Decalcified Bone Sections in Animal Models of Rheumatoid Arthritis

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Histology

Specimen Collection:

At necropsy, the bilateral joint of both ankles are removed and the medullary and subchondral regions. The biopsies are fixed in 10% buffered formalin and embedded in paraffin.  Sections 5 microns thick are cut and stained with hematoxylin and eosin (H&E), and with safranin-O for the cartilage.  The histopathologic scores are entered into an excel spreadsheet that will summarize and tabulate means and standard error for each treatment group on each of the parameters scored.  Statistical analysis can be performed by using either a two-tailed students t-test or a non-parametric test with a significance set at p<0.05.  Graphical representation of the data is also prepared in excel.  Knee and ankle data is tabulated and graphed separately.  Percent inhibition of each of the histologic parameters is calculated by the following equation:

\[ \text{Percent Inhibition} = 100 \times \frac{\text{B} - \text{A}}{\text{B}} \]

\[ \text{A} = \text{Mean of Disease Control - Mean of Normal Control} \]

\[ \text{B} = \text{Mean of Treated - Mean Normal Control} \]

Conclusion

The collection and histological evaluation of the knee and ankle joints in animal models of rheumatoid arthritis can help determine the efficacy of potential clinical candidates.  The low bone phenotypes of body weight and paw swelling can help determine the effectiveness of a drug candidate.  The effects of a candidate's on bone formation and resorption can be monitored by comparing the area of bone formation and resorption to the area of bone destruction.  The effects of a candidate’s on the cartilage can be monitored by comparing the area of cartilage destruction to the area of normal cartilage.  The histopathologic scores are entered into an excel spreadsheet that will summarize and tabulate means and standard error for each treatment group on each of the parameters scored.  Statistical analysis can be performed by using either a two-tailed students t-test or a non-parametric test with a significance set at p<0.05.  Graphical representation of the data is also prepared in excel.  Knee and ankle data is tabulated and graphed separately.  Percent inhibition of each of the histologic parameters is calculated by the following equation:

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Literature Cited


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Histopathology

Adjuvant A vs Adjuvant B

A = Mean of Disease Control - Mean of Normal Control

B = Mean of Treated - Mean Normal Control

Results

The histopathologic scores are entered into a spreadsheet that will summarize and standard error for each treatment group on each of the parameters scored.  Percent inhibition of each of the histologic parameters is calculated by the following equation:

\[ \text{Percent Inhibition} = 100 \times \frac{\text{B} - \text{A}}{\text{B}} \]

\[ \text{A} = \text{Mean of Disease Control - Mean of Normal Control} \]

\[ \text{B} = \text{Mean of Treated - Mean Normal Control} \]

Adjuvant Arthritis

Hematoxylin and eosin stained sections of both ankles are given scores of 0 to 5 for inflammation and bone erosion according to specific criteria.  Cartilage damage is not scored in the adjuvant model because it has been found to be a minor feature.  Hematoxylin and eosin stained sections of the spleen are also evaluated for inflammation, increased extramedullary hematopoiesis and lymphoid atrophy.

Type II Collagen Arthritis

Toluidine blue stained slides of both ankles and knees are given scores of 0 to 5 for cartilage damage, inflammation, periarthritis and periostitis according to specific criteria.