Hyperalgesia Post Constriction Injury: An Animal Model of Neuropathic Pain

Shaikhah Aljumiah, Maryam AL-Qahtani, Wissam AL-Suwailm, Kholoud Al Harbi

Abstract— Patients with insults to the somatosensory system may suffer from the most debilitating pain with presence of sharp and burning neuropathic pain that is largely resistant to treatment. This pain is spontaneous in most patients but may manifest as both hyperalgesia and / or allodynia. The aim of the current research was to investigate changes in the latency of paw withdrawal to noxious heat stimuli after nerve injury. The experiment was done in 6 male Lewis rates (Charles River, UK). Chronic Constriction Injury (CCI) was performed on 3 rats and the other 3 rats were subjected to sham operation. The rats underwent three rounds of habituation and baseline measurements of latencies of ipsilateral paw withdrawal were taken one day before the operation (day -1). After the operation, heat latencies of ipsilateral paw withdrawal where measured in CCI and sham rats on days 1, 3, 6, 8, 10, 13, 15 and 21. The results show that sham rats did not develop hyperalgesia but in CCI rats there was a significant decrease in the heat latencies between baseline and day eight. Over the rest of time points, the mean of different latency started to increase indicting recovery. Also, there was some variability in both groups. These changes in the time-course of hyperalgesia may be related to immune cell activation and cytokine production at different time-points. This was an animal study, yet, it may pave the road for understanding similar conditions in humans. Nevertheless, the subject will need further experimental study whether in vivo as animal models that are so difficult with the strict rules of animal studies or in vitro as a cell line or computerized simulators conditions.

Index Terms — Neuropathic pain, Hyperalgesia, allodynia, Rats, Chronic Constriction Injury, latency, withdrawal.

1 Introduction

Pain is an unpleasant sensory experience induced by harmful stimuli. Physiological pain is important for humans to avoid tissue injury [1]. However, several patients with insults to the somatosensory system suffer from most debilitating pain with presence of sharp and burning neuropathic pain that is largely resistant to treatment [2]. The Iinternational Association for the Study of Pain defines neuropathic pain as "Pain initiated or caused by primary lesion or dysfunction of the nervous system" This neuropathic pain is unpleasant, lasts for prolonged time after injury and is characterized by a heightened responsiveness to both noxious and non-noxious stimuli [1], affecting the patients' life style such as sleep, mood, work, social and recreational capacities [3].

Neuropathic pain develops from a lesion or disease affecting the central or peripheral nerve system. Lesion means the directly damage to the somatosensory system, while disease refers to indirectly injury by metabolic stress, autoimmune conditions or inflammatory conduction [4]. This pain is spontaneous in most patients but can manifest both as an increased pain with harmful stimulation (hyperalgesia) and as pain in response to previously non-harmful stimuli (allodynia) [5-7].

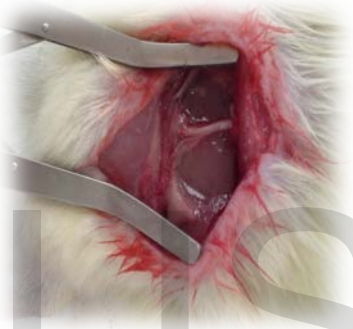
It is very difficult to evaluate neuropathic pain in humans, because only stimuli that do not produce irreversible harm can be used in these subjects. It can also be difficult to find a large number of volunteer patients needed for a clinical trial. Therefore, animal models are important to understand the mechanism of neuropathic pain and development of effective therapy. Many animal models using mechanical peripheral nerve injury are currently described [8]; a well-established model developed by Bennett and Xie (1988) Chronic Constriction Injury (CCI) model, is one of the most common models for peripheral nerve injury, done in rodents [9].

Nowadays a neuropathic pain patients have little response to commonly used pain reducing drugs, such as NSAIDS and Opiates, so there is a need to develop a new successful treatment [10]. To develop a new successful treatment animal research must done to understand the mechanisms and the development of the disease. The aim of the current research was to investigate changes in the latency of paw withdrawal

to noxious heat stimuli after nerve injury.

2 MATERIALS AND METHODS

All experiments complied with king Faisal University and international ethical guidelines for conduct of research on animals. Male Lewis rats (Charles River, UK) at 11- 13weeks of age were used. All efforts were made to reduce the number of animals used and their suffering. CCI was performed on rats anesthetized with isoflurane as described [9]. The left sciatic nerve was exposed at the level of the middle of the thigh and the adhering tissue was separated from the nerve. Four ligatures (4.0 chromic gut), with about 1 mm spacing, were tied loosely around the nerve only slight constriction of its diameter. Finally, the muscle layer was closed by suturing and the skin with wound clips. For control (sham) rats, the left sciatic nerve was exposed, but not ligated. Under aseptic conditions, the surgery was performed.



2.1 Behavioral tests

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All experimental rats were in good health and showed normal level of exploratory and feeding activity. The rats were placed individually in a clear plastic chamber on a glass floor at room temperature, for about 10 min of acclimatization. Their postures for standing, walking, and resting were monitored daily up to one week post-CCI.

Heat hyperalgesia was indicated by a decrease in the latency of paw withdrawal from a noxious heat stimulus. The paw withdrawal latency to a noxious heat stimulus (heat hyperalgesia), Hargreaves apparatus [11] was applied to the planter surface of the hindpaw to measure the latency of response to a noxious heat stimulus. Each rat was positioned on glass floor under which the laser radiant heat source was placed. When the evoked paw withdrawal was detected by a photocell, the stimulus onset activated timer was automatically stopped. Through each session of testing three latency measurements were taken and averaged for each hindpaw. Between sequential stimuli on the same hindpaw, measurements were taken with at least 5 min interval. The term hyperalgesia throughout refers to heat hyperalgesia.

2.2 Experiment design

The experiment was done in 6 rats, 3 rats were subjected to CCI and 3 rats were subjected to sham operation. The rats underwent three rounds of habituation and baseline measurements of latencies of ipsilateral paw withdrawa were taken one day before the operation (day -1). After the operation, heat latencies of ipsilateral paw withdrawal where measured in CCI and sham rats on days 1, 3, 6, 8, 10, 13, 15 and 21.

2.3 Statistic analysis

Unpaired T test was used to compare CCI and sham rats at each time point. Also, the paired T test was used to compare the baseline with each time points in CCI rats. The significance was set at P value less than 0.05. The Graphpad prism 5 edition (California, USA) was used for data analysis.

3 RESULTS

3.1 General behavioral

The rats show good health and normal level of activity after CCI injury. After injury, the rats put their weight on contralateral side at rest. Also, they did not stick their injury leg fully during walk.

The development of hyperalgesia in relation to time course:

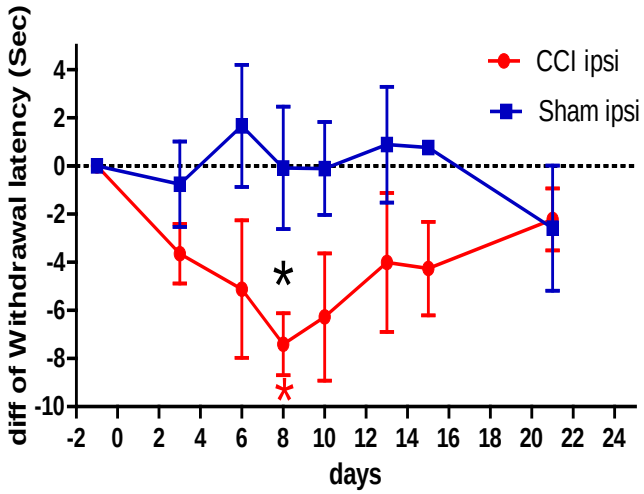

Figure 1: Changes in the differences between withdrawal latencies post CCI and baseline in seconds over specific days in sham and CCI rats. Blue line indicates mean different withdrawal latency in sham rats. Red line indicates mean different withdrawal latency in CCI rats.*significant ($p<0.05$) in day eight.* significant ($p<0.05$) between day 8 and baseline day in CCI group.

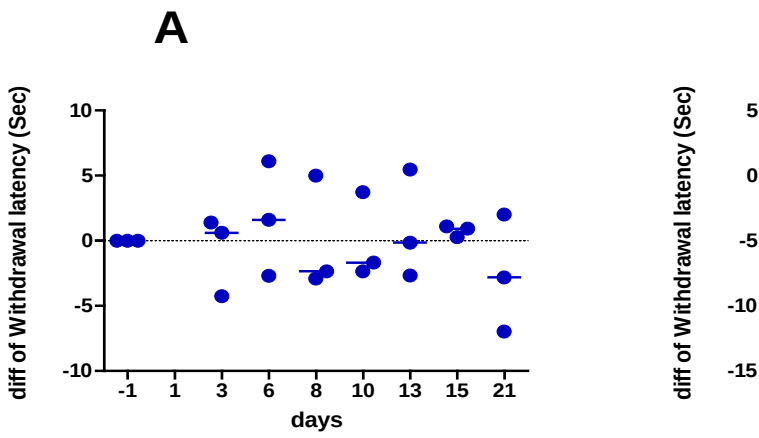

Figure 2: Individual difference of withdrawal latencies post CCI from baseline in seconds over days post CCI in sham and CCI rats. A: Blue scatter shows individual difference of withdrawal latencies in sham rats. B: Red scatter shows individual differences of withdrawal latencies in CCI rats.

In Sham rats, the difference latency post CCI did not change significantly from the baseline. The difference latency from the baseline only change around zero. This indicates that sham rats did not develop hyperalgesia. (See figure 1).

Early on day 3 post-CCI, the main difference of heat latencies decreased. There was continuous decrease of main latency on day six and further decrease on day eight (< 0.05) compared to sham rats. Also, in CCI rats, there was a significant difference between day baseline and day eight (< 0.05).

Over the rest of time points (10, 13, 15, and 21) post-CCI, The main of difference of latencies started to increase which indicted recovering. (See figure 1).

There was some variability in both groups. In sham rats, most of the difference latencies were around zero (see figure 2A). In CCI rats, most of the differences latencies were below the zero (see figure 2B).

4 Discussion

In this study, CCI model of neuropathic pain was produced in Lewis rats to measure the withdrawal heat latency of the injured paws. Measurements were taken one day before the operation (baseline) and also different time points after the CCI operation. The study was to investigate how hyperalgesia changes after peripheral nerve injury in the CCI model of neuropathic pain. The experience results showed that CCI rats developed heat hyperalgesia which started to develop early after CCI, with signs of recovery towards the end of the study. The results show a decrease of the withdrawal latencies in CCI rats starting from day3 post – CCI and further decrease in day 8 post-CCI, followed by a tendency for increasing back in paw withdrawal latencies indicating that rats were recovering (see figure 1). Researches demonstrated that immune cells have a rule in hyperalgesia development. Neutrophils are one of the immune cells that are responsible for the inflammatory

stage. In the site of nerve lesion significant infiltration of neutrophils was observed in a number of neuropathy models including CCI [12]. Perkins and Tracey have shown substantial endoneurial neutrophil leakage at the site of sciatic nerve injury, during 24h after nerve injury [13]. These authors also shown that depletion of circulating neutrophils, after systemic administration of a selective cytotoxic antibody, was preventive, rather than curative way to reduce the development of thermal hyperalgesia. The mediator releasing from neutrophils such as chemokinse in the injury site during the early stage of neuropathic pain is impotent to initiate macrophage infiltration and activation [14]. Several groups demonstrated that a reduction of neuropathic pain behavior correlated with reduction of macrophage recruitment into the damaged nerve. For example, in the C57BL/Wld mouse, which has delayed recruitment of nonresident macrophages so there was also delayed Wallerian degeneration after nerve injury [15;16], there is a lack of thermal hyperalgesia after CCI [17;18]. More importantly, after CCI hyperalgesia was attenuated in congenitally athymic nude rats, which lack mature T cells [19]. They note reduction of pain sensitivity when they transfer a Type 2 helper T cells (which produce anti-inflammatory cytokines) into nude rats produced compared with their heterozygous littermates [19]. Several types of immune cells have been implicated in the pathogenesis of peripheral neuropathic pain. In the beginning of hyperalgesia development, the neutrophils release a variety of proinflammatory factors, including cytokines and chemokines, which activate macrophages [20;21], which play a critical role in removing injured and dying tissue debris during Wallerian degeneration, which corresponds to timing of decrease in withdrawal latency of CCI rats from day 3. After day 8, type 2 helper T-cells may play an important role of recovering which show an increase of withdrawal latency after day 8 as shown in figure [1].

The increase in hyperalgesia seen in the current study, from day 1 to day 8 post-CCI. The cytokines might contribute to changes in the heat latencies. TNF-α has been proved to be directly involved in the production of pain in many models of nerve injury. Injury-induced increases in TNF-α mRNA [22] and protein expression [18;23] have been shown to correlate with the development of allodynia and hyperalgesia in several neuropathic pain models. Impairment of TNF-α signaling attenuates hyperalgesia and allodynia after spinal nerve ligation (SNL) [24], CCI [25; 26], and partial transaction of the sciatic nerve [27]. In addition, IL-1β has been identified as one of many algogenic agents that may play a role in neuropathic pain. In the periphery, IL-1β itself results in extended hyperalgesia and allodynia after intraplantar [28; 29], intraperitoneal [30], and intraneural [31] administrations. There is an upregulation of IL-1β mRNA in the injured sciatic nerve after transection [32], crush [33], and CCI [34;35]. There is also compelling proof that IL-6 is involved in the mechanisms of neuropathic pain after both CCI [36;37] and partial nerve ligation (PNL) [36]. Importantly, IL-6 quietus mice exhibit a reduction of thermal hyperalgesia and mechanical allodynia after CCI compared with wild-type mice [37]. Therefore, the changes in the time-course of hyperalgesia may be conducted with relation to changes in inflammatory cytokines that are produced in response to peripheral nerve injury.

Withdrawal latencies to heat stimuli were measured in CCI and sham rats. There was some degree of variability in both groups (see figure B). Human studies revealed that there are individual differences noted in sensitivity to experimental [38;39] and clinical pain[40]. There is evidence of familial similarities of pain characteristics, which were reasonably obtained from twin studies [41]. However individual differences to shared environmental variance and/or familial modeling have been reported [42]. Variation of pain sensitivity, and even susceptibility to more common pain pathologies (e.g. low back pain) in the "normal" range are unlikely to be mediated by single genes [43], Where adaptive or random processes have mutated or altered the allelic frequencies of genes relevant to pain in various subpopulations as in animal models of naturally occurring genetic differences. In the current study, variability in measurements of paw withdrawal to heat stimuli may be due genetic differences.

5 Conclusion

In conclusion, withdrawal latencies to heat stimuli were measured of injured paws in CCI and sham rats. The results showed that CCI rats developed heat hyperalgesia. The changes of hyperalgesia during the time course of the study have been explained by the role of immune cells which are responsible for the inflammatory response to nerve injury. The role of these immune cells may have been exerted through cytokines which may have conducted the production of hyperalgesia after peripheral nerve injury. In the current study, variability in measurements of paw withdrawal to heat stimuli may be due to genetic differences.

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