samples.

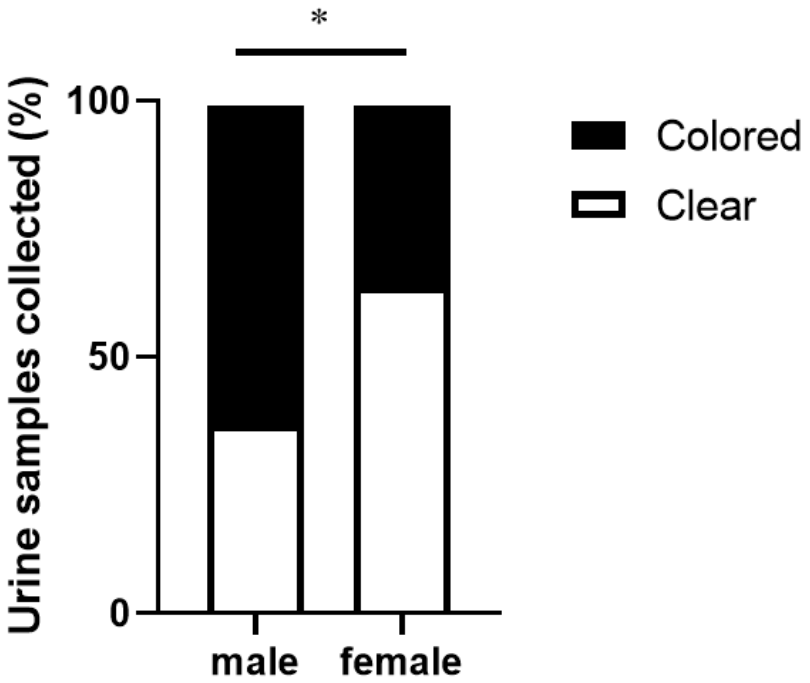

Figure 7. Differences in male and female urine color following SCI. The percentage of clear urine samples compared to colored urine samples of male (n=47) and female (n=37) rats. Fisher's exact test was used to compare samples and differences were considered significant at $p<0.05$.

Figure 8 depicts a representative example of a standard calibration curve with a series of five dilutions of standard BSA solution ranging from 250

µg/mL to 1000 µg/mL. The curve is used to predict the protein concentration in urine samples through interpolation of known data sets. To avoid deviations, a standard calibration curve was created on each day before any experimental measurements were performed. The linear equation equates to y=0.0003x+0.1964, where x is the concentration of BSA solution and y is absorbance at a wavelength of 595nm. The coefficient of determination (R^2) of 0.9811 indicates a good fit, 98.11% of points fall within the regression line (Figure 8.). Protein concentrations that were predicted as negative values using linear regression are assumed to be non-detectable. We managed to detect some values by not diluting supernatants of centrifuged urine samples.

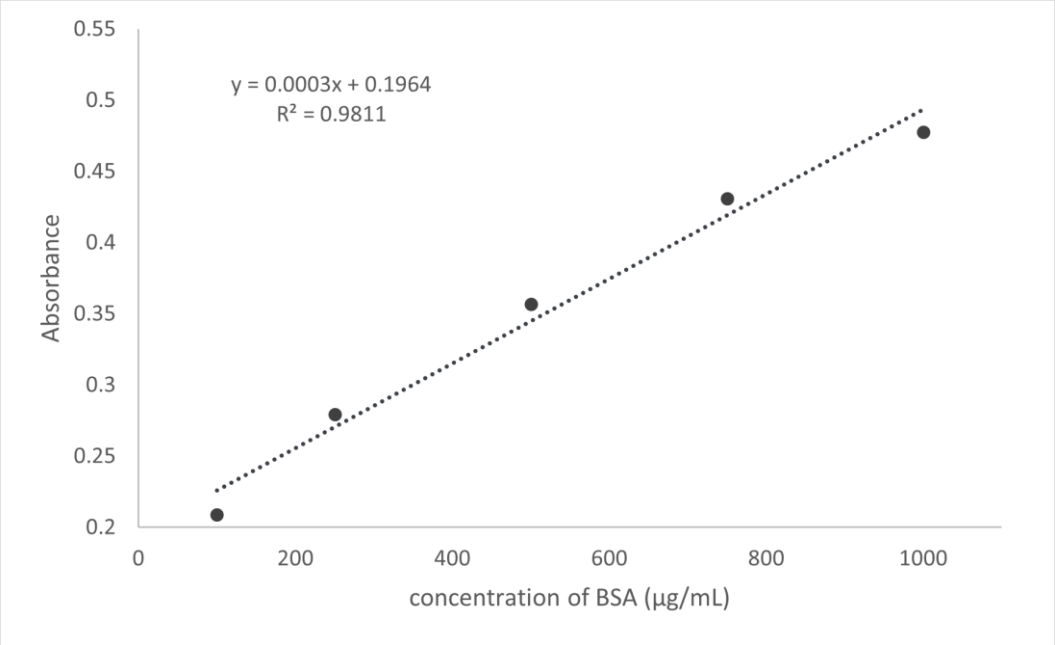

Figure 8. A Bradford protein assay standard curve generated using BSA at triplicate points of 0, 250, 500, 750 and 1000 µg/mL. The data are fit with a linear regression by the line y = 0.0003x + 0.1964 with an R^2 value of 0.9811.

We wished to test if there will be differences in urine protein concentration in relation to gender. Protein levels in the urine of male rats were significantly higher compared to those in the urine of female rats at all three timepoints (Figure 9). Those levels remained high, while protein levels in female samples decreased at a higher rate and became

undetectable at 72h post SCI. Protein concentration in male samples also decreased as time post SCI increased, but at a lower rate than females.

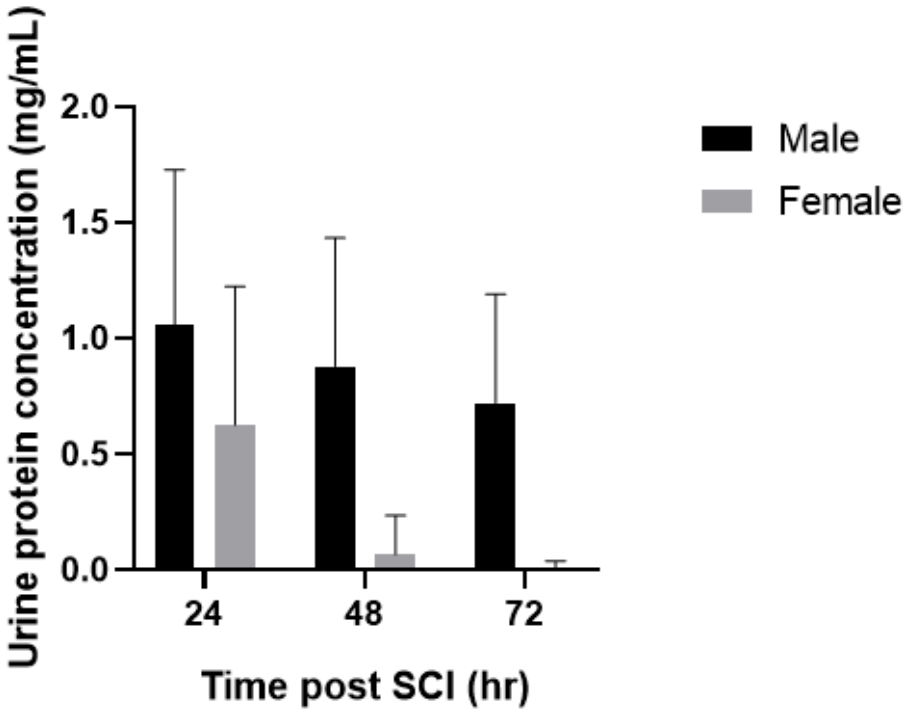

Figure 9. Sex differences in urine protein concentration post-SCI. Protein levels expressed in mg/ml in urine of male rats compared to female rats in 24h, 48h and 72h periods post-SCI. Šídák's multiple comparisons test was used to compare samples and differences were considered significant at $p<0.05$. Error bars represent standard deviation.

Since male rats had higher concentrations of protein in the urine compared to female rats, we compared data of meloxicam treated and control males to see how meloxicam administration affects the urine of male rats. Meloxicam treated males had a significantly higher percentage of colored samples in comparison to clear samples of urine. Out of 12 urine samples of meloxicam treated rats, 83.3% of them were colored. Out of 15 control group urine samples, 60% of them were colored. Both male groups had a higher ratio of colored urine samples to clear (Figure 10).

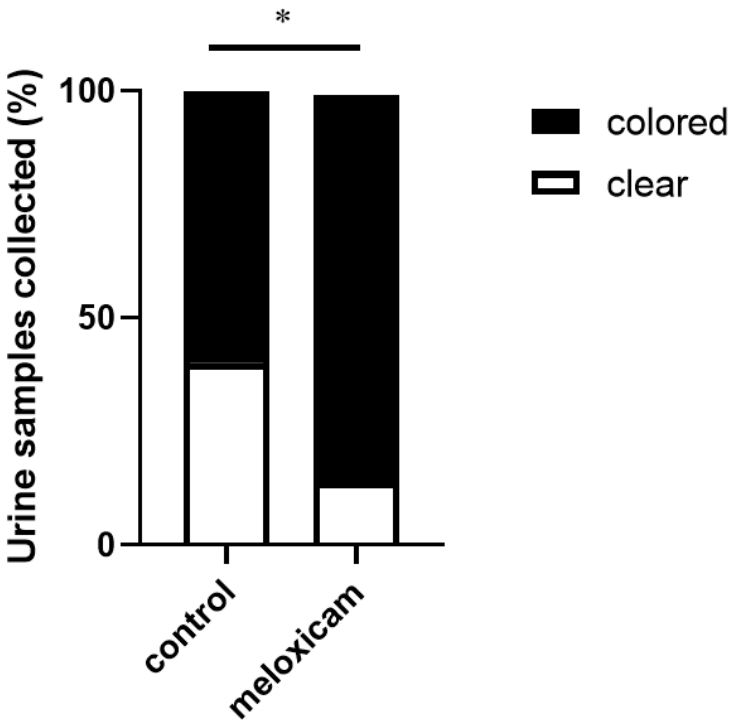

Figure 10. Differences in urine color of control and meloxicam treated male animals. The percentage of clear urine samples compared to the number of colored urine samples of control (n=15) and meloxicam treated (n=12) rats. Šídák's multiple comparisons test was used to compare samples and differences were considered significant at $p<0.05$.

Along with urine color, we compared the concentration of protein in urine samples of control males and meloxicam treated males. The concentration of protein in the urine of meloxicam treated males was significantly higher.

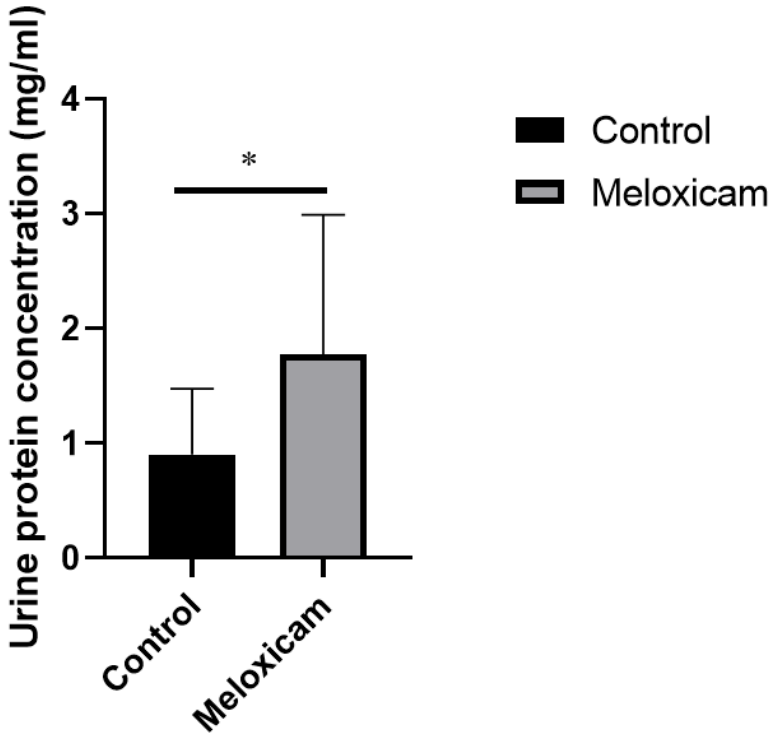

Figure 11. Differences in urine protein content of control and meloxicam treated male animals following SCI. Urine protein levels of male control rats compared to meloxicam treated male rats are expressed in mg/ml. Statistical analysis was performed using unpaired t-test. Differences were considered significant at $p>0.05$.

4. Discussion

The overarching goal of this study was to test the effect of meloxicam on development of proteinuria in rats following spinal cord injury which indicates altered renal-urinary handling and is possibly the cause of mortality associated with post operative administration of drugs.

The data of the present thesis suggest that administration of meloxicam following experimental upper thoracic SCI surgery increases bladder complications, impedes recovery and reduces overall health condition of the animals. Urine protein levels of rats treated with meloxicam were more elevated compared to those in the vehicle control group. Interestingly the urine protein concentrations were particularly elevated 72 hours following the surgery (data not shown). These data suggest that meloxicam administration had negative effect on the bladder function possibly by further damage to the uroepithelium, leading to barrier failure in the bladder. This increases permeability and allows large molecules like proteins to leak into the urine (16). The proteins found in these urine samples are of pathological significance since very little protein is present in the urine in normal conditions.

4.1. Gender differences

Female rats are usually lighter in weight and smaller in comparison to male rats. After SCI, food and water consumption decline and muscle atrophy increases (31), so weight loss is expected. Males lost a higher percentage of weight compared to females, which is consistent with the more difficult recovery that male animals experienced.

Male rats in the control group exhibited significantly higher levels of protein in the urine in relation to their female counterparts. This means that SCI alone causes a more severe proteinuria in males. Sex differences in the excretion levels of urinary proteins are present. Urinary excretion of total protein usually tends to be higher in male rats but decreases after orchiectomy, so testosterone could be one of the factors responsible for these differences (32). The renal and urinary systems of female rats

return to normal voiding occurred by 24 hours after the injury, and urine protein levels in dropped each consecutive day. The same happened with the administration of meloxicam. In females, 24h after SCI is when protein concentration was the highest, since it is a shock for the whole system.

Urinary albumin is the predominant urinary protein in most proteinuric renal diseases (33). In normal conditions, the levels of protein in urine are very low. As blood passes through healthy kidneys, they remove waste and extra fluid, while albumin and other proteins pass through and return into circulation. The three mechanisms by which protein can be present in urine: 1) disruption of the urothelium, 2) disruption to the Bowman's capsule of the kidney and altered filtration into the proximal tubule, 3) altered pressures at the level of the afferent arteriole in the kidney. Alterations in the urinary function during the acute stage post-SCI are accompanied by structural changes in both the bladder and the renal system.

4.2. Hematuria and urine color

A high percentage of urine samples were visibly colored, indicating gross hematuria. Hematuria is associated with a cellular inflammatory response early post-SCI and since patients with SCI are at a higher risk for infections of the bladder, can be result of a bladder infection.

The detrusor muscle is thicker in males than females because a higher voiding pressure is needed to empty the bladder through the longer urethra and narrower bladder neck found in males, so they are more dependent on burst contractions (7). In females, the involuntary bladder neck and voluntary external sphincters are not distinct structures and both sphincters have less strength. The female urinary tract has a shorter urethra in comparison to the male one, and therefore requires less pressure for voiding. This anatomy also increases the frequency of infections. The weaker sphincter mechanisms being more dependent on burst contractions along with the significantly shorter urethra make aided

voiding easier (7)(34). This could explain the increased quantities of blood or other metabolites that caused discoloration in the urine of male rats and explain the quicker return to voiding. It is also possible that this difference is partially due to a potential neuroprotective effect of ovarian hormones, as beta estradiol has been shown to have such an effect in spinal cord injury (35).

In normal conditions, the color of urine ranges from pale yellow to deep amber, primarily determined by its concentration. In a healthy individual, urine typically has little to no odor (36). Visual inspection of patients' urine can provide valuable information. Intrinsic pigments in urine like hemoglobin, porphyrin, or myoglobin generally cause black/brown discoloration of urine (37). Brown urine could be caused by the presence of melanocytes in the urinary system, acute kidney injury may generate melanin accumulation in the tubular system (38). Hematuria had occurred in most samples labeled as colored, staining the urine a pink or red color depending on the concentration of red blood cells. The proportion of males who exhibited hematuria was greater than females. Blood in the urine can originate anywhere in the urinary tract system, from the kidneys to the urethra. Gross hematuria is expressed with a deep red staining of urine (12). In humans, hematuria and exposure of the tubular epithelial cells to free hemoglobin and iron are marks of an underlying kidney disorder (39). Some samples had a green hue to them which could be caused by the presence of biliverdin, a waste product of heme degradation. This could indicate damage to the liver, as liver failure leads to impaired metabolism and excretion of bilirubin. Urine can also turn a green color when blue compounds excreted by the kidneys mix with yellow pigments found in urine (40). Many of our samples were stained red, indicating hematuria and thus damage to the urinary tracts and kidneys. The protein content of some clear samples could indicate microscopic hematuria.

4.3. Neurological assessment of spinal cord injured animals

On day three post-surgery, the rats showed functional recovery, gaining the ability to use their front paws and regaining energy. After a successful laminectomy, permanent paralysis of the hindlimbs occurs with a corresponding impairment of sensory and autonomic system functions.

Hind paw withdrawal reflex was absent until 72h post SCI surgery. This was tested by brief cutaneous stimuli, i.e. squeezing the paw. This behavior is not due to conscious pain perception, but it is a symptom of spasticity rather than pain (41). Spasticity is a state that results in involuntary and sustained contractions of muscles. It is defined as an abnormal increase in muscle tone, clonus, exaggerated deep tendon reflexes and muscle spasms. In humans, spasticity develops gradually over several months after injury (42).

Meloxicam inhibits the synthesis of PGs, which participate in normal renal functioning and aid in healing. Traumatic SCI triggers a series of complex events immediately and over time. Events that occur immediately include damage to the neurons and oligodendrocytes, disruption of the vasculature and the blood-spinal cord barrier. Blood vessel injury exposes the cord cells and signaling molecules of the immune system, causing inflammation within the spinal cord and also within organs (1). When injury occurs, PGs are often involved in response to that injury. PG synthesis is stimulated in pathophysiological situations such as inflammation and pain. They participate in a variety of physiological processes in the kidney which include renal hemodynamics and regulation of the body water metabolism. Vasodilatory prostaglandins enhance renal blood flow and glomerular filtration rate during conditions of reduced actual or effective circulating volume. Prostaglandin E2 (PGE2), which is produced by all renal cells, is the most prevalent prostaglandin in the kidneys and plays crucial roles both physiologically and pathologically. It is key in regulating sodium and water reabsorption. Others increase potassium secretion by stimulating renin secretion (43)(44). COX enzymes are expressed and distributed in the kidney. COX-2 is normally

expressed at low levels in the kidney under normal physiological conditions, but its expression spikes in response to inflammation and renal injury (45). Meloxicam is a NSAID that preferentially inhibits the COX-2 enzyme, and thus inhibits the production of PGs. Since prostaglandins are important for proper kidney function, COX-2 inhibiting NSAIDs like meloxicam cause an interference. In mice, COX-2 related events are dominant in kidney disease compared to COX-1. Gender differences are also present, with males having a stronger tendency for development of kidney disease (45). This aligns with our findings. The COX pathway partakes in eicosanoid production. Other eicosanoids can be found in various other organs and tissues. PGs aid in healing and participate in normal function of the kidney but are also involved in pain and inflammation. While NSAIDs such as meloxicam are used to reduce pain and inflammation, they simultaneously impair the healing process or cause further adverse effects by inhibiting the synthesis of PGs.

4.4. Effect of SCI on the kidneys

Renal dysfunction is a hallmark of SCI that is associated with increased mortality. Therefore, preservation of kidney function is important in patients with SCI. Chronic kidney disease (CKD) is associated with SCI and causes premature mortality and decreased quality of life. In CKD, irreversible structural or functional kidney damages are occur, which increases the chances for other complications such as altered mineral metabolism, anemia, metabolic acidosis, and increased cardiovascular events (46). It is often characterized by hematuria and exposure of the tubular epithelial cells to free hemoglobin and iron. The free hemoglobin may affect tubular epithelial cells by generating reactive oxygen species (ROS) and increased lipid peroxidation and therefore, increased oxidative stress (39). Renal dysfunction after SCI can indirectly affect blood pressure and cardiovascular health since the kidney is one of the main regulators of blood pressure. SCI usually initiates an inflammatory response at the site of injury or near it that spreads and affects the

function of peripheral organs. Immune cells release pro-inflammatory cytokines and cause renal inflammation shortly after SCI (47). Hypotension induced during the SCI operative procedure can alter renal blood flow, inducing renal functional disturbances (48). The loss of supraspinal control of autonomic input to the kidney from the injury causes dysregulated sympathetic activity. SCI also causes structural changes in the renal parenchyma and vasculature.

4.5. Effects of spinal cord injury (physiology and pathophysiology)

The clinical expression of SCI is determined by the severity of neurological damage and preservation of spinal cord tissue. SCI can lead to a partial or complete loss of sensorimotor function beneath the site of injury. The pathophysiology of traumatic spinal cord injury occurs in two stages: the primary and secondary phase. The secondary phase is divided temporally into acute (0-48 hours injury), subacute (48 hours to 14 days after injury), intermediate (14 days to 6 months after injury) and chronic (>6 months after injury) phases (49). The primary injury refers to the initial, sudden, traumatic impact on the spine. Hallmark mechanisms of primary injury include impact with persistent compression, impact with transient compression, distraction, and transection. The mechanical trauma of the primary injury initiates the significant secondary injury cascade by activating resident astrocytes and micro glia. The infiltration of blood-borne immune cells results in a strong neuroinflammatory response (50). Initiation of the secondary injury phase is followed by a series of events, including damage to neurons and oligodendrocytes and disruption of the blood vessels which results in further spinal cord damage and dysfunction of the nervous system. In the acute phase of the secondary phase of the injury cascade cell dysfunction and death occur. The vascular supply is compromised soon after injury causing ischemia and severe hemorrhages. This makes the spinal cord exposed to inflammatory cells, cytokines and vasoactive peptides. The inflammatory response and disrupted blood-spinal cord barrier leads to swelling of the spinal cord,

leading to compression and worsening of the injury. The acute phase is also characterized by necrosis and apoptosis of neurons and glial cells, causing demyelination and the loss of neural circuits. Signaling from the activated glial cells causes the secretion of proteins that are inhibitory to axonal growth. These proteins condense with proliferating astrocytes to form glial scars. Glial scars can limit the spread of neuroinflammation (50). The described events are illustrated in figure 12. In the subacute phase inflammatory cells continue to infiltrate the site of injury causing further death of neurons and glial cells and the formation of cystic microcavities. Occurrences in the subacute phase include fibroblast and microglial infiltration, further demyelination, axonal dieback, astrogliosis, vasospasms, ongoing oedema, release of radical oxygen species and inhibitory proteoglycan deposition. In the intermediate and chronic phases axons degenerate further and maturation of the glial scar and upregulation of matrix chondroitin sulfate proteoglycans inhibit axonal regeneration and cell differentiation (1).

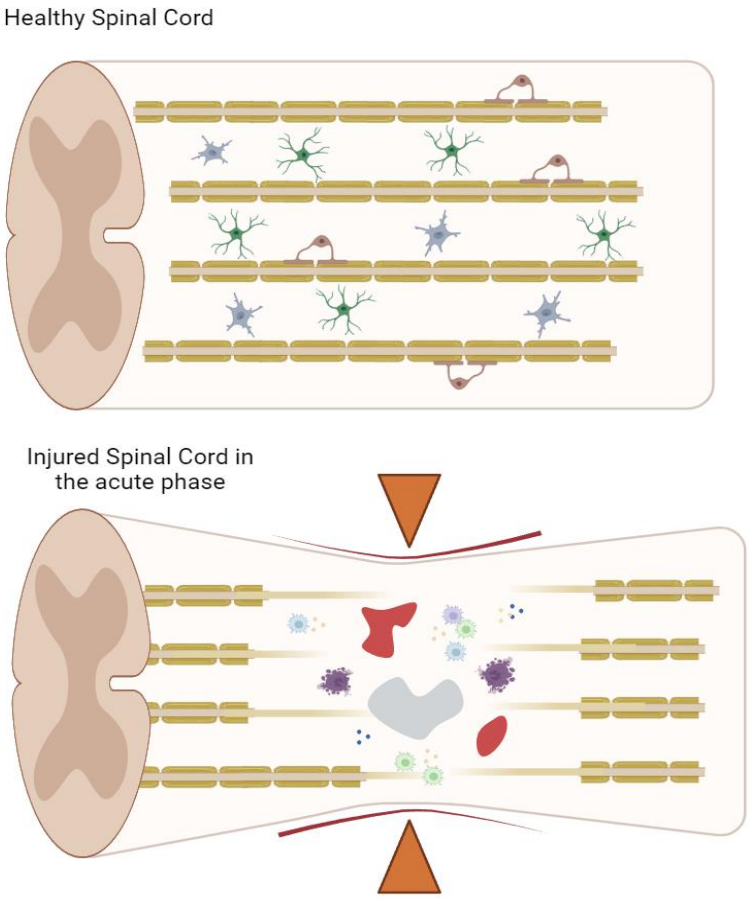

Figure 12. Pathophysiology of traumatic SCI**.** This figure illustrates the events and differences in healthy spinal cord and injured spinal cord in the acute phase of the secondary phase of SCI.

4.6. Limitations

It must be noted that the number of subjects who received meloxicam as an analgesic is small, so we interpret these results with caution.

5. Conclusion

In conclusion, using a rodent model of spinal cord compression we tested the hypothesis that administration of meloxicam exacerbates urinary and renal function following spinal cord injury and leads to proteinuria and hematuria in male and female rats.

The results of this thesis suggest that:

(i) there are significant differences in recovery and pathophysiology in response to experimental SCI between male and female Wistar rats

(ii) the use of meloxicam for pain relief in male rats following SCI leads to an increased concentration of protein in urine, which may indicate increased stress to the renal and urinary system by the drug following the injury.

SCI affects physiology of renal and urinary system of male rats and leads to hematuria in these subjects and a greater weight loss. Female subjects are affected to a lesser degree. The severity of hematuria between sexes is most probably partially due to physiological differences such as hormone levels.

The study showed that administration of meloxicam following experimental SCI surgery had tendency to increase the stress on the kidneys and bladder resulting in proteinuria. These data suggest that meloxicam or other COX-2 inhibiting drugs are a suboptimal analgesic choice of postoperative treatment regimen, especially in male rats. Meloxicam disrupts the normal function of the kidneys and urinary tract. Since SCI alone has an adverse effect on the renal and urinary physiology, treatment with meloxicam aggravates the symptoms. To confirm our findings, a subsequent study with more subjects who are treated with meloxicam should be conducted.

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7. Resume

Eva Mihelec

Nationality: Croatian Date of birth: 07/04/1999

WORK EXPERIENCE

Master Thesis Experimental work

Faculty of Biotechnology and drug research, Faculty of Medicine City: Rijeka | Country: Croatia

- spinal injury surgery on rats
- post-operative care
- urine analysis
- spectrophotometry

Internship

Centre for Micro and Nano Sciences and Technologies (NANORI), University of Rijeka City: Rijeka | Country: Croatia

- UV-VIS spectrophotometry
- DLS
- PALS

Bachelor Thesis Experimental work

Laboratory of Behavioural Genetics, Department of Biotechnology City: Rijeka | Country: Croatia

- Working with *Drosophila melanogaster*

Student Jobs

Sensapharm, Sancta Domenica, Rijeka Airport, Food Truck Festivals [2017 – 2023]

City: Rijeka, Istria | Country: Croatia

- Sales
- Teamwork
- Working in a professional kitchen
- Pharmacy
- University elections

Student Mentor

Faculty of Biotechnology and Drug Research, University of Rijeka

Graphic Design - Posters, graphics for printing purposes or social media

EDUCATION AND TRAINING

Master of Biotechnology in Medicine, mag. biotech. in med.

Faculty of Biotechnology and Drug Research, University of Rijeka [2020 – Current]

Address: Radmile Matejčić 2, 51000 RIJEKA (Croatia)

University Bachelor of Biotechnology and drug research, univ. bacc. biotech. et pharm. inv.

Department of Biotechnology, University of Rijeka [30/09/2017 – 23/09/2020]

Adobe Illustrator Online Course
Udemy

Das Deutsche Sprachdiplom (DSD I)
Kultusministerkonferenz
Address: 51000 Rijeka (Croatia)

French Diploma A2
French Alliance Rijeka
Address: 51000 Rijeka

NETWORKS AND MEMBERSHIPS

Member of Rotaract Club Rijeka
- community service, organizing events and actions

Member of Biotechnology Student Association at University of Rijeka

PROJECTS

[02/2022 – 06/2022]
Project Manager - Rijeka Carnival Organizing and coordinating logistics for student carnival group through the Biotechnology Student Association at University of Rijeka

DIGITAL SKILLS

Molecular design software (PyMol, Avogadro, Marvin) / Statistical softwares (GraphPad, SPSS, MedCalc) /
Adobe (Illustrator, Photoshop) / Microsoft Office (Excel, Powerpoint, Word, Access, Outlook) /
Python Language - Basic knowledge

PUBLICATIONS

[2020]
Measurement of Redox States of Drosophila melanogaster Circadian Mutants
Bachelor Thesis, Mentor: dr.sc. Rozi Andretić Waldowski

LANGUAGE SKILLS

Mother tongue(s): Croatian
Other language(s):

English LISTENING C2 READING C2 WRITING C2 SPOKEN PRODUCTION C2 SPOKEN INTERACTION C2
Italian LISTENING C1 READING C1 WRITING C1 SPOKEN PRODUCTION C1 SPOKEN INTERACTION C1
German LISTENING B1 READING B1 WRITING B1 SPOKEN PRODUCTION B1 SPOKEN INTERACTION B1
French LISTENING A2 READING A2 WRITING A2 SPOKEN PRODUCTION A2 SPOKEN INTERACTION A2
Spanish LISTENING A2 READING A2 WRITING A1 SPOKEN PRODUCTION A2 SPOKEN INTERACTION A2
Levels: A1 and A2: Basic user; B1 and B2: Independent user; C1 and C2: Proficient user

CONFERENCES AND SEMINARS

Department of Biotechnology, University of Rijeka
Conference "Budućnost i perspektiva studija" *Passive participant*

DRIVING LICENCE

Driving Licence: AM
Driving Licence: B