Introduction:

- 20% of adults in the U.S. have chronic pain.6
- Most people never get sufficient pain relief.3
- The mechanisms behind the transition from acute to chronic pain is still not fully understood7 which is why treatment can be difficult.
- A recent study6 found that males and females have different cellular responses to chronic pain.
- Males use the innate immune system whereas females use the adaptive immune system.
- In this study we examined macrophages, an innate immune cell, and cytotoxic T cells, an adaptive immune cell, to compare the differences between sexes.
- The presence of macrophages peaks (day 3) before the presence of cytotoxic T cells do (day 21).1
- We also investigated the effect a Celecoxib loaded nanoemulsion had on pain relief and cell presence in the nerve.5

Methods:

Experimental Timeline. Behavior tests begin 2 days before surgery that constricts the sciatic nerve (CCI). Behavior continues before the presence of cytotoxic T cells do (day 21).1

Embed
Nerve

Tissue Workflow. Tissue was embedded in OCT, sectioned on a cryostat, and double immunostained. Images from the stains were analyzed and quantified.

Nanoemulsion Results:

Quantification of Macrophage and CD8 Positive Cells in Sciatic Nerve at Day 12.

For macrophages, 7 regions of interest (ROI) were examined per tissue and individual cells were counted. The average number of cells per ROI are shown above. For CD8 positive cells, cells were counted and divided by area of nerve examined. Statistical significance was tested for using multiple t tests and two-way ANOVA with a Tukey honest significance test. There is a statistical significance (p<.05) in the amount of macrophages between CCI and CXBNE and between CCI and CXBNE for both sexes. There was no statistical significance between conditions and sex for CD8 positive cells. Males and Females sham N=4. Males CCI N=6, DFNE N=5, CXBNE N=6. Females CCI N=5, DFNE N=4, CXBNE N=7.

Results:

At day 12, while there appears to be a trend that distinguishes macrophages and T-cell infiltration in females compared to males, there is no statistical significance between the sexes.
- Both males and females have inflammation at day 12 evident through the large presence of macrophages in CCI and DFNE conditions compared to the sham.
- There was no sex differences in the presence of macrophages for any condition.
- There was no sex or condition differences for presence of CD8 positive cells.
- The nanoemulsion was found in macrophages but not in CD8 positive cells.
- CD8 positive cells were also found in the epineurium.

Future Directions:

- Increase the sample size for each condition
- Examine Cytotoxic T cells and Macrophage presence at Day 18 in CCI, Drug-free, and Drug conditions.
- Compare Helper T Cells in males and females
- Co-stain CD8 and CD3 to identify cytotoxic T cells as compared to CD8 positive cells
- Identify time point when females have maximum pain relief from COX-2 inhibiting nanoemulsion.

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