Implementation of the Comet Assay in Eight Target Organs and Tissues of the Sprague-Dawley Rat

F. Merah, G. Marceau, M. Ghoussoub, S. Lavallée, N. Boudjadja C. Gauthier and A. Nelson

ITR Laboratories Canada Inc., Montreal, Canada

Abstract

The comet assay utilizes single-cell gel electrophoresis for the detection of DNA strand breaks in the nuclei of cells isolated from a variety of animal tissues. It is used as part of the genetic toxicology battery of tests to identify potential genotoxic compounds in pre-clinical research. Various target organs may be assessed for genotoxic analysis as required by the nature of the studied test item, target tissue, dosing method and metabolism. Here we detail the implementation of the comet assay in eight target organs or tissues of rats for the purpose of in vivo genotoxicity assessment.

Two groups of Sprague-Dawley rats were treated, in six independent replicates at 24 hours intervals of each other. In each replicate, the first group of animals was treated twice by oral gavage with ethyl methanesulphonate (EMS) at 200 mg/kg and the second group of animals treated with water by the same route. Tissues were collected (liver, stomach, kidney and skin in the first three replicates; and brain, spleen, lung and bladder in the last three replicates) in mincing buffer. Single cell suspensions were prepared, embedded in agarose on glass slides, lysed, and then subjected to alkaline unwinding and electrophoresis. The migrated DNA slides were then randomized, and stained with ethidium bromide before scoring. The DNA migration was measured with Comet Assay IV (Perceptive Instruments) scoring system and % DNA intensity was compared between groups within each replicate.

The mean percentage values of DNA intensity obtained in this study were significantly increased (p<0.01) between negative (water) controls (from 6.2% to 11.9%) and EMS-treated tissues (from 33.2% to 38.9%). More specifically, DNA intensity increased in the liver from 7.1% to 33.2%, in the lung from 6.2% to 36.0%, in the stomach from 7.6% to 33.4%, in the kidney from 6.6% to 33.3%, in the brain from 7.6% to 35.5%, in the skin from 11.9% to 38.9%, in the bladder from 8.1% to 36.1% and in the spleen from 7.6% to 35.0%.

The procedures used in this study are reliable and the organs selected are sensitive to genotoxic compounds such as EMS at 200 mg/mL which induced positive response in all these organs.

Objective

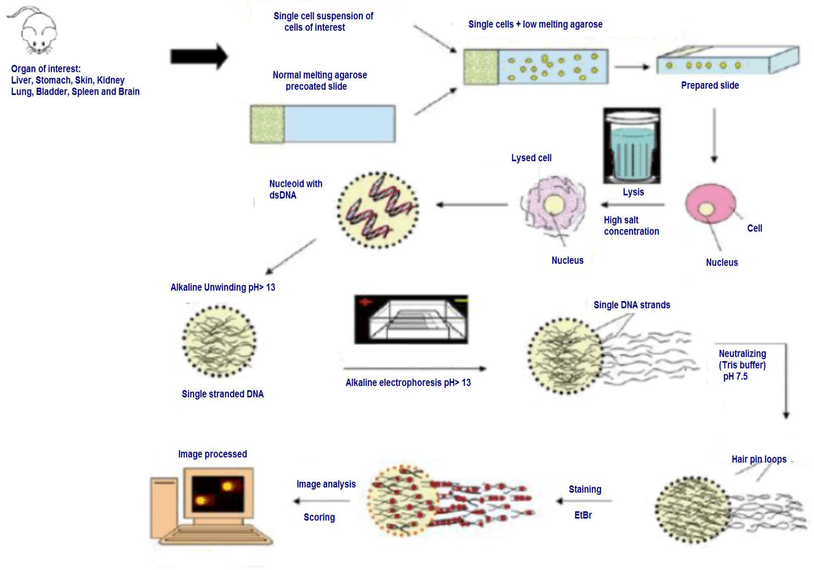
The objective of this work was to develop at ITR Laboratories Inc. the comet assay in different organs that could be considered as first site of contact for a variety of administration routes.

Introduction

The comet assay utilizes single-cell gel electrophoresis for the detection of DNA strand breaks in the nuclei of cells isolated from a variety of animal tissues. It is used as part of the genetic toxicology battery of tests to identify potentially genotoxic compounds in pre-clinical research. Various target organs may be assessed for genotoxic analysis as required by the nature of the studied test item, target tissue, dosing method and metabolism. Here we detail the implementation of the comet assay in eight target organs or tissues of rats for the purpose of in vivo genotoxicity assessment [1].

Experimental Design

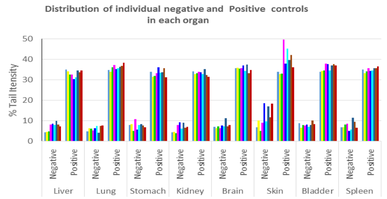
Six independent replicates of Sprague-Dawley rats were treated at 24 hours intervals of each other. Each replicate contains two groups with 3 animals in each group. The first group was treated twice by oral gavage with ethyl methanesulphonate (EMS) at 200 mg/kg and the second group was treated with water by the same route. Tissues were collected (liver, stomach, kidney and skin in the first three replicates; and brain, spleen, lung and bladder in the last three replicates) in mincing buffer. Single cell suspensions were prepared, embedded in agarose on glass slides, lysed, and then subjected to alkaline unwinding and electrophoresis. The migrated DNA slides were then randomized, and stained with ethidium bromide before scoring [2 and 3]. The DNA migration was measured with Comet Assay IV (Perceptive Instruments) scoring system and % DNA intensity was compared between groups within each replicate.



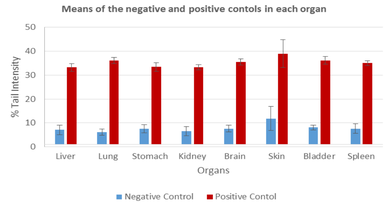
(Figure modified from : Gharsalli T. 2016 *J Environ Anal Toxicol)*

Results and Discussions

The mean % of DNA intensity calculated in the vehicle and positive controls in each organ are respectively: liver 7.1% and 33.2%, stomach 7.6% and 33.4%, skin 11.9% and 38.9%, in the kidney 6.6% and 33.3%, in the lung 6.2% and 36.0%, in the bladder 8.1% and 36.1%, in the spleen 7.6% and 35.0%, and the brain 7.6% and 35.5%.



Overall, the % DNA intensity obtained with vehicle controls ranged from 6.2% to 11.9% and the positive controls from 33.2% to 38.9%. The difference between the two groups in each organ was statistically significant (p<0.01).



Conclusion

The procedures used in this study are reliable and reproducible. The organs selected are sensitive to genotoxic compounds such as EMS at 200 mg/mL which induced positive response in all organs tested.

References

1. OECD guideline for the testing of chemicals . In vivo mammalian alkaline comet assay. TG487 adopted 09-2014.
2. Hartmann A., E. Agurell, C. Beevers, S. Brendler-Schwaab, B. Burlinson, P. Clay, A. Collins, A. Smith, G. Speit, V. Thybaud and R.R. Tice. Recommendations for conducting the in vivo alkaline Comet assay. *Mutagenesis* (2003) vol.18 no.1 pp.45–51,
3. Toyoizumi T., R. Ohta, Y. Nakagawa, Y. Tazura, M. Kuwagata, S. Noguchi, K. Yamakage. Use of the in vivo skin comet assay to evaluate the DNA-damaging potential of chemicals applied to the skin. *Mutation Research* (2011) vol.726 pp. 175–180.
4. Gharsalli T.. Comet Assay on Toxicogenetics; Several Studies in Recent Years on Several Genotoxicological Agents J Environ Anal Toxicol (2016) 6:6 418