Development of the *In Vivo* Alkaline Comet Assay for Use With Sprague-Dawley Rat Skin

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

ITR Laboratories Canada Inc., Montreal, Canada

Abstract

The *in vivo* alkaline comet assay (Single cell Gel Electrophoresis) is used for the detection of DNA strand breaks in the nuclei of cells isolated from different animal tissues. It is part of the genetic toxicology battery for the detection of potential genotoxic effect of substances. Here we report on the reproducibility of the comet assay in rat skin tissue and building a historical control database.

Five independent experiments were performed with Sprague-Dawley rats. Each experiment included two groups. One group was treated with water (negative control) and a second group was treated twice with a positive control substance, ethyl methanesulphonate (200 mg/kg, 10 mL/kg), at 24 and 3 hours before euthanasia. All the animals were treated via oral gavage. At termination a section (3x3 cm) of dorsal skin was shaved and removed. The skin was sliced into strips, immersed in a cold EDTA/PBS solution, transferred into a trypsin/HBSS solution overnight at 4°C and then rinsed again in cold PBS. The epidermis was peeled from the dermis and transferred into a cold RPMI with 10% FBS solution. The epidermal strips were gently stirred to prepare single cell suspensions, which were then passed through cell strainers. The isolated cells were laid over agar-coated slides, lysed, and subjected to electrophoresis. The prepared slides were then stained with ethidium bromide and evaluated under a fluorescent microscope. The DNA migration was measured with the Comet Assay IV scoring system (Perceptive Instruments) and % DNA intensity was compared between groups within each experiment.

The results obtained indicated that there were significant increases in DNA migration in the positive controls versus the negative controls (P< 0.01); and the results were reproducible between the five experiments. The mean values of the negative and positive controls were (19.91, 37.74) and (13.03, 32.47), (7.37, 33.22), (12.49, 44.29) and (15.75, 39.33) in the five experiments.

In summary, this technique can be used to prepare single cell population to perform Comet assay on skin for dermal studies in rats.

Objective

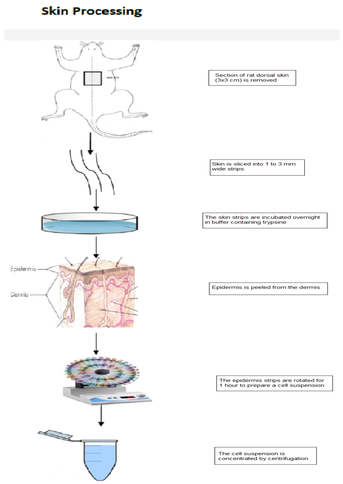
The objective of this work was to develop the comet assay in rat skin for use in dermal studies at ITR Laboratories Inc.

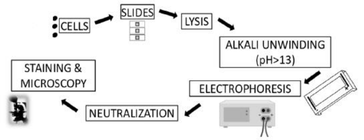
Introduction

The comet assay, alternatively called *in vivo* alkaline comet assay or single cell Gel Electrophoresis) is used to detect DNA damage and it is widely used in genotoxicity testing of substances such as industrial chemicals, biocides, agrochemicals, food additives and pharmaceuticals. It could be used as a second in vivo assay along with the *in vivo* micronucleus assay or as a follow-up to positive in vitro cytogenetic assay. It could be used in variety of target tissues with direct or/and indirect exposure to the test substance. Due to the scarcity of comet procedures and data with rat skin, the purpose of this work was to adapt and apply the comet assay in rat skin tissue and to build a historical control database.

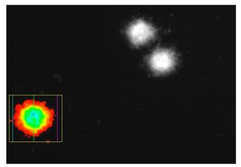
Experimental Procedures

Five independent experiments were performed with Sprague-Dawley rats. Each experiment included two groups (vehicle and positive controls) and all the animals were treated via oral gavage. The vehicle control was treated once with water at 10 ml/kg. The positive control was treated with ethyl methanesulphonate (EMS) at 200 mg/kg and 10 mL/kg) at 24 and 3 hours before euthanasia. At termination, section (3x3 cm) of dorsal skin was shaved and removed. The skin was sliced into 1 to 3 mm wide strips. These strips were immersed in a 0.02%w/v EDTA/PBS solution for approximately 10 minutes, transferred into a 0.25% w/v trypsin/HBSS solution overnight at 4°C and rinsed again in cold PBS. The epidermis was peeled from the dermis and transferred into a 10 mL cold RPMI with 10% FBS solution. The epidermal strips were gently stirred for approximately 1 hour using a rotator at 4°C to prepare single cell suspensions, which were then passed through cell strainers and concentrated by centrifugation (300g, 5 min at 4°C)[1]. The isolated cells were mixed with 0.5% low melting agarose and laid over 1% normal melting agarose coated slides. The cells were lysed overnight in lysing solution. The slides were submerged in electrophoresis tank with alkaline buffer for 20 minutes. The slides were electrophoresed for 20 min at 0.7V/cm). The prepared slides were then stained with ethidium bromide and evaluated under a fluorescent microscope. The DNA migration was measured with the Comet Assay IV scoring system (Perceptive Instruments) and % DNA intensity was compared between groups within each experiment.[2,3 and 4]

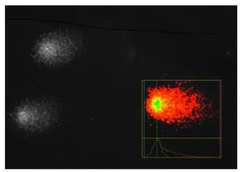




Comets samples from negative and positive control scored with Comet system IV



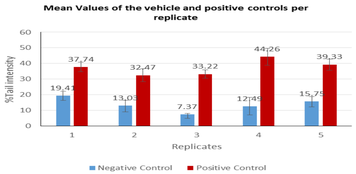
Comets from negative controls



Comets from positive controls

Results and Discussions

There was clear increase in DNA migration between the negative and the positive controls. The mean values of the negative and positive controls were (19.91, 37.74) and (13.03, 32.47), (7.37, 33.32), (12.49, 44.26) and (15.75, 39.33) respectively in the five experiments.



To confirm if this increase was significant, the results obtained in the two groups within each replicate were subjected to statistical analysis using analysis of variance (ANOVA). The significance level between the control and EMS treated groups was below 0.01 and it was consistent and reproducible between the five experiments.

Conclusion

Based on these results, it is considered that the technique used in this research is reliable and reproducible. It could be used for the studies where the test substance is administered topically.

References

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