Comparative Cytotoxicity of Equine Non-Steroidal Anti-Inflammatory Drugs

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Chinese Hamster Ovary Cells

Chinese Hamster Ovary (CHO) cells are an immortalized cell line isolated in 1958 by Dr. Theodore Puck.[1] They are frequently utilized for biomedical research due to their mammal-specific protein glycosylation.[2] The structural and functional protein similarity to other mammalian cells make CHO cells an ideal model for mammalian cytotoxicity research.[2] A CHO cell culture was generously donated to NHTI, and a CHO cell line was successfully established for educational use (fig. 1).

Figure 1: T_{25} flasks containing CHO cells cultured in 90% Dulbecco's Modified Eagle Medium (which contains phenol red, a pH indicator) and 10% fetal bovine serum.

Phenylbutazone and Firocoxib

Figure 2: Phenylbutazone (left) and firocoxib (right).

Phenylbutazone and firocoxib are both non-steroidal anti-inflammatory drugs (NSAIDs) used to treat pain and inflammation in horses (fig. 2).[3,4] Both drugs work by inhibiting cyclooxygenase, an enzyme responsible for synthesizing pro-inflammatory chemicals.[4,5] The goal of this study was to assess and compare the cytotoxicity of these two NSAIDs. In a rat model, the LD_{50} is around ten times lower for phenylbutazone than firocoxib. For this reason, it was hypothesized that phenylbutazone would be more cytotoxic to CHO cell cultures than firocoxib would.[5,6]

Methodology

CHO cells were seeded in a 24-well microplate and incubated at 37° C and 5% CO_2 for 48 hours. NSAID tablets were ground to a powder and dissolved in dimethyl sulfoxide (DMSO), then diluted with cell culture water. Concentrations from 500 µM to 3000 µM of each drug were prepared with culture media and applied to the cells, then the plate was incubated at 37° C and 5% CO_2 for 24 hours (fig. 3). A trypan blue dye-exclusion assay was then used to assess the mortality rate of each treatment group.

Figure 3: Various concentrations of treatments (back) were applied to CHO cells seeded in a 24-well microplate (front).

Results

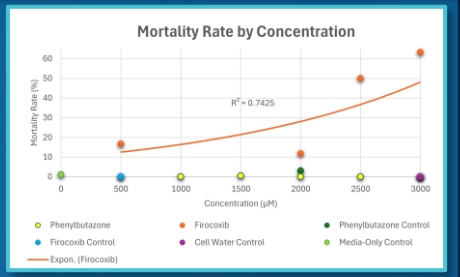

Figure 4: A line graph comparing average mortality rates of each drug and control at each dosage.

The mortality rate of cultures treated with firocoxib was consistently higher than that of cultures treated with phenylbutazone, DMSO controls, a cell water control, and a media-only control (fig. 4). As the dosage of firocoxib increased, the mortality rate of the treated CHO cells increased exponentially (R^2 = 0.7425). In addition, the pH indicator in the media of wells treated with firocoxib had turned yellow, indicating in increase in acid waste product production, which was not observed in phenylbutazone or control groups.

Discussion and Conclusions

The increased mortality rate in firocoxib experimental groups compared to control groups suggests that this drug had a cytotoxic effect on CHO cell cultures at dosages 500 µM and greater. The uptick in acid byproduct production in cultures treated with firocoxib could be attributed to an increase in cell metabolism stimulated by the addition of a toxin, as cellular metabolic rate generally increases with increasing drug concentrations.[7] Unfortunately, a comparison of the cytotoxicity of firocoxib and phenylbutazone could not be confidently made. Sediment was observed in the phenylbutazone drug stock at the time of treatment application; as DMSO was dosed accurately for dissolution of the drug, this sediment was proposed to be insoluble drug fillers, but the unaltered mortality rate seen in phenylbutazone groups suggests that the active ingredient was not present in the applied treatments. Despite the in vitro cytotoxicity of firocoxib observed during this experiment, oral administration of firocoxib at a rate of 0.1 mg/kg has been established as a safe and effective treatment for pain and inflammation in horses (fig. 5).[4]

Figure 5: NSAID treatment can help injured or arthritic horses return to work. Photo courtesy of NKG Photo.

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