Cytotoxicity of 5,6-Dihydroxytryptamine in Dimethylhydrazine-induced Carcinomas of Rat Colon

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SUMMARY

Male Sprague-Dawley rats were given weekly s.c. injections of 1,2-dimethylhydrazine (21 mg/kg) for 20 weeks. The injections were then discontinued, and, after an interval of 2 to 8 weeks, experimental animals were given i.p. injections of 5,6-dihydroxytryptamine (5,6DHT) at a dose of 40 mg/kg and sacrificed at intervals of 1, 2, 6, 16, and 48 hr later. Specimens of descending colon and carcinomas of the descending or transverse colon from sacrificed animals were examined using light microscopy and transmission electron microscopy.

The results show that 5,6DHT at a dose of 40 mg/kg is cytotoxic to malignant colonic epithelial cells but not cytotoxic to adjacent nonmalignant colonic epithelial cells. In malignant colonic epithelial cells, ultrastructural changes in cytoplasmic membranes and mitochondria were evident at 1 hr after 5,6DHT treatment. At 6 hr after 5,6DHT treatment, light microscopy of sections of tumor showed areas of cell necrosis and disrupted tumor morphology. Sections of specimens taken 16 hr after treatment showed widespread destruction of malignant cells.

INTRODUCTION

Numerous studies have shown that biogenic amines are important regulators of cell proliferation in a variety of nonneoplastic and neoplastic tissues, including colonic crypt epithelium and colonic carcinomas. In 1 recent study, it was observed that inhibition of tissue monoamine oxidase stimulated cell proliferation in DMH-induced colonic carcinomas but not in the adjacent, nontumorous colonic epithelium. Since monoamine oxidase is important only for the degradation of amines taken into cells and has no role in the degradation of amines acting on cell membrane receptors, this was taken as preliminary evidence that colonic tumor cells, unlike their nonneoplastic counterpart, take up biogenic amines. Cell kinetic studies have now demonstrated that 5HT is an important amine acting to stimulate cell proliferation in experimentally induced colonic carcinomas (P. J. M. Tutton and D. H. Barkla, unpublished observation). Hence it was decided to investigate the effect of a cytotoxic congener of 5HT, 5,6DHT, on DMH-induced carcinomas of rat colon.

MATERIALS AND METHODS

Male Sprague-Dawley rats were fed Clark King Nu-pig pellets and tap water ad libitum and housed at 21–24° with artificial light from 7:00 a.m. to 9:00 p.m. and darkness from 9:00 p.m. to 7:00 a.m. Rats were given weekly s.c. injections of DMH (Aldrich Chemical Co., Inc., Milwaukee, Wis.) at a dose of 21 mg/kg as previously described (4, 15). After 20 weeks the DMH injections were discontinued. Following an interval of 2 to 8 weeks, the animals were used in the experiments described below.

Thirteen experimental animals were given i.p. injections of 5,6DHT, (Labkemi AB, Stockholm, Sweden) at a dose of 40 mg/kg (1) and were subsequently killed by decapitation at intervals of 1, 2, 6, 16, and 48 hr after treatment. This dose of 5,6DHT was chosen because it had been shown to be a safe but effective dose in other experiments (1). Eight of the experimental animals also received an injection of phentolamine (Regitine; Ciba-Geigy, Sydney, Australia) at a dose of 10 mg/kg to reduce the pressor effects of 5,6DHT treatment. Control animals received the same regimen of DMH injections as the experimental animals but did not receive injections of 5,6DHT or phentolamine.

Light Microscopy. Specimens of descending colon and of tumors of the transverse or descending colon were fixed in Bouin’s solution, dehydrated through alcohols, and sectioned at 4 μm. Electron microscopic thin sections were then excluded from the study.

Electron Microscopy. Specimens of descending colon and of carcinomas of the descending or transverse colon were cut into 1- x 5-mm segments and fixed in a solution containing formaldehyde (3%), glutaraldehyde (4%), and trinitroresol (0.5%) in 0.05 M cacodylate buffer (pH 7.4) for 6 hr at 4°. The tissue was then rinsed in 0.1 M cacodylate buffer (pH 7.4) and postfixed for 2 hr in 1% osmium tetroxide. The tissue was treated with 1% uranyl acetate in 0.2 M maleate buffer (pH 5.15) for 1 hr and then dehydrated through graded ethanol and embedded in Epon-Araldite. The thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens 1A electron microscope at 80 kV.
RESULTS

Following injection of 5,6DHT the rats became blanched in appearance and somewhat lethargic; both these reactions were more conspicuous in animals not treated with phenolamine. These effects lasted some 3 hr, and during this time 1 rat treated with 5,6DHT but not phenolamine died; tissues from this animal are not included in the study. All other animals survived, and no further sign of generalized toxicity was noted.

Macroscopic Appearance of Colonic Mucosa and Tumor. Many tumors examined at 1, 2, 6, 16 hr after 5,6DHT treatment were hyperemic in appearance and showed areas of surface hemorrhage. Tumors examined 48 hr after 5,6DHT treatment were usually pale gray.

In all experimental animals the non-tumor-bearing colonic mucosa retained a normal appearance except for 1 animal showing an area of brown mucosa measuring approximately 1 x 1 cm surrounding a tumor 48 hr after 5,6DHT treatment.

Light Microscopy. Typical DMH-induced tumors not treated with 5,6DHT were moderately well-differentiated adenocarcinomas closely resembling human colorectal tumors (Fig. 1). Histological sections of tumors taken at 1 and 2 hr after 5,6DHT treatment showed dilated blood vessels and increased numbers of leukocytes. Sections of tumors taken at 6 and 16 hr after 5,6DHT treatment showed areas of necrosis where tumor glandular formations were disrupted and tumor cells were degenerating (Fig. 2). However, not all of the tumor mass was equally affected, and some glandular formations remained evident in the majority of the tumors (Fig. 2). In 1 animal sacrificed 48 hr after 5,6DHT treatment, there were few tumor cells remaining in sections examined, and the tumor mass was formed by a surface layer of amorphous eosinophilic material and a deeper layer of connective tissue containing densely basophilic granules. Dilated blood vessels and areas of hemorrhage were no longer apparent. In sections taken from other animals sacrificed 48 hr after treatment, the changes were less marked.

The histological appearance of adjacent nontumorous colonic epithelium in all animals examined following 5,6DHT treatment was similar to that seen in control tumor-bearing animals not treated with 5,6DHT.

Electron Microscopy. Changes in the ultrastructural appearance of tumor cells were evident in specimens taken 1 hr after 5,6DHT treatment (Figs. 3 and 4). The most obvious changes were seen in mitochondria and intracellular membranes. Many mitochondria were swollen, showed disrupted cristae, and were filled with flocculent material. The cytoplasm of tumor cells was frequently filled with vacuoles that were either discrete, round, and membrane bounded or irregularly shaped dilations of the endoplasmic reticulum and nuclear envelope membranes (Fig. 3). The lateral plasma membrane was frequently discontinuous, allowing cell organelles to pass into the intercellular space (Fig. 3). At 1 hr, the nuclei of some malignant cells showed margination of chromatin, but changes in nuclear morphology were not a conspicuous feature at this time (Figs. 3 and 4). In addition, the microvilli, apical plasma membrane, and apical junctional complexes were usually intact. Similar ultrastructural changes were evident in specimens taken 2 hr after 5,6DHT treatment.

The ultrastructural appearance of adjacent nontumorous colonic epithelium in all animals examined was similar to that seen in control animals.

DISCUSSION

The effects of 5HT, 5HT precursors, and 5HT antagonists upon tumor growth do not appear to have been widely investigated during recent years. Growth of a transplanted sarcoma in rats has been reported to be stimulated by 5HT (11) and inhibited by the 5HT-blocking effect of lysergic acid derivatives (12). In addition, 5HT has been reported to be cytotoxic and inhibitory to growth of transplanted melanomas in mice (3). The observation in the present study that 5,6DHT at a dose of 40 mg/kg is cytotoxic to malignant epithelial cells in DMH-induced tumors but nontoxic to the antecedent epithelium of rat colon suggests that further investigation into the role of 5HT and related compounds upon tumor cell biology should be undertaken. At present, the possibility that 5,6DHT toxicity is mediated by vasoconstriction cannot be disproved.

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REFERENCES


Fig. 1. Light micrograph of a section of a DMH-induced adenocarcinoma of the descending colon showing the typical morphology of a DMH-induced tumor not treated with 5,6DHT. H & E, × 320.

Fig. 2. Light micrograph of a section of tumor 16 hr after 5,6DHT treatment showing disruption of normal tumor morphology and necrotic debris. H & E, × 320.

Fig. 3. Electron micrograph of several tumor cells in a specimen taken 1 hr after 5,6DHT treatment. Intracellular vacuole formation, changes in mitochondrial morphology, and disruption of cell-cell contacts are evident. × 2500.

Fig. 4. Higher power electron micrograph showing in more detail abnormal mitochondria and isolated desmosomes (arrowheads) in tumor cells 1 hr after 5,6DHT treatment. × 10,000.
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