Effects of Sample Processing on the Interpretation of *In Vivo* Comet Assay Data

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ABSTRACT

Among the various mechanistic-based techniques for evaluating the genotoxic activity of a compound, the comet assay is increasingly regarded as one of the more valuable approaches. Applicable to either *in vitro* or *in vivo* methodologies, this assay is a sensitive technique for the detection of DNA damage (e.g., direct single and double strand breaks, DNA adducts and other classes of damage that result in alkali-labile sites, DNA-DNA and DNA-protein crosslinks, and single strand breaks associated with incomplete excision repair events) in individual eukaryotic cells (**1-5**).

Current guidelines for *in vivo* comet assay genetic toxicology testing do not address specific technical methodologies such as sample processing that may have significant effects on the data. If sample processing techniques are not taken into consideration during comet assay study design and conduct and/or during data interpretation, there is a significant risk of the misinterpretation of data when determining the genotoxicity of tested compounds. Therefore, the ability of the assay to detect genotoxicity is dependent on the control of variability in methods used during sample processing.

This paper includes data from an *in vivo* comet study performed at Helix3. The data is presented as an example of the possible effects of cell sample handling on DNA migration and the interpretation of comet assay data.

INTRODUCTION

Tissue samples for *in vivo* comet assay analysis are preferably processed fresh immediately after harvest. However, as demand increases for special exposure conditions and/or test systems requiring comet assay analysis, the ability to appropriately process freshly harvested samples becomes limited .

To address this limitation, freshly harvested comet tissue samples have been minced in buffer containing 2mM Na_2EDTA and 10%DMSO, flash frozen, and stored at –70±10°C until they can be thawed and processed to slides. This method has proven to be effective for many samples with only a slight increase in DNA migration when compared to freshly processed samples. However, this paper demonstrates that the use of frozen samples in the determination of DNA damage using the comet assay can be grossly affected by the handling and transport of these samples.

METHODS & MATERIALS

Animals

The study was conducted with 8 week old male Sprague Dawley rats obtained from Charles River Laboratories, Raleigh, NC. Animals were maintained single housed in polycarbonate cages with absorbent bedding and at a controlled temperature of 18-26°C, 30-70% relative humidity, and with an air exchange rate of 70 exchanges per hour. Lighting was controlled to provide 12 hours of light and 12 hours of dark. Purina Rodent Chow 5002 (Purina Mills) and water were provided *ad libitum*.

METHODS & MATERIALS (CONT.)

Dose Administration

Each dose group consisted of 6 animals. Dose groups received a single oral administration in a dose volume of 10 mL/kg and 4 hours prior to necropsy The EMS dose group received 300 mg/kg body weight (bw) of ethyl methanesulfonate (EMS; CAS No. 62-50-0) prepared in dH_2O. The vehicle control dose group received dH_2O.

Comet Sample Processing and Analysis

Animals were anesthetized by CO_2 before being euthanized by exsanguination 4 hours after the final dose administration. Following exsanguination, portions of the liver, duodenum, and urinary bladder were removed for processing and analysis.

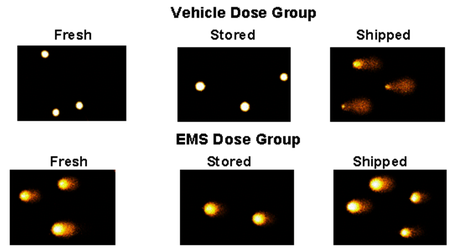
To compare the differences in migration induced by the freezing and/or the transport of frozen comet samples, comet slides were prepared from freshly harvested and minced tissue samples. Immediately after slides were prepared, two aliquots of the remaining minced cell suspension were flash frozen in liquid nitrogen and stored at –70±10°C for at least 24 hours. One frozen aliquot from each sample remained stored at –70±10°C (stored samples) while the other frozen aliquots were shipped on dry ice overnight by air to another facility (shipped samples). Immediately after receipt, the shipped samples were returned to the Helix3 facility by the same method. Upon arrival, it was confirmed that the shipped samples remained frozen and in good condition with an ample supply of dry ice still present in the shipment container.

The frozen stored and shipped samples were thawed quickly at room temperature and processed to slides. All comet slides were prepared, lysed, electrophoresed, and scored using the standard Helix3 procedures(6).

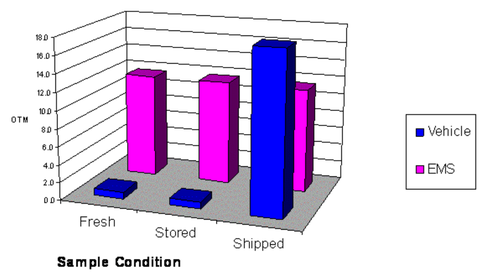
RESULTS

Based on visual examination and image analysis, there were no significant differences in DNA migration when comparing the freshly prepared tissue samples to the stored tissue samples from the same dose groups. However, there were significant differences when comparing the shipped samples to the freshly prepared or stored samples.

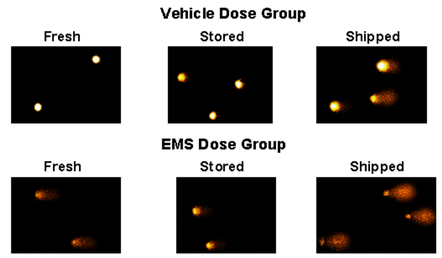
Liver Samples



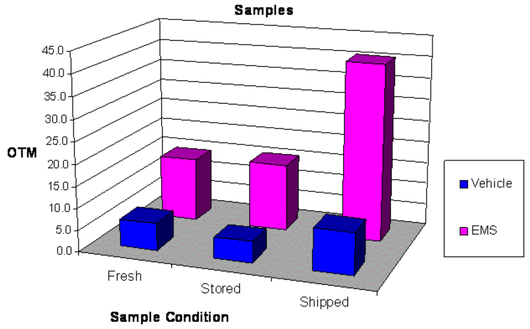
Comparison of DNA Migration Levels in Processed Liver Samples



Duodenum Samples

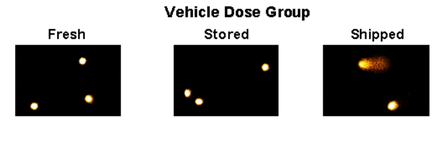


Comparison of DNA Migration Levels in Processed Duodenum Samples

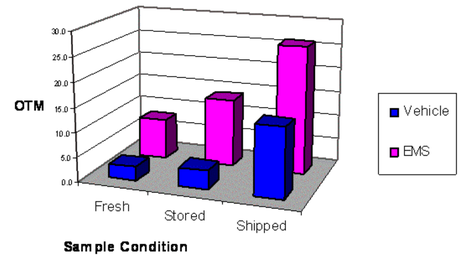


RESULTS (CONT.)

Urinary Bladder Samples



Comparison of DNA Migration Levels in Processed Urinary Bladder Samples



CONCLUSIONS

Based on the increase in DNA migration induced by shipping frozen samples by air, this method is not sufficient for maintaining comet assay sample integrity. Increased levels of migration induced in all of the tissues from the negative control animals significantly reduces the sensitivity of the assay. In the liver, the common target organ for genotoxins, this effect is most pronounced with DNA migration in the vehicle control samples exceeding migration detected in the samples from EMS treated animals. Therefore, the best method for processing comet samples is to process them to slides fresh and immediately after harvest. Shipping of frozen samples is not recommended.

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