In Vivo Comet Assay: Integration with Repeat Dose Toxicity Study Provides Concurrent DNA Damage Information in Potential Target Organs

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Figure 1. Comparison of Individual Vehicle Control Animal %Tail DNA to Legacy Historical Control Limit Values

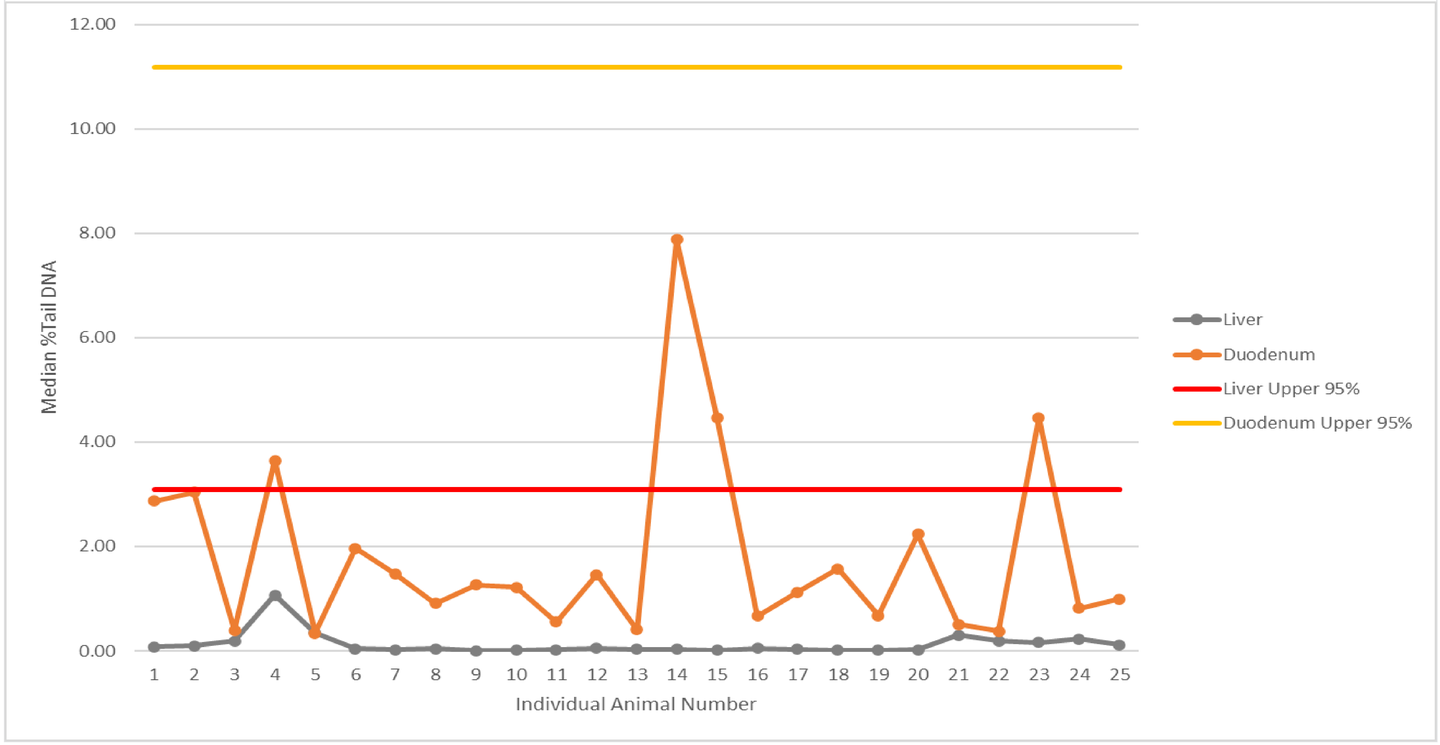


Figure 2. Group Mean Duodenum Comet Data

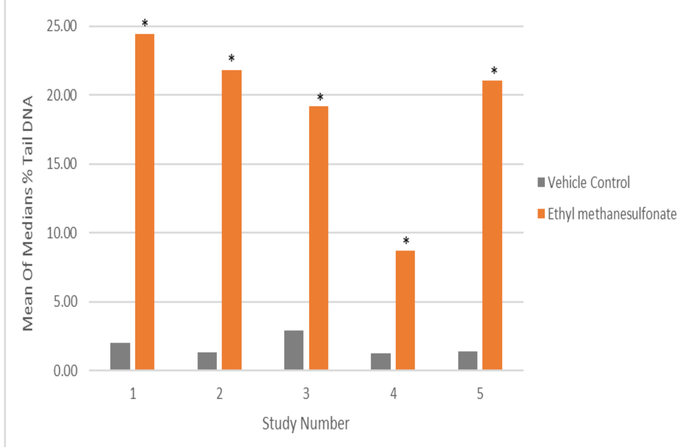
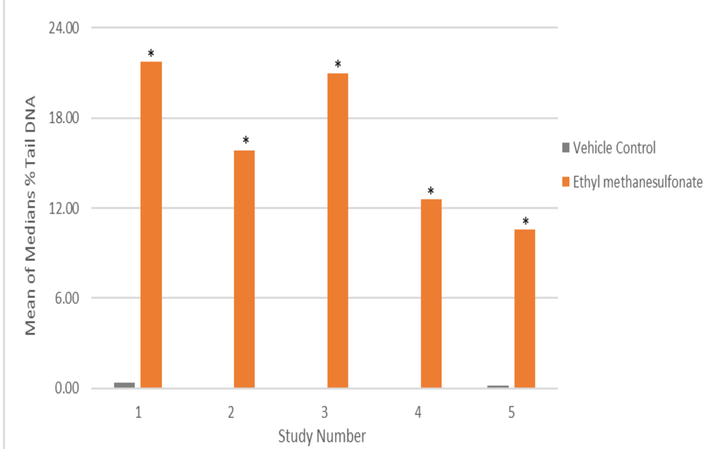


Figure 3. Group Mean Liver Comet Data



Introduction

The rodent alkaline comet assay is increasingly used as a second in vivo assay to mitigate in vitro positive results as recommended by the ICH S2(R1) guideline. Although an independent test guideline exists for this assay (OECD 489), integration of a comet endpoint within a general toxicology study may provide additional DNA damage information in potential target organs, which could be used as a screening or GLP assay. This approach not only minimizes the number of animals used (3R concept), but it also considers aspects of a living system which are more relevant for risk assessment with pharmacokinetic and/or pharmacodynamic considerations. We have evaluated multiple proprietary compounds using this integrated screening approach. In each study, female rats were subcutaneously or orally administered test compound for 6 days. Positive control animals were intraperitoneally or orally administered ethyl methanesulfonate twice. All animals were dosed at 5 mL/kg body weight. Slides for comet evaluation were prepared from duodenum and liver. For vehicle control, the mean %Tail DNA ranged from 1.26 to 2.95 for duodenum and 0.02 to 0.36 for liver. For positive control, the mean %Tail DNA ranged from 8.71 to 24.39 for duodenum and 10.57 to 21.77 for liver. The positive control group showed significant induction in %Tail DNA compared to vehicle control. These data demonstrate significant value for integrating comet assay with repeat dose toxicity studies. This study demonstrates our capability of conducting in vivo alkaline comet assay in multiple tissues at our newly established genetic toxicology division within Inotiv.

Materials

Female Sprague-Dawley rats (8 weeks, 150 grams at initiation) were obtained from Charles River Breeding Labs (Raleigh, NC).

Dimethyl sulfoxide (DMSO), ethyl methanesulfonate (EMS), methylcellulose (400 cPs), Tween 80, sodium citrate, citric acid, 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD), 0.9% saline, sterile water for injection, and 200 proof ethanol were purchased from Sigma Aldrich (St. Louis, MO). Lysis solution and Flare slides were purchased from R&D Systems (Minneapolis, MN). Low melting point agarose, 10N Sodium hydroxide (NaOH), Deionized water were purchased from VWR (Bridgeport, NJ). Ethylenediaminetetraacetic acid (EDTA) 200 mM solution and neutralization buffer were purchased from Molecular Toxicology (Boone, NC). Tris–borate–EDTA 1X buffer solution and SYBR Gold Nucleic Acid Gel Stain were purchased from Fisher Scientific (Hudson, NH). All other reagents and media were of the highest available grade.

Methods

Vehicle control groups consisted of 5 female animals. Positive control groups consisted of 3 to 5 female animals. EMS was administered at 200 mg/kg body weight. The vehicles were 30% w/v 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) in water, pH 8.0 ± 0.2 or 0.5% methylcellulose and 0.1% Tween 80 in 30mM citrate buffer, pH 3.5 ± 0.2. The dose volume was 5 mL/kg. Vehicles were subcutaneously or orally administered for 6 days. EMS was intraperitoneally or orally administered twice. Vehicle controls were administered subcutaneously in studies 1 to 3 and were administered orally in studies 4 and 5. EMS was administered intraperitoneally in studies 1 to 3 and were administered orally in studies 4 and 5.

The last dose administration to animals occurred 3 to 4 hours prior to euthanasia on study day 6. All animals were euthanized by CO_2 inhalation and then the liver and duodenum were collected. Liver was minced with scissors to prepare a single cell suspension and processed to slides for the comet assay after filtration. Duodenum was rinsed and scraped with a Teflon scraper to prepare single cell suspend and processed to slides for the comet assay after filtration. Cells were lysed overnight. Alkaline electrophoresis (pH > 13) was performed at 0.7 V/cm for 30 minutes after 20 minutes of unwinding. Slides were neutralized and dehydrated prior to staining with SYBR gold for evaluation. Slides were scored for number of hedgehogs and 150 cells were scored for evaluation at 200X magnification.

Results

Figure 1: Comparison of individual animal %Tail DNA to legacy upper 95% control limit for each tissue examined. The legacy upper 95% control limit for liver is 3.10%. The legacy upper 95% control limit for duodenum is 11.18%.

Figure 2: Group mean %Tail DNA values for duodenum in the 5 studies performed. All studies had significant increases in %Tail DNA observed between the vehicle and positive control per a pairwise T-test ($p < 0.05$).

Figure 3: Group mean %Tail DNA values for liver in the 5 studies performed. All studies had significant increases in %Tail DNA observed between the vehicle and positive control per a pairwise T-test ($p < 0.05$).

Conclusions

- Data collected at Inotiv's newly established genetic toxicology laboratory was found to be within legacy historical control limits for each organ examined.
- The significant response of ethyl methanesulfonate was observed irrespective of the route of administration used in the assay.
- A Comet assay endpoint can be incorporated into repeat dose toxicity studies to concurrently obtain and assess DNA damage in potential target tissues with proper planning and execution.

References

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OECD Guidelines for Testing of Chemicals, Guideline 489 (Genetic Toxicology: In Vivo Mammalian Alkaline Comet Assay), adopted, 29 July 2016.