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Target Organ Selection for the I*n Vivo* Comet Assay: Genotoxicity Versus Tumorigenesis

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ABSTRACT

Target organ selection in the *in vivo* comet assay is one of the most critical processes for assessing the genotoxicity of a compound. However, the site of tumorigenisis may not be the site of genotoxicity due to the mechanistic pathway of the compound. For example, 7, 12 dimethyl benz[a]anthracene (DMBA) is known to induce mammary tumors; but DNA damage has not been detected in the mammary tissue. DMBA induced mammary tumors may be a result of DNA damage induced in a component of the endocrine system, such as the pituitary gland. Using the comet assay to measure DNA damage induced in the endocrine system may provide insight into the mechanisms of tumorigenisis for diseases such as breast cancer and prostate cancer. Data is presented to demonstrate how the comet assay can be used on the components of the endocrine system.

INTRODUCTION

DMBA is commonly used as a positive control for mammary tumor induction; however the exact mechanism by which tumors are induced is not understood. According to the American Cancer Society, breast cancer is the most common form of cancer among women, accounting for about 25 percent of all cancer diagnoses among women. Up to this point, most research has been directed specifically at the mammary glands as the site of tumorigenisis and does not focus on other tissues that may contribute to tumorigenisis. Using the comet assay to test the tissues of the endocrine system may provide valuable information for diseases such as breast cancer. The hormones created by the endocrine system affect almost every tissue in the body including the mammary glands. Damage in one of these tissues may affect the hormones that signal cellular activities in other tissues.

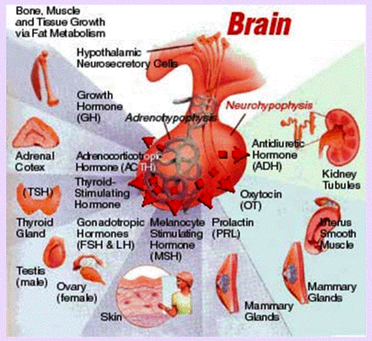


Figure 1: Hormones Produced by the Endocrine System and Tissues Affected.

INTRODUCTION (CONT.)

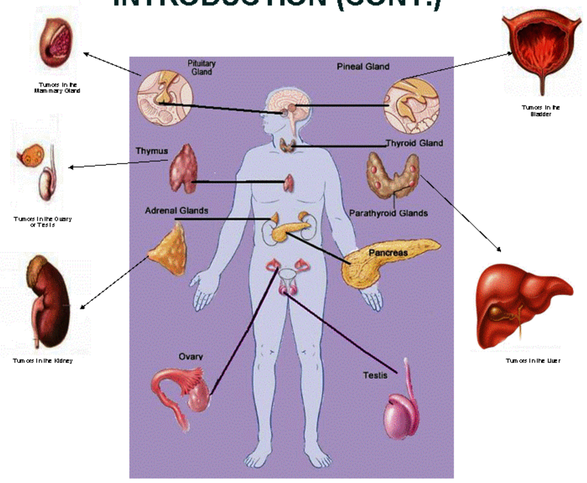


Figure 2: Components of the Endocrine System that May Cause Tumorigenisis in Other Organs.

To determine if 7,12 dimethyl benz[a]anthracene (DMBA) induced tumors are the result of damage induced to the pituitary gland or other tissues of the endocrine system, female sprague dawley rats were orally exposed to DMBA and sampled for comet analysis.

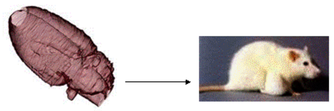


Figure 3: Damage to the Pituitary Gland May Cause Mammary Tumors.

METHODS & MATERIALS

This experiment was conducted with 8 week old female Sprague Dawley rats. Because of limited pharmacokinetic data available, the standard comet assay experimental design was selected. Animals received DMBA at 100 mg/kg BW or the vehicle control on two consecutive days, 20±0.5 hours apart. 4±0.5 hours after the final dose administration, animals were anesthetized by CO2 before being euthanized by exsanguination.

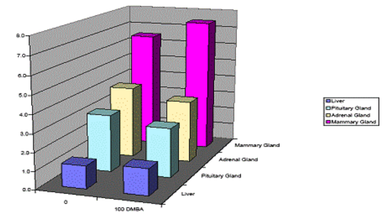
METHODS & MATERIALS (CONT.)

Prior to exsanguination, portions of an inguinal mammary gland were removed for processing and analysis. Following exsanguination portions of the liver, the pituitary gland and the adrenal gland were removed for processing and analysis. All comet slides were prepared, lysed, electrophoresed, and scored using the standard Helix3 procedures (1).

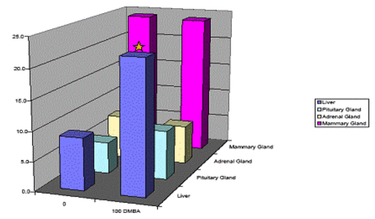
RESULTS

Four hours after the final oral administration of DMBA at 100 mg/kg bodyweight, DMBA did not induce a significant increase in the level of DNA migration or cytotoxicity in cells of the adrenal gland, pituitary gland or mammary gland. DMBA also did not cause in an increase in DNA migration in cells of the liver; however, based on a one- tailed trend test, DMBA induced a significant increase in cytotoxicity in cells of the liver (☆ =p<0.05).

Table 1: DNA Damage & LMW Diffusion in Rats Exposed to DMBA and Sampled 4 Hours After Final Administration



DNA Damage



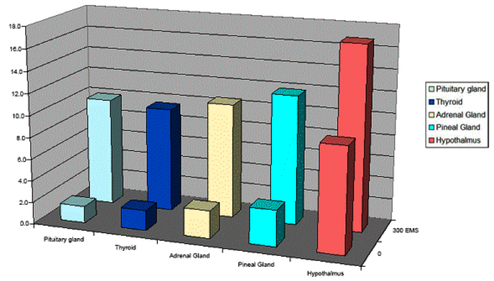
LMW Diffusion

CONCLUSIONS

Under the current experimental conditions, DMBA did not induce an increase in DNA migration in the target tissue (mammary gland), the pituitary gland, the adrenal gland, or the liver. However, an increase in cytotoxicity was observed in the liver four hours after the final administration. Because pharmacokinetic data has not been obtained for DMBA in any of the tissues tested, a time course may be necessary to determine the optimal dosing and sampling times to observe genotoxicity in the sampled tissues (2). In addition, other tissues of the endocrine system may need to be tested.

Many of the current methods for assessing genotoxicity only look at specific primary tissues such as liver or bone marrow. To take advantage of the sensitivity and the flexibility of the comet assay, other tissues should be investigated to determine true risk assessment. Looking at the liver or only the site of tumorigenisis may not be sufficient when testing compounds such as DMBA.

Table 2: DNA Damage in Rats Exposed to EMS in Different Tissues of the Endocrine System.



REFERENCES

1. Vasquez, M. and S. Pfuhler (2006) Effects of cytotoxicity on the interpretation of in vivo comet assay data. Environ. Mo. Mutagen Poster Presentation.
2. Vasquez, M. (2008) Comet Assay: Breaking from Tradition. Environ. Mo. Mutagen Poster Presentation.