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Advances in Brief

Suppression of Preneoplastic Changes in the Intestine of Rats Fed Low Levels of Polyamines

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Abstract

Administration for 7 days of an enteral diet that is naturally deficient in polyamines strikingly reduces the preneoplastic changes observed in the intestines of adult Wistar rats previously treated with the carcinogen 1,2-dimethylhydrazine. On the contrary, supplementing the enteral diet with spermidine favors preneoplastic development. The effects of the low-polyamine diet included a 40% decline in the putrescine content of the intestinal mucosa, a significant decrease in the turnover rate of the epithelial cells from the crypts to villus tip in the ileum, and a 2-fold reduction in the number of abnormal colonic crypts. The experimental data support the view that it might be of interest to control the dietary intake of polyamines in the clinical management of cancer patients.

Introduction

The polyamines putrescine, spermidine, and spermine are a ubiquitous group of low molecular weight polycationic molecules that play a central role in cellular growth and differentiation (1, 2). Polyamines are involved in many steps of DNA, RNA, and protein synthesis. Tumor cells exhibit a very high requirement for these molecules in order to sustain cellular growth. This is achieved by *de novo* synthesis and enhanced captation from the extracellular environment (3, 4). The importance of polyamines from the intestinal lumen, originating from the colonic bacterial microflora or from the diet, has been increasingly recognized (5, 6). It has been shown that intraluminal polyamines support, in part, tumor growth in tumor-bearing rats (7). In this regard, two different therapeutic strategies may be developed to inhibit tumor progression: (*a*) the first one is based on the use of specific inhibitors of enzymes involved in intracellular polyamine metabolism (8); and (*b*) the second is to reduce the intracellular polyamine content by reducing all exogenous and endogenous sources of polyamines *in vivo* (9). The availability of the exogenous source seems crucial for tumor development because the system for polyamine uptake is enhanced in tumor cells when *de novo* polyamine synthesis is inhibited (10). Furthermore, it has been reported that polyamines present in food are absorbed to a greater extent by the intestinal mucosa of tumor-bearing animals as compared to healthy ones (9). Thus, polyamine requirements are higher during tumoral growth, and although these can be satisfied to some extent by increased biosynthesis, the contribution of polyamines supplied by the diet might have an important role to play.

We report herein data showing that lowering the polyamine content of the diet without further inhibition of the endogenous sources of polyamine is already efficient in reducing preneoplastic changes occurring in the intestinal tract of rats treated with the carcinogen DMH[2] and, conversely, that supplementing the low-polyamine diet with spermidine promotes preneoplastic development.

Materials and Methods

Animals and Diets. The experiments were conducted according to the National Research Council Guide for use and care of laboratory animals with the authorization (No. 00573) of the French Ministry of Agriculture.

Male Wistar rats (n = 36) weighing 300–350 g were housed under standardized conditions (22°C; 60% relative humidity; 12 h light/12 h dark cycle; 20 air changes/h). All animals received i.p. injection of 20 mg DMH/kg body weight once each week for 12 weeks. The DMH solution, adjusted to pH 6.5 with NaOH, was freshly prepared each week. After 12 weeks, the rats received no additional injections of DMH. All animals were fed a standard pelleted diet (UAR A04, Villemoisson/Orge, France) and had free access to drinking water.

After 17 weeks, the rats were randomly divided into three groups that received controlled isoenergetic diets (234 Kcal/kg/day) for 7 days. The reference group (n = 12) was kept on the standard diet (22% protein as casein and fish protein, 69% carbohydrates as cornstarch, and 9% lipids as soya and fish oil). The animals of the two other groups (n = 24) were fed enterally via an intragastric catheter (11). They received a nutritive mixture (Normoreal Na40; Laboratoires Sodietal, Ploudaniel, France) containing 15% protein (50% casein and 50% soya protein), 55% carbohydrate (maltodextrin), and 30% soya oil. One group (n = 12) received the nutritive mixture alone, and the other (n = 12) was given the nutritive mixture supplemented with 0.1 mmol spermidine/day (Sigma-Aldrich, Saint Quentin Fallavier, France).

At the end of the 7 days of controlled feeding, the entire colon and a 20-cm segment corresponding to the terminal part of the ileum were collected under ether anesthesia. Immediately after collection, the ileal segment was flushed with ice-cold 0.9% NaCl.

Assessment of Aberrant Crypts and Crypt Foci in the Colon. The determination was performed on a segment of 6 cm in length corresponding to the distal part of the colon. The segment was cleansed with physiological saline, cut open, pinned flat, and fixed in 10% buffered formalin. The colon was stained with 0.2% methylene blue for 5 min, rinsed in Krebs-Ringer buffer, placed onto a glass slide, and examined with a low-power objective (×40) for assessment of the presence and number of aberrant crypts as described by Bird (12).

The criteria used to identify an aberrant crypt topographically included: (*a*) increased size; (*b*) a thicker epithelial cell lining; and (*c*) an increased pericryptal zone relative to normal crypts.

Determination of the Polyamines. Samples were homogenized in 10 parts (w/v) of perchloric acid (200 mM), and the homogenates were centrifuged at 3000 × g for 10 min after standing for 16 h at 2°C. The clear supernatants were diluted with perchloric acid (200 mM), and 200-μl aliquots were applied on a reversed-phase column for separation. The polyamines (putrescine, spermidine, and spermine) were determined by separation of the ion pairs formed with n-octanesulfonic acid, reaction of the column effluent with o-phtalaldehyde/2-mercaptoethanol reagent, and monitoring of fluorescence intensity (7).

Nuclear DNA Labeling. Epithelial cell migration rate from crypt base to villus tip was measured in 5 animals from each group. The rats were injected i.p. with [^{3}H]thymidine (300 μCi/kg body weight; 81 Ci/mmol; Amersham, Les Ulis, France) 20 h before sacrifice. Labeling of nuclear DNA was revealed *in situ* in ileal samples. Tissue sections (5 μm) embedded in paraffin were coated with the photographic emulsion EM-1 (Amersham) for high-resolution microautoradiography and exposed for 4 weeks in the dark. The villus–crypt

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[2] The abbreviation used is: DMH, 1,2-dimethylhydrazine.

Table 1 *Daily intake of putrescine, spermidine, and spermine in rats fed the standard pelleted diet or the enteral nutritive mixture*

Values are the mean ± SE of food samples collected in triplicate at four different days during the 7 days of controlled feeding. The diet composition is given in "Materials and Methods."

Groups	Putrescine (nmol)	Spermidine (nmol)	Spermine (nmol)
Pelleted diet	3080 ± 210	5980 ± 430	6577 ± 539
Enteral diet	115 ± 12	93 ± 8	1910 ± 205

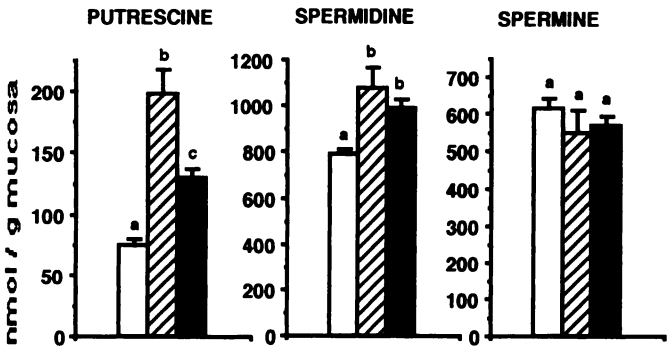

Fig. 1. Polyamine content of the ileal mucosa of DMH-treated rats fed for 7 days with the low-polyamine enteral mixture (□), the same enteral mixture supplemented daily with 0.1 mmol spermidine (▨), or the standard laboratory diet (■). Values are means ± SE of 7 animals/group. Columns not sharing a common superscript differ significantly ($P < 0.05$).

height and the position of the silver grains in relation to the crypt base were determined with an image analyzer (BIOCOM, Les Ulis, France).

Statistics. Data are reported as means ± SE. Statistical differences between groups were evaluated by one-way ANOVA, and specific differences were identified using the Student-Newman-Keuls multiple comparisons test.

Results

Polyamine Content of the Diet. As illustrated in Table 1, the polyamine content of the enteral nutritive mixture is very low as compared with that of the standard pelleted diet. The daily intake of polyamine is reduced by approximately 85% in animals consuming the enteral mixture. Among the polyamines, the intake of putrescine and spermidine was reduced by 95%, and the intake of spermine was reduced by 70% These results show that such an enteral mixture, commonly given in clinical nutrition, meets the criteria to be used as a low-polyamine dietary source.

Polyamine Content of the Intestinal Mucosa. The rats treated with the chemical carcinogen and fed for 7 days with the low-polyamine enteral mixture showed a significant reduction in their mucosal content of putrescine and spermidine as compared to those kept on the standard diet (Fig. 1). The amount of putrescine and spermidine was reduced by 40 and 20%, respectively. The amount of spermine was not modified under the various dietary conditions. Supplementing the enteral diet with spermidine significantly enhanced the mucosal content of spermidine and especially that of putrescine. The content of spermidine was similar to that measured in animals fed with the standard pelleted diet, whereas the putrescine content was increased by 60% in the polyamine-supplemented rats as compared to that of the group receiving the standard diet.

Effect of Diet on Epithelial Cell Migration. The migration rate of the ileal epithelial cells was examined in rats treated with the carcinogen, fed for 7 days with the various diets, and injected with [^{3}H]thymidine 20 h before sacrifice (Table 2). The crypt–villus height was significantly reduced in rats receiving the low-polyamine enteral diet. Spermidine supplementation exerted a trophic effect, and the height of the crypt–villus unit was similar to that measured in rats fed the standard diet. Lead-labeled epithelial cells were positioned at 41% up to the crypt base in the ileal mucosa of rats fed with the low-polyamine enteral diet. The position reached 54% up to the crypt base after spermidine supplementation; similar results were obtained with the standard diet. These data indicate that feeding rats the low-polyamine diet caused a decline in the turnover of intestinal epithelial cells.

Effect of Diets on the Formation of Aberrant Colonic Crypts. Regardless of the dietary treatment, all rats injected with DMH developed abnormal and hyperplasic colonic crypts. However, the administration of a low-polyamine diet for 7 days resulted in a 2-fold drop in the number of abnormal colonic crypts or crypt foci when compared with those of animals fed either the standard diet or the enteral mixture supplemented with spermidine. Spermidine supplementation completely inhibited the antiproliferative effect of the enteral mixture and led to the development of aberrant crypts in a similar way as the standard pelleted diet (Fig. 2).

Discussion

Systemic administration of the procarcinogen DMH to rodents is a well-established experimental model of colon carcinogenesis. DMH selectively induces colonic adenomas and adenocarcinomas and also, to a lesser extent, small intestine tumors (13). Administration of DMH causes a continuum of morphological changes from normal intestinal epithelium to carcinoma. One injection of DMH/week for 6 weeks typically causes grossly visible adenomas and carcinomas of the colon (14) within 16–24 weeks. The hyperproliferative lesions, aberrant crypts, adenomas, and adenocarcinomas of the colon induced by DMH are biologically and histologically quite similar to those seen in humans (15). Because of the potential progression of early changes to malignancy, the study of premalignant hyperproliferative lesions and

Table 2 *Effect of diet on epithelial cell migration in the ileum of rats treated with a chemical carcinogen*

The rats were injected i.p. with [^{3}H]thymidine (300 μCi/kg body weight) 20 h before sacrifice. The villus–crypt height and the position of the silver grains in relation to the crypt base were determined with an image analyzer. Values are the mean ± SE for 30 villus–crypt units/animal counted in 5 animals/dietary group.

Dietary groups	H[a] (μm)	h[b] (μm)	H/h (%)
Standard diet	465 ± 10	242 ± 15	52
Enteral mixture	425 ± 12[c]	176 ± 12[c]	41
Enteral mixture + spermidine	461 ± 8	248 ± 10	54

[a] H, crypt–villus height.
[b] h, position of the lead-labeled cells in relation to the crypt base.
[c] $P < 0.05$.

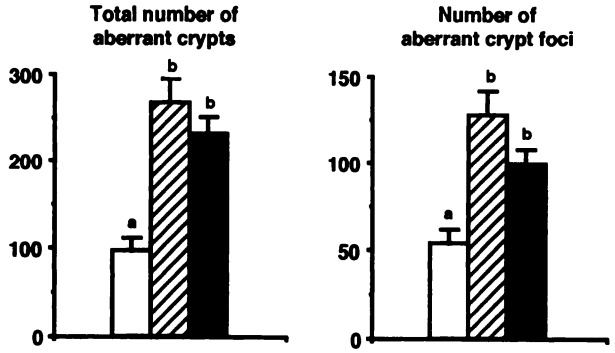

Fig. 2. The number of aberrant crypts and aberrant crypt foci in the distal colon (6-cm length) of DMH-treated rats fed for 7 days with the low-polyamine enteral mixture (□), the same enteral mixture supplemented daily with 0.1 mmol spermidine (▨), or the standard laboratory diet (■). Values are means ± SE of 7 animals/group. Columns not sharing a common superscript differ significantly ($P < 0.05$).

aberrant crypts is crucial to understanding the pathogenesis of colon cancer.

In this regard, the identification of dietary factors that are able to modulate such a premalignant process might have important consequences on the management of anticancer therapy. Among those factors, dietary polyamines might represent an interesting tool for dietary manipulation of neoplastic proliferation because polyamine requirements are very high during intensive growth. We present evidence that the amount of polyamine present in the diet has a direct influence on the premalignant changes occurring in the intestine of DMH-treated rats.

At present, the reduction of tumor progression in tumor-bearing rats has been obtained by a complex combination including the blockade of endogenous polyamine sources with specific drugs, the decontamination of the gastrointestinal tract, and the administration of a polyamine-free diet (7, 9). The observation that the administration of a low-polyamine enteral diet strikingly reduces the hyperproliferative changes observed in the preneoplastic intestine might represent a new approach in the clinical management of cancer patients.

It is noteworthy that numerous nutritive mixtures commonly used for clinical nutrition have a low polyamine content.[3] It has been reported that the average daily polyamine consumption in the Western diet is in the range of 350–700 μmol/person (16). In comparison, it can be estimated that the daily polyamine intake will be reduced 8–10-fold in patients receiving a polymeric enteral mixture.

Under our experimental conditions, the low polyamine level of the enteral diet seems directly responsible for the observed antiproliferative effects. Such effects included a significant reduction in the number of abnormal crypts in the colon, a decrease in the turnover rate of epithelial cells in the ileum, and a drop in the intracellular content of putrescine. All these events were reversed by spermidine supplementation of the enteral diet. These results also indicate that in preneoplastic intestine, the endogenous synthesis of putrescine and spermidine seems insufficient *per se* to support preneoplastic growth. This might be related to the intensive breakdown of spermidine and putrescine occurring through the action of amine oxidases, which are highly active enzymes in intestinal tissues (17, 18). This is corroborated by the lower spermidine and putrescine content of the intestinal mucosa of rats receiving the enteral diet and by the increased amount of putrescine observed in the intestinal mucosa after spermidine supplementation of the enteral diet. Thus, polyamine requirements that cannot be met by biosynthesis have to be satisfied from diet to sustain preneoplastic growth.

[3] F. Raul, personal communication.

In conclusion, the experimental data support the view that the dietary intake of polyamines should perhaps be reduced in the clinical management of cancer patients to slow the growth and progression of the tumor. Alternatively, when periods of intensive growth are required, such as during the initial period of radio- or chemotherapy, switching from a low-polyamine diet to a standard diet or to an enteral diet supplemented with polyamines might represent a way to enhance the efficiency of the antitumoral treatment.

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