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COMPARISON OF THE MUTAGENIC RESPONSE IN SMALL INTESTINE EPITHELIUM AND IN BONE MARROW OF CHIN. HAMSTERS BY CHEMICAL MUTAGENS

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Abstract

When chromosomal aberrations induced by different mutagens were compared in bone marrow and in small intestine epithelium, the primary target cells of the small intestine exhibited remarkably less mutagenic damage. Tissue-specific sensitivity may be involved.

Introduction

In vivo mutagenicity tests using cytogenetic methods require target cells of proliferative tissues. These tissues are characterized by a short cell-cycle and therefore contain a high proportion of dividing cells. Bone marrow cells fulfill these preconditions in an ideal manner. The lining cells of the villi in the epithelium of the small intestine exhibit the same fast cell cycle of about 10 hours as bone marrow cells (LIPKIN, 1981). These intestinal cells represent the primary target for mutagens ingested with food or generated during gastro-intestinal metabolism, and it should therefore be possible to detect clastogenic effects in this tissue.

Contributions to the literature on this topic deal mostly with aspects of cell preparation for cytogenetic investigation (MILTENBURGER et al., 1980; BLAKEY, 1985), with problems of the possible transformation of promutagens in this part of the intestinal tract (NEAL and PROBST, 1984; MÜNZNER and WEVER, 1987) or with the absorption, distribution and excretion of orally administered potential mutagens (HENSCHLER and WILD, 1985; MÜNZNER and WEVER, 1984). Although bone marrow is a comparatively convenient tissue for chromosome preparation it

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may not be a suitable target organ for the detection of all mutagens. There is thus a distinct need to monitor genotoxic effects in various other organs or tissues of the treated animal (ASHBY, 1983). Some authors have avoided the difficulties of chromosome analysis in the target organs selected (small intestine, colon, liver, or spleen) and have evaluated instead the incidence of micronuclei or nuclear anomalies using procedures more closely related to histological techniques (RONEN and HEDDLE, 1984; TATES et al., 1980; PROUDLOCK and ALLEN, 1986).

The following bioassays were designed to detect possible clastogenic effects on the intestinal epithelium using a number of different mutagens. A comparison was then made with chromosomal aberrations induced in bone marrow cells by the same mutagen. It was thus possible to compare the local genotoxic effects in the primary target organ due to direct contact with ingested mutagens with the effects of mutagens which have passed through the intestine-blood barrier and have been transported, metabolised and diluted by the blood stream.

Experimental

Chinese hamsters from our own breeding colony (aged 12 - 16 weeks, weighing 30-35 g and housed in climatised rooms) were used. Feed (Herilan laboratory chow) and tap water were available ad lib. throughout the experiments. Six animals (3 males and 3 females)/test were used and 100 well-spread metaphases/animal were evaluated. Bone marrow cells were flushed out from both femora and intestinal cells were obtained from a 4 cm part of the upper intestine.

The chromosome aberration test was performed using the commonly accepted technique (SCHWARZACHER and WOLF, 1974). To score bone marrow cells 1 mg/kg colchicine (Demecolcin, Serva) and to investigate intestinal epithelial cells 5 mg/kg colchicine were injected s.c., in each case 2 hours before sacrifice of the animals by carbon dioxide asphyxiation.

To find out the most effective oral doses as well as the times of the maximum response for each tissue different doses of the test substances and different sampling times were checked. As a consequence investigations on bone marrow and intestinal cells could not be performed on the same animals.

The preparation of the epithelial cells of the small intestine was performed according to the procedure described by BLAKEY (1985), with some modifications. After hypotonic treatment and centrifugation steps the cells, suspended in fixative, were dropped on chilled slides followed by flame fixation and staining with 2% aceto-orcein solution for 7 min.

All compounds were administered by stomach tube as a single treatment in a volume of 1 ml/100 g animal body weight; (1) as an aqueous suspension of 0.5% carboxymethylcellulose, (2),(5),(6) suspended in plant oil, (3), (4) as as aqueous solution. Dosages are given in Tab. 1.

	bone marrow cells				small intestine epithelial cells			
Substance	dose (mg/kg)	sampling time after dosing (h)	aberrant metaphases*) %	±SD	dose (mg/kg)	sampling time after dosing (h)	aberrant metaphases*) %	± SD
negative control	---	--	0.2	0.4	---	--	0	0
6-mercaptopurine	125	30	3.3	0.6	125	30	3.5	1.1
		36	3.7	0.7		36	6.0	0.9
		48	1.5	1.2		48	1.5	0.6
busulfan	50	30	9.5	2.2	75	20	7.3	1.1
		36	12.5	1.4		24	7.8	1.9
		48	7.3	1.3		30	6.5	1.1
thiotepa	10	24	8.0	1.0	15	20	2.3	0.5
		30	9.8	1.2		24	2.8	1.0
		36	5.8	1.6		30	2.3	1.0
cyclophosphamide	18	16	7.0	0.6	100	16	1.8	1.0
		20	12.6	2.2		20	3.2	0.8
		24	12.0	1.4		24	2.8	0.4
benzo(a)pyrene	100	24	0.2	0.4	100	20	0.5	0.5
		30	0	0		24	1.8	0.5
		36	0.5	0.5		30	1.5	0.5
7,12 DMBA	75	30	0.5	0.6	100	30	0.3	0.5
		36	2.8	1.3		36	1.0	0.8
		48	0.8	0.4		48	0.6	0.5

Tab. 1 Induction of clastogenic effects in bone marrow and small intestine epithelial cells of Chinese hamsters after oral administration of different mutagens

Numbers of aberrant metaphases represent mean values of 6 x 100 metaphases ± standard deviation

*) excluding gaps

The following test mutagens were selected for these investigations:

Compound	Type of compound type of action	Supplier
(1) 6-mercaptopurine	purine analogue, antimetabolite	Serva
(2) busulfan	methane sulfonic acid ester, direct-acting alkylant	Serva
(3) thiotepa	ethylenimine derivative, direct-acting alkylant	Lederle
(4) cyclophosphamide	N-mustard gas derivative, indirect-acting alkylant	Degussa/Asta
(5) benzo(a)pyrene	PAH model substance carcinogen, weak mutagen	Sigma
(6) 7,12 dimethylbenz(a) anthracene	PAH indirect-acting muta-carcinogen	Aldrich

Results

Of the six substances under test, four compounds represent strong mutagens and two compounds are well known mutagenic carcinogens. As shown in Tab. 1 chromosomal damage observed in the intestine in no case reached the magnitude of the clastogenic effects induced in bone marrow. The times for the maximum response in the small intestine were equal to or shorter than those observed in bone marrow. The mutagen 6-mercaptopurine, a potent antimetabolite of nucleic acid metabolism (FROHBERG and SCHULZE-SCHENCKING, 1975) produced a higher aberration rate in the intestine epithelium than in bone marrow whereas busulfan induced the strongest chromosomal damage of all in the intestine. Of the potent muta-carcinogens benzo(a)pyrene (BASLER and RÖHRBORN, 1976) and DMBA (JERINA et al., 1980) only the latter showed elevated break rates in bone marrow and slight effects in the intestine.

Discussion

The cell material used for our cytogenetic investigations originated from the duodenum and the upper jejunum. The monolayered epithelium of this part of the gut covers the villi and the crypts. In this region the epithelial cells migrate from the crypts to the top of the villi and exhibit most frequent mitoses in the crypts (MOOG, 1981). Because of this process it may be assumed that the observed chromosome damage in the small intestine occurs mainly in the epithelium of the crypts. However, about 48 hours later, these damaged cells reach the top of the villi and are sloughed off into the gut lumen, resulting in a permanent regeneration of the small intestinal epithelial surface.

The small intestine is one of the organs of the body least likely to develop cancer (LOWENFELS, 1973). Using MNU and benzo(a)pyrene, compounds known to induce tumours in the small intestine of mice, GOLDBERG and CHIDIAC (1986) were able to provoke tissue-specific effects, namely nuclear aberrations, in the small intestinal epithelium of mice. Similarly, WARGOVICH et al. (1983) found five large bowel carcinogens to be genotoxic in a colonic nuclear aberration short-term test.

The high rate of replication of the small intestinal epithelial cells, the fluid nature of the small bowel contents and their rapid transit through the small intestine may contribute to the apparent low tumourigenic potency of ingested genotoxins or carcinogens in this tissue (HARTMAN, 1983 and 1986, LOWENFELS, 1973) and to the relatively low mutagenicity observed in this study.

Therefore there appears to be no advantage in using the epithelial cells of the small intestine instead of the bone marrow when trying to establish the genotoxicity of a compound, especially when the technical difficulties of mucosal cell preparation are taken into consideration. The weak intestinal response of orally administered mutagens and mutagenic carcinogens contrasts with their distinct effects on bone marrow. Tissue-specific properties of the intestinal epithelium may lead to physiological protection against mutagenic and carcinogenic attacks on this site. However, this does not apply to the possible actions of ingested genotoxins in the colon or after absorption and metabolisation in other organs.

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REFERENCES

ASHBY J., 1983. Mutation Res. 115: 177-213.

BASLER A. and G. RÖHRBORN, 1976. Mutation Res. 38: 327-332

BLAKEY D.H., 1985. Cancer Lett. 28: 299-305.

FROHBERG H. and M. SCHULZE-SCHENCKING, 1975. Arch. Toxicol. 33: 209-224.

GOLDBERG M.T. AND P. CHIDIAC, 1986. Mutation Res. 164: 209-215.

HARTMAN P.E., 1983. Environ. Mutagen. 5: 139-152.

HARTMAN P.E., 1986. In: Shankel et al. (Eds.) Antimutagenesis and anticarcinogenesis mechanisms, pp. 169-179. Plenum Press.

HENSCHLER D. and D. WILD, 1985. Arch. Toxicol. 57: 214-215.

JERINA D.M., J.M. SAYER, D.R. THAKKER, H. YAKI, W. LEVIN, A.W. WOOD and A.H. CONNEY, 1980. In: Pullman et al. (Eds.) Carcinogenesis: Fundamental Mechanisms and Environmental Effects, pp. 1-12. D. Reidel, Dordrecht.

LIPKIN M., 1981. In: Johnson (Ed.) Physiology of the gastrointestinal tract, pp. 145-168. Raven Press.

LOWENFELS A.B., 1973. Lancet 1: 24-26.

MILTENBURGER H.G., P. METZGER and CH. KRAUSE, 1980, Mutation Res. 79: 257-262.

MOOG F., 1981. Scientific American. November: 116-125.

MÜNZNER R. and J. WEVER, 1984. Cancer Lett. 25: 225-230.

MÜNZNER R. and J. WEVER, 1987. Arch. Toxicol. 60: 328-330.

NEAL S.B. and G.S. PROBST, 1984. Environ. Mutagen. 6: 407.

PROUDLOCK R.J. and J. ALLEN, 1986. Mutation Res. 174: 141-143.

RONEN A. and J.H. HEDDLE, 1984. Cancer Res. 44: 1536-1540.

SCHWARZACHER H.G. and U. WOLF (Eds.), 1974. Methods in human cytogenetics. Springer Verlag.

TATES A.D., I. NEUTEBOOM, M. HOFKER and L. den ENGELSE, 1980. Mutation Res. 74: 11-20.

WARGOVICH M.J., M.T. GOLDBERG, H.L. NEWMARK and W.R. BRUCE, 1983. J. Natl. Canc. Inst. 71: 133-137.

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