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Sex Differences in Sepsis Survival Involve Effects of Gonadal Steroid Hormones and Activation of Inflammatory Pathways that Contribute to Disease Progression

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T3: TEXAS A&M TRIADS FOR TRANSFORMATION
A President's Excellence Fund Initiative

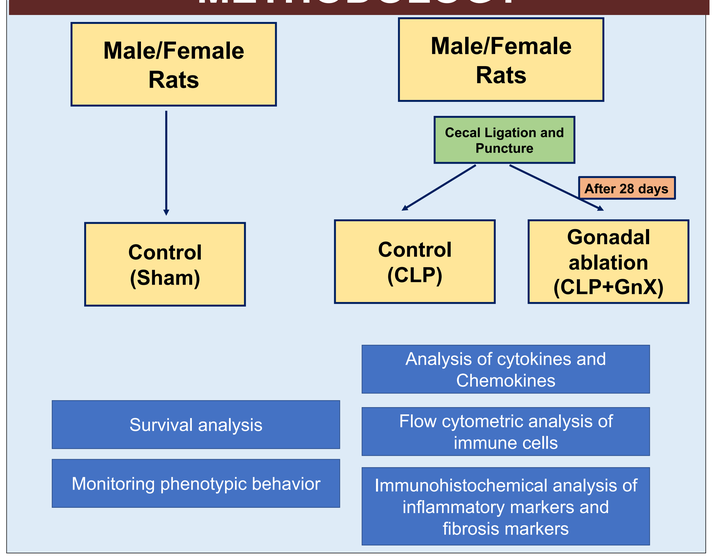
INTRODUCTION

Sepsis is a complex clinical condition that results from the inability to of the host to regulate inflammatory responses against infection and causes severe adverse outcomes such as shock, organ dysfunction, and death. Studies collectively suggest that male gender and age are risk factors for the development of sepsis and multiple organ failure following trauma. However, the precise mechanisms that contribute to progression of this disease or sex based differences that accounts for this increased susceptibility remains ill-defined. Several clinical studies indicate a differential immune response in patients following sepsis that corelates with gender. Sex-specific expression of inflammatory cytokines have also been found in surgical patients at molecular level. The differences in survival rate may involve effects of the gonadal steroid hormones on inflammatory and immune function in males versus females. However, these mechanisms remain completely unknown and thus preclude the development of effective targeted therapies to mitigate sepsis associated mortality. In this study, we carried out a detailed analysis to investigate these unknown factors and delineate the impact and effect of sex differences on sepsis survival rates and the role(s) of the gonadal steroid hormones in mediating cellular, molecular and immunological pathways that corelate with disease progression.

OBJECTIVE

The objective of this experiment was to *investigate the sex based differences in sepsis onset and progression using a rat model of cecal ligation and puncture (CLP) at the cellular and molecular levels to define therapeutic interventions*. We used three experimental groups; Sham or control, CLP- standard sepsis model in rats, and the CLP+GnX -sepsis induced group with a gonadectomy to see the sex specific effects on sepsis.

EXPERIMENTAL DESIGN AND METHODOLOGY



SIGNIFICANT SEX DIFFERENCES ARE OBSERVED IN SURVIVAL AFTER ONSET OF SEPSIS

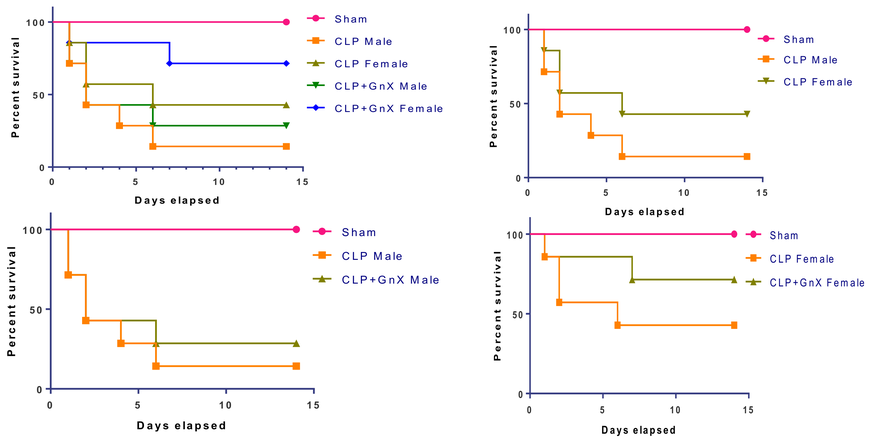


Figure 1. Survival Plots showing the difference in survival times between males and females after onset of sepsis post cecal ligation and puncture as well as after ablation of gonadal hormones.

FEMALES EXHIBIT LOWER FIBROSIS AND COLLAGEN DEPOSITION COMPARED TO MALE SEPSIS MODELS

Figure 2. Immunohistochemical analysis showing Sirius Red Staining and Masson Trichrome staining showing significant alterations in fibrosis and collagen deposition in liver sections from male and female septic animals with or without gonadal ablation. Females show significantly lower levels of fibrosis compared to male animals with cecal ligation puncture.

ALTERATIONS IN SEVERAL INFLAMMATORY MEDIATORS ASSOCIATE WITH GONADAL HORMONES IN SEPSIS

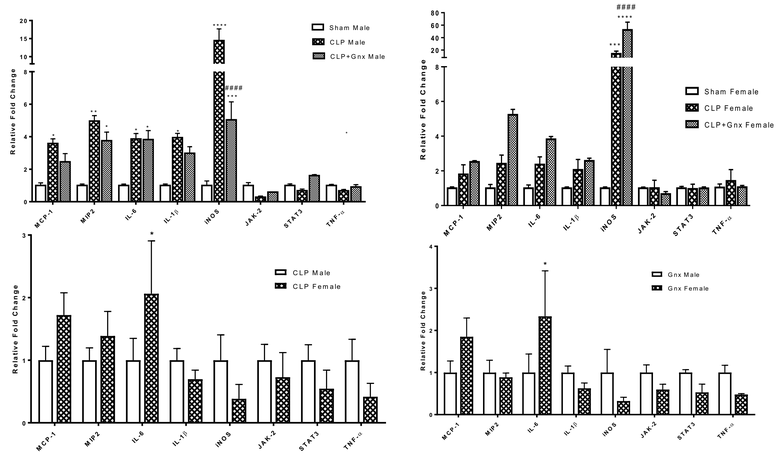


Figure 3. Expression of Inflammatory cytokines in Mesenteric Lymph Node after Cecal Ligation and punture. mRNA levels of inflammatory cytokines were analyzed using real-time PCR. Data is presented as fold change over Sham (RPL19) and presented as Mean ± SEM. *, **, *** and **** respectively represent values significantly when compared to Sham at p ≤0.05, p ≤0.01 at p ≤0.001 and at p ≤0.0001. # represent value significantly different when compared to CLP at at p ≤0.05. Analyzed using Two-way ANOVA.

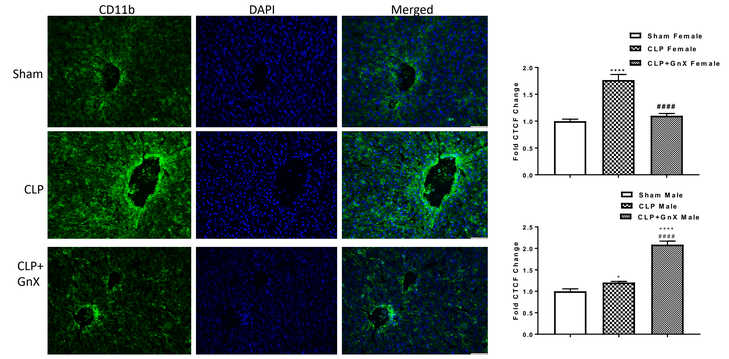


Figure 4. Immunofluorescence analysis indicates significant alterations in the macrophages in a sex dependent manner. Liver sections were stained with CD11b(known to stain monocytes and macrophages). CD11b (green) and DAPI(blue). Inflammatory reactions are increased in liver sections from female animals with onset of sepsis while gonadal ablation seems to reduce the inflammation. Data are presented as Mean ± SEM. *, **** respectively represent values significantly when compared to Sham at p ≤0.05, or p ≤0.0001. # represent value significantly different when compared to CLP at at p ≤0.05. Analyzed using Two-way ANOVA.

CONCLUSIONS

- Male CLP and CLP+GnX groups showed lower survival rates females
- Both male and female CLP groups had lower survival than their respective CLP+GnX groups indicating that sex hormones affect sepsis survival
- CLP male groups had the highest fibrotic and collagen area percentages indicating sepsis had a greater effect on this group
- Inflammatory mediators and cytokines were upregulated for the CLP and CLP+GnX groups. However, CLP+GnX groups showed a decreased expression for some of these underscoring a direct link with sepsis and sex hormones

ACKNOWLEDGEMENT

Triads for Transformation President's Excellence Fund 2019