SHORT COMMUNICATION

Unsymmetrical DMH – An isomer of 1,2 DMH – Is it potent to induce gastrointestinal carcinoma in rats?

Pamela Christudoss\textsuperscript{a,*}, R. Selvakumar\textsuperscript{a}, Anna B. Pulimood\textsuperscript{b}, J.J. Fleming\textsuperscript{a}, George Mathew\textsuperscript{c}

\textsuperscript{a}Department of Clinical Biochemistry, Christian Medical College, Vellore 632004, Tamil Nadu, India
\textsuperscript{b}Department of G.I. Sciences, Christian Medical College, Vellore 632004, Tamil Nadu, India
\textsuperscript{c}Department of General Surgery, Christian Medical College, Vellore 632004, Tamil Nadu, India

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Abstract

Very few animal studies have used 1,1-dimethyl hydrazine (unsymmetrical dimethyl hydrazine – UDMH) as a carcinogen. This study was designed to investigate the carcinogenicity of UDMH in the gastrointestinal tract in a rat model. We wanted to observe if there were any changes in tissue zinc levels and tissue copper zinc superoxide dismutase (CuZnSOD) enzyme activity during the carcinogenic process, and to compare these values with those of control rats in the medium- and long-term. Six-week-old Wistar rats were given a subcutaneous injection of UDMH (30 mg/kg body wt) twice a week for 20 weeks, and sacrificed after 5 and 9 months of treatment. Tissue zinc levels showed a significant decrease \( (p < 0.05) \) in the large intestine at 9 months, whereas in the stomach and small intestine there were no significant changes at 5 and 9 months. Tissue CuZnSOD enzyme activity in the stomach, small intestine and large intestine showed no significant decrease at 5 and 9 months as compared to controls. Histologically, the large intestine was normal at 9 months.

This study suggests that UDMH administered at the above dosage was not carcinogenic in this model.

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Introduction

Most published rat studies have been done on animals initiated with symmetrical dimethyl hydrazine (1,2-DMH) or its metabolite Azoxymethane (AOM) (Corpet and Tache, 2002). The carcinogenicity of 1,1-DMH (unsymmetrical dimethyl hydrazine – UDMH), which is an isomer of 1,2-DMH has been the subject of recent investigation by several national and international organizations as there have been very few animal studies using UDMH. However, these studies have reported an increase in the incidence of cancers after exposure to UDMH.

Rats and mice have almost no spontaneous gastrointestinal cancer. To test diets and agents, which could prevent cancer, one needs animals with tumors. That is why rodents are given a carcinogen.

Commonly used carcinogens are DMH derivatives. DMH \( (\text{C}_2\text{H}_8\text{N}_2) \) exists in two forms, namely 1,1-DMH (UDMH) where both methyl groups are attached to the
same nitrogen atom and 1,2-DMH (symmetrical dimethylhydrazine) where one methyl group is bonded to each of the two nitrogen atoms. 1,2-DMH is metabolized in the liver to AOM and then to the methyl carbonium ion – the ultimate carcinogen, which binds to the DNA of stem cells in the colon.

There is evidence for the carcinogenicity of UDMH in organs other than the gastrointestinal tract in experimental animals (International Agency for Research on Cancer, IARC, 1974). When administered by gavage at a dose of 0.5 mg daily for 20 weeks, UDMH increased the incidence of lung tumors in female Swiss mice by 30 weeks (IARC, 1974). Female mice given UDMH at 20 mg/kg body wt, subcutaneously (s.c) once a week for 20 weeks exhibited multiplicity of lung adenoma (Tanura et al., 1999). Hamsters injected with 37 mg/kg s.c UDMH once a week for 30 weeks developed hepatocellular carcinoma or adenocarcinoma of the stomach especially in females (Ernst et al., 1987). In one study, mice rats, and hamsters were administered UDMH in their drinking water. Both the mice and hamsters showed a significant increase in tumors of blood vessels, lungs, kidneys and liver, the rats developed liver cancer and the hamsters also developed vascular and caecal tumors (Cincinnati, 1991). Rats given 1.3 mg/kg/day UDMH, via drinking water for 1 year had an increase in lung tumors (US Environmental Protection agency, 1989).

However, it is not known to date, to the best of our knowledge whether UDMH induced gastrointestinal carcinoma in rats.

This study was designed to investigate the carcinogenicity of UDMH in the gastrointestinal tract in a rat model. We wanted to observe if there were any changes in tissue zinc levels and tissue copper zinc superoxide dismutase (CuZnSOD) enzyme activity during the carcinogenic process, and to compare these values with those of control rats in the medium- and long-term.

Materials and methods

Six-week-old adult Wistar rats (100–120 g) were housed in polypropylene plastic cages, in an animal holding room under controlled conditions with 25±2 °C, 50±10% humidity, and 12-h light–dark cycles. The rats were allowed water and food ad libitum, observed daily and weighed weekly. This study was approved by the Animal Experimentation Ethics Committee of the Institution.

Experimental design

Twenty-four rats were randomly assigned to four groups of six each and were fed the same diet. Groups 1–2 were experimental groups and received an s.c. dose of 30 mg/kg body wt of UDMH, twice a week for 20 weeks, and were euthanized after 5 and 9 months, respectively. Groups 3 and 4 were control groups and received a s.c. dose of 0.25 ml of saline for 20 weeks and were also euthanized after 5 and 9 months, respectively. All groups were euthanized by chloroform inhalation.

The colon, small intestine and stomach of the rats were harvested. One part of each tissue was fixed in 10% buffered formaldehyde and processed for Histology studies as per standard methods. The second portion was used for tissue zinc estimation. The mucosa of the stomach, small intestine and large intestine were used for cytotoxic homogenate preparation, using phosphate-buffered saline pH 7.4. CuZnSOD enzyme activity in the homogenate was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction (Kahnke, 1966). Zinc determination was carried out using a Perkin Elmer Atomic Absorption Spectrophotometer model-100 (Kim et al., 2000). Small intestine and large intestine were examined macroscopically for tumors.

Statistical analysis

Data are expressed as mean ± SD. Differences between groups were analyzed using the Mann–Whitney test and the ANOVA statistics program. A difference was considered statistically significant when the probability was less than 0.05 (p<0.05).

Results and discussion

Tissue zinc levels in stomach and small intestine did not show any significant changes over the time period as

Fig. 1. Tissue zinc levels in stomach, small intestine and large intestine in control and test rats at 5 months (a) and 9 months (b). Value represents mean ± SD from six rats, *p<0.05, when compared to control.
compared to control (Fig. 1a and b). There were no significant changes in CuZnSOD activity in stomach and small intestine at 5 months and 9 months (Fig. 2a and b). Of all the parts of the gastrointestinal tract studied, only the colon showed a significant decrease \((p<0.05)\) in zinc concentration at 9 months (mean% decrease is 30%) after administration of UDMH (Fig. 1b). Tissue CuZnSOD activity was not significantly decreased in the colon at 9 months (Fig. 2b). There were no mucosal changes from normal to precancerous transformation in the histological specimens of colon at 5 months or 9 months.

This is in contrast to our earlier studies in the AOM model which showed a significant decrease in tissue zinc and zinc-related enzyme (CuZnSOD) activity along with the progression of mucosa from normal to the precancerous stage in colon at various time periods (Christudoss et al., in press).

It was noted that UDMH administered at the above dosage was not carcinogenic in this model.

Thus from the above, we conclude that unsymmetrical dimethylhydrazine (1,1-DMH, UDMH) is not a good agent for inducing gastrointestinal carcinoma in this rat species.

References


