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Cathepsin K Knockout Mice do not Exhibit Mechanical Hypersensitivity after Injection of an Inflammatory Insult

Andrea Falk

ABSTRACT

Cathepsin K, a cysteine peptidase found primarily in osteoclasts, has traditionally been studied in the context of bone degradation. However, cathepsin K has also been identified in other tissues and linked to the inflammatory process, but nothing is known regarding cathepsin K and chronic inflammation. Consequently, the goal of this project was to determine whether cathepsin K mediates chronic peripheral inflammation, which may provide new pharmacological targets for the treatment of chronic and neuropathic pain. We tested our hypothesis utilizing cathepsin K knockout ($CstK^{-/-}$) mice, which lack cathepsin K from birth. Both wild-type and knockout mice were injected with complete Freund's adjuvant (CFA), a chronic inflammatory insult, into the right hind paw and tested for changes in mechanical sensitivity with von Frey filaments. With the results of this experiment we were able to show that $CstK^{-/-}$ mice did not develop mechanical hypersensitivity to the CFA-injection, suggesting that cathepsin k plays a role in mediating inflammatory-induced nociception. These results will provide the laboratory with a solid platform to further investigate cathepsin K as a possible target for chronic pain, a disease which effects millions of people which currently has no successful clinical treatment.

Keywords: *cathepsin K, inflammation, chronic pain*

INTRODUCTION

Cathepsins are a broad family of peptidases that have typically been associated with lysosomes, which degrade intracellular proteins. Within the human body there are three distinct groups of cathepsin proteases: serine (A and G), aspartic (cathepsin D and E), and cysteine (B, C, F, H, K, L, O, S, V, W and X) (Turk et al., 2000). Cathepsin K, a cysteine peptidase, primarily expressed in osteoclasts play a major role in bone metabolism by degrading insoluble collagen of the matrix (Turk et al., 2000). Thus, inhibitors of cathepsin K activity have been under pharmaceutical investigation for the treatment of osteoporosis (Saftig et al., 1998; Gauthier et al., 2008; Boggild et al., 2015). However, studies show that cathepsin K contributes to other pathologies including arthritis, periapical disease, an oral inflammatory disease, and inflammation associated with bone irritation (Hao et al., 2015; Suzuk et al., 2015; Bonnet et al., 2015). The common thread between these studies is the role of cathepsin K in mediating inflammatory processes but little else is known about its role outside the arthritic, endodontic, and bone degenerative models. In addition to inflammation, a few studies have shown that cathepsins B, S, X may have a role in modifying nociceptive signaling (Irie et al., 2008; Leichsenring et al., 2008; Sun et al., 2012). However, whether cathepsin K may play a role in nociception not related to bone degradation has not been studied.

The goal of this experiment was to determine whether mice lacking the cathepsin K protein would express mechanical hypersensitivity after injection of an inflammatory insult, CFA. We hypothesized that $CstK^{-/-}$ mice would not show mechanical hypersensitivity after CFA-injection. This hypothesis was formulated based upon our understanding of the literature and a preliminary experiment done with an acute inflammatory insult (formalin) in the laboratory. Consequently, my contribution to the overall working hypotheses in the laboratory was to expand what the laboratory knew about the role of cathepsin K in acute inflammation and determine whether cathepsin K also mediates chronic inflammation.

MATERIALS AND METHODS

Animals

Eight WT male mice and nine $CstK^{-/-}$male mice were utilized for the experiment. The mice were run in two separate groups: group one, which was not blinded, had five WT and six $CstK^{-/-}$ and the second group, which was blinded, contained three WT and three $CstK^{-/-}$. Mice were then habituated to the test table, measuring 36 (l) x 22 (w) x 24 (h) with a wire mesh surface, for one hour every day for three consecutive days prior to von Frey testing.

Von Frey Testing

Each mouse was weighed and placed in an assigned single 400ml plastic beaker and then placed on a test table, containers were separated by white paper dividers. Mice were left undisturbed for one hour in order to habituate. After completion of habituation the plantar surface of the right hind paw of each mouse was poked ten times with varying degree of forces, via von Frey filaments. Responses were observed and recorded. A positive response included at least one of the following: fast paw withdrawal or flinching, licking, biting, or toe spreading. All of the

right hind paws were tested before proceeding to test the left hind paws. After both paws were tested the procedure was repeated with the next strength of filament. This was repeated until the threshold was reached (threshold was determined when mouse exhibited five positive responses out of ten pokes).

A baseline von Frey was completed the day before injection and then again post CFA-injection day 1, 2, 3, 4 and 7. Following the von Frey test both hind paws were measured with a caliper to assess paw edema.

Injection

The right hind paw was injected with CFA, an agent that inflicts inflammatory injury which persists for 1 to 2 weeks (Ren and Dubner, 1999).

The CFA was suspended with saline (1:1) until the mixture appeared "milky". Mice were then put under with isoflurane and the right hind paw of each mouse was injected with CFA using an insulin needle. Mice were placed back in their cage to recover for 24 hours.

RESULTS

To determine whether cathepsin K activity mediates CFA-induced inflammation both wild-type (n=8) and cathepsin K knockout mice (n=9) were injected with CFA into the right hind paw and mechanical hypersensitivity was assessed daily for seven days. There was a significant genotype effect on mechanical sensitivity ($F_{(1, 15)}$ = 12.19, p<0.01), and a significant day effect ($F_{(5, 75)}$ = 5.44, p<0.01) but no genotype and day interaction p>0.05 (Figure 1). The cathepsin K knockout mice did not express mechanical hypersensitivity to the CFA injection, compared to the WT mice, suggesting cathepsin K activity plays a significant role in mediating the nociceptive response to the peripheral inflammation. Additionally, there was no significant difference in CFA-induced edema between WT and KO mice (data not shown) indicating that inflammation was still present in the periphery within both genotypes.

DISCUSSION

We tested the hypothesis that mice lacking cathepsin K protein would not develop mechanical hypersensitivity after injection of an inflammatory insult. Both WT and CstK$^{-/-}$ mice were injected with CFA, an inflammatory insult that could induce chronic inflammation within the injected tissue and tested for the development and persistence of mechanical hypersensitivity with von Frey filaments daily for 7 days. Our results show that there was a significant difference between WT and CstK$^{-/-}$ thresholds while inflammation remained unchanged between the two groups, indicating that cathepsin K plays a role in nociceptive signaling. However, experimental interpretations are limited since the cathepsin K protein was completely knocked out from the entire

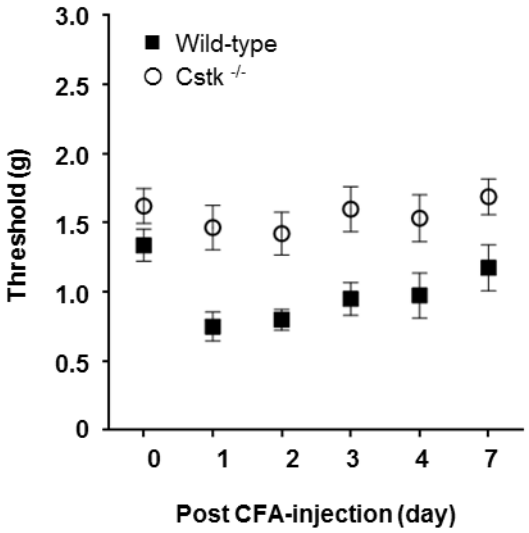

Figure 1. CstK$^{-/-}$ mice do not develop CFA-induced mechanical hypersensitivity. WT (n=8, closed squares) and CstK$^{-/-}$ mice (n=9, open circles) were injected with CFA into the hind paw prior to daily testing with von Frey filaments. CstK$^{-/-}$ mice did not develop CFA-induced mechanical hypersensitivity.

mouse from birth. Consequently, we are unable to determine where the nociceptive information is being modified (peripheral vs central) and cannot rule out compensatory changes that could be influencing are interpretation. Further experiments utilizing pharmacological and tissue-directed knockout mice are needed to determine whether the signal was not being transmitted correctly to the spinal cord or if it is interrupted at the higher level of central processing.

With these results and the previous results produced by the lab it is strongly suggested that cathepsin K plays a significant role in the inflammatory process at both the acute and chronic stages. This preliminary data will allow the lab the opportunity to further investigate cathepsin K as a possible target for the treatment of chronic pain, which effects over a 100 million Americans annually (Loeser, 2012). Currently little is known about the underlying pathophysiological mechanism of induction and persistence in regards to chronic pain making it rather difficult to treat.

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