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Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats

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It has been demonstrated for the first time that a wheat germ extract prevents colonic cancer in laboratory animals. Four-week-old inbred male F-344 rats were used in the study. Colon carcinogenesis has been induced by azoxymethane (AOM). Ten rats served as untreated controls (group 1). For the treatment of the animals in group 2, AOM was dissolved in physiologic saline and the animals were given three subcutaneous injections 1 week apart, 15 mg/kg body weight (b/w) each. In two additional groups Avemar (MSC), a fermented wheat germ extract standardized to 2,6-dimethoxy-*p*-benzoquinone was administered as a tentative chemo-preventive agent. MSC was dissolved in water and was given by gavage at a dose of 3 g/kg b/w once a day. In group 3, animals started to receive MSC 2 weeks prior to the first injection of AOM daily and continuously thereafter until they were killed 32 weeks later. In group 4 the basal diet and MSC were administered only. At the end of the experiment all the rats were killed by exsanguination, the abdominal large vessels were cut under a light ether anesthesia and a complete autopsy was performed. Percentage of animals developing colon tumors and number of tumors per animals: group 1 – 0 and 0; group 2– 83.0 and 2.3; group 3 – 44.8 ($P < 0.001$) and 1.3 ($P < 0.004$), group 4 – 0 and 0. All the tumors were of neoplastic nature also histologically. The numbers of the aberrant crypt foci (ACF) per area (cm^2) in group 2 were 4.85 while in group 3 the ACF numbers were 2.03 only ($P < 0.0001$).

Introduction

Wheat kernel is one of the staple foods of mankind. It contains 2–4% germ, also called embryo, which is separated from the endosperm by milling operations like rolling, sieving, etc. In the wheat grain, most nutrients with the exception of starch, are concentrated in the germ (1). Even though it is nutritious, wheat germ is mainly used as an animal feed. Besides its proteins of high biological value (2) and its oil of favorable fatty acid pattern (3), wheat germ is the richest known natural source of tocopherols and also abundant in B-group vitamins (4). The most significant antinutrients of wheat germ are the lectin WGA (wheat germ agglutinin) (5) and a group of trypsin inhibitors (6), which can be destroyed by heat treatment. Other remarkable non-nutrients of wheat germ are the methoxy-substituted benzoquinones which are present as glycosides of the corresponding methoxyhydroquinones (7). These compounds have been reported to exert anticancer effects in experimental systems (8).

Our team has produced a per os applicable standardized complex of multiple, biologically active molecules obtained from the aqueous extract of fermented wheat germ. The code name of the preparation was MSC. It has been registered as an over-the-counter dietary supplement under the trade name of Avemar (MSC). The preparation did not show toxic effects in acute and subacute oral toxicity studies. Oral administration of MSC enhanced blastic transformation of splenic lymphocytes in mice (9). The same treatment shortened the survival time of skin grafts in a co-isogenic mouse skin transplantation model, pointing to the immune-reconstructive effect of the preparation (10). A highly significant antimetastatic effect of MSC has been observed in three metastasis models (Lewis lung carcinoma, B16 melanoma, HCR-25 human colon carcinoma xenograft) (11). Combination of MSC and 5-fluorouracil or dacarbazine exhibited a dramatically enhanced antimetastatic effect in C38 mouse colon carcinoma and B16 mouse melanoma models, respectively (9). MSC also showed clinically significant therapeutic effects in experimental autoimmune models, such as rheumatoid arthritis (G.Falkay, unpublished results) and systemic lupus erythematosus (12). In cyclophosphamide treated mice, MSC significantly increased the regeneration of platelets (13). Because this product seems to be a potent immunomodulator, and it is active upon per os administration, in order to further characterize its anti-neoplastic nature we designed an experiment in which the chemopreventive effect of MSC was studied in azoxymethane (AOM)-induced colonic carcinogenesis in rats. This specific tumor has been chosen as in a phase II clinical trial in patients with advanced colorectal cancer MSC strongly inhibited progression of the malignant disease and significantly prolonged the survival (14).

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; LPS, lipopolysaccharide; PMA, phorbol ester.

Materials and methods

Animals and diet

One hundred 4-week-old inbred male F-344 rats were used in this study. The animals were housed in an air conditioned Animal Care Facility at our Institute under controlled humidity (55 ± 5%) and temperature (25 ± 2°C). They were caged in polyethylene boxes (5/cage) and all had free access to CRLT/N standard pelleted laboratory rodent chow (Charles River Ltd, Hungary) and tap water. This basal diet contained 86% dry material, 19% crude protein, 17% digestible protein, 4.5% crude fat, 6% crude fiber, 6% crude ash, 40% non-protein nitrogen material, 0.8% calcium, 11 000 IU/kg vitamin A, 600 IU/kg vitamin D3, plus amino acids as lysin, methionin and cystein.

Chemicals

Colon carcinogenesis has been induced by AOM (Sigma; A 9517). MSC was produced and supplied by Biromedicina Co., Hungary, by means of wheat germ fermentation with *Saccharomyces cerevisiae* (at 30°C for 18 h). The

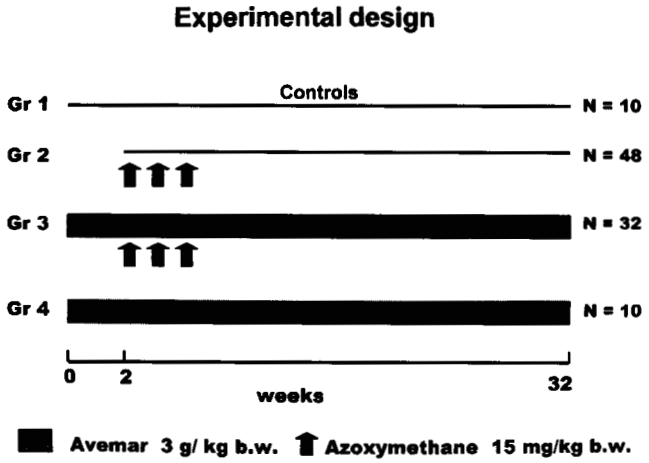

Fig. 1. Experimental schedule. Colon carcinogenesis was induced by three consecutive s.c. doses of AOM 1 week apart in F-344 rats. Oral administration of MSC was started 2 weeks before the carcinogen treatments. All the animals were killed at the end of the experiment, e.g. on the 32nd week.

end product was a dried, standardized extract containing 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ) in 0.4 mg/g concentration. (9)

Experimental protocol

Following randomization the animals were divided into 4 groups as shown in Figure 1. Ten rats served as untreated controls (group 1). For the treatment of the animals in group 2 AOM was dissolved in physiologic saline and the animals were given three subcutaneous injections 1 week apart, 15 mg/kg body weight (b/w) each (n = 48). In two additional groups the effect of the MSC was investigated. The fermented wheat germ was freshly dissolved in tap water and was given by gavage at a dose of 3 g/kg b/w once a day, between 09:00 and 10:00. In group 3 (n = 32) animals started to receive MSC 2 weeks prior to the first injection of AOM daily and continuously thereafter (including weekends and holidays) until killed 32 weeks later. In group 4 basal diet plus MSC were administered only (n = 10), but no carcinogen was applied.

At the end of the experiment all the rats were killed by exsanguination: the abdominal large vessels were cut under a light ether anesthesia and a complete autopsy was performed. All organs, especially the colons were carefully inspected and abnormalities were recorded. The removed intestines were flushed with physiologic saline, excised, longitudinally cut open and thoroughly washed with saline. The tumorous lesions were counted along the total length of the bowel, their diameters were recorded and upon removal they were fixed in 8% buffered formalin, and three other non-tumorous sections (~3 cm each) were placed into formalin between filter papers for 24 h for ACF (aberrant crypt foci) studies. Liver and lung tissues were also routinely processed for histology.

The fixed colon sections were dipped into 0.02% methylene blue solution for 5 min, then washed in distilled water, placed onto a microscopic glass and the number of ACFs were determined in a light microscope at a magnification of ×32. The area of each field was 0.32 cm^2; the final count of ACFs was expressed as the number of lesions per 1 cm^2. For statistical analysis either a Student's t-test or the χ^2 test were used.

Results

No disease-related death occurred during the experiment. The animal weights did not significantly differ (data not shown). In group 2 one rat died after administration of the third AOM shot apparently as a result of acute toxicity. In groups 3 and 4 some animals died during gavage (Table I).

Macroscopical findings

The results are presented in Table I. No macroscopically visible lesions occurred in the intestinal tract of the untreated controls or in rats receiving MSC alone (groups 1 and 4). Out of 47 AOM-treated rats (group 2) 39 animals (83%) developed grossly identifiable polypoid tumors in the colon. In addition, in one rat a small intestinal tumor was discovered and in another animal colon, tumors appeared together with a renal mass. No other organs were affected. In group 3, where MSC was administered before and following AOM doses the number of animals with neoplastic lesions in the colon has significantly dropped (13/29 rats; 44.8%, $P < 0.001$).

The average number of colon tumors per individual animal has also significantly decreased upon the effect of the administration of MSC. AOM treatment resulted in a 2.3 ± 0.21 tumors (mean ± SEM), while this value was 1.3 ± 0.17 in group 3 animals ($P < 0.004$). In group 2 (AOM alone) the number of polypoid colonic lesions varied between 1 and 8, while in MSC + AOM group there were 1–3 tumors in the large bowel. The diameter of these neoplastic lesions, however, did not differ significantly (2.21 ± 0.12 mm in group 2; 2.35 ± 0.25 mm in group 3).

ACF studies

The number of ACF per area (cm^2) was significantly lower in group 3 receiving MSC + AOM than that of group 2 treated with AOM alone. In the latter animals 4.85 ± 0.43 ACFs per cm^2 developed, while in the MSC-supplemented rats this value was 2.03 ± 0.28/cm^2 only ($P < 0.0001$).

Histological evaluation

All the grossly visible intestinal and renal lesions were evaluated histologically by two well-trained pathologists (A.Z., B.Sz.). Colon tumors were classified as adenomas, adenomas with severe dysplasia and adenocarcinomas. All neoplastic lesions observed by gross examination proved to be of neoplastic nature histologically, too.

In the AOM-treated animals 51.7% of the polypoid lesions proved to be adenomas (mostly of villous type). Adenomas with severe dysplasia were found in 6.9% of cases, while adenocarcinomas (ordinary or signet-ring carcinomas) developed in 41.4%. The corresponding figures in the MSC + AOM group were 61.5, 7.7 and 30.8%, respectively. Although in the latter group more adenomas and less carcinomas occurred, but the differences were not statistically significant.

Liver and lung tissues were also evaluated microscopically, but no metastases were found in these organs.

In the AOM-treated group 1 renal neoplasm developed, but it proved to be independent from the intestinal lesions: histologically, a typical Wilms' tumor was seen with a predominating blastemal pattern.

Discussion

The majority of colon carcinomas, besides their genetic-familiar background, develop as a result of multiple hits by various exogenous/endogenous carcinogenic compounds. The human environment, especially the diet seems to play a major role in the etiology of these tumors. For studying the natural history of colon carcinogenesis in rats, AOM is one of the standard chemical carcinogens. In this model the progression from the ACF towards the microadenomas, polyps and carcinomas is clearly established by several studies, but the distribution of the ACFs and adenocarcinomas do not always correlate (15). As the ACFs are regarded as putative pre-neoplastic conditions and may serve as intermediate markers for colon cancer in rat, in most studies the efficacy of chemopreventive agents has been assessed by using ACF-assay. The idea of chemoprevention comes from the assumption that the noxious effects of carcinogenic materials can be

Table I. Macroscopic findings in the large intestines of F-344 rats treated with MSC or MSC + AOM

	No. of animals having colonic tumors	Average number of colonic tumors per animal*	Average diameter of the colonic tumors	Remark
1. Untreated controls (n = 10)	0/10	0/10	–	
2. AOM (n = 47)	39/47 (83.0%)	2.3 ± 0.21 (range 1–8)	2.35 ± 0.25	1 Wilms' tumor
3. MSC + AOM (n = 29)	13/29 (44.8%)**	1.3 ± 0.17***[2] (range 1–3)	2.21 ± 0.12	
4. MSC (n = 9)	0/9	0/9	–	

*The number of grossly visible colonic polypoid lesions in the individual animals (mean ± SEM). **$P < 0.001$; *** $P < 0.004$.

antagonized by a continuous presence of dietary factors and these agents are able to reverse, suppress or prevent the progression to cancer. Intensive investigations revealed that chemoprevention of colon carcinomas in experimental animals can be achieved by hundreds of synthetic and natural compounds, but their chemical structure is highly variable and their mode of action is different (16). Some chemicals were effective when administered during the initiation phase, while some others just during the post-initiation period (16). These findings underline the fact that the chemoprevention is not linked to either a common chemical structure or a universal mechanism.

One of the most intensively studied nutrients with colon cancer preventive properties is the group of dietary fibers, particularly those of cereal origin (17). Actually, this is a main reason why whole grains are considered as an essential part of the healthy diet. Wheat bran and its fractions have recently been reported to be strong anticarcinogens in AOM-induced experimental colon carcinogenesis in rats (18). Similarly, different barley bran preparations have been shown to reduce 1,2-dimethylhydrazine-induced intestinal tumors in rats (19). In the same experiments it was found that outerlayer barley bran fraction including the germ was less effective than the pure bran fraction. Rice germ has been found effective in reducing tumor incidences in both AOM- and 4-nitroquinoline-1-oxide-induced colon carcinogenesis in rats (20). In the same experiments it was found that outlayer barley bran including the germ was less effective in reducing tumor incidences in both AOM- and 4-nitroquinoline-1-oxide-induced colon carcinogenesis in rats (20,21).

In comparison, no relevant data on wheat germ can be found in the literature. For the first time it has been demonstrated in the present work that a wheat germ preparation can prevent cancer in laboratory animals. In F-344 rats, fed with MSC in addition to the basal diet, significantly less ACFs per area have developed, the number of rats with colon tumors has been significantly reduced and, the colon tumor multiplicity has also decreased. Although, the histological spectrum represented more adenomas and less carcinomas in the MSC plus AOM-treated group than in the AOM-only group, these differences were not statistically significant.

Natural products, similar to MSC, were also capable of successfully preventing the formation of ACF or colon neoplasms. Among them caffeine, quercetin and morin resulted in a significant reduction of the frequency of ACF formation (22). Similarly, a xanthine oxidase inhibitor (1'-acetoxychavicol acetate) has significantly suppressed the incidence of colon carcinoma by a dose-dependent manner both in the initiation and in the post-initiation phase (23). It has also been reported that naturally occurring flavonoids, diosmin and hesperidin, both alone and in combination, inhibited the formation of ACF and the multiplicity of neoplasms in AOM-induced colon carcinogenesis in rats (24).

Summing up, although the chemoprevention of colon cancers (and their pre-neoplastic lesions) has well and long been established and could be achieved by totally different compounds, the mechanisms have still remained to be clarified. This is also true for MSC.

The exact mechanism by which the fermented wheat germ extract with standardized methoxy-substituted benzoquinone concentration can prevent colon cancer is still partly unknown. MSC did inhibit the AOM-induced ACF and colon neoplasm formation, the multiplicity of the tumors, apparently acting in the initiation phase. Regarding this, we can hypothesize that MSC acts as an immunomodulator. In *in vitro* studies, macrophages activated by lipopolysaccharide (LPS) and/or phorbol ester (PMA) exhibited very high sensitivity to MSC. The preparation showed synergism with LPS and PMA in activating myeloid cell lines and in inducing the transcription of cytokine genes and release of inflammatory cytokines (25). MSC increased the extravasation potential of tumor infiltrating lymphocytes by increasing the expression of ICAM-1 molecules on the surface of microvascular endothelial cells. MSC treatment of human tumor lymphocytes (leukaemic T cell line, Jurkat) resulted in the down regulation of MHC class I molecules thus, exposing the tumor cells to natural killer cell activity (26). Significant induction of apoptosis was also observed in leukemic T-cell line (Jurkat) and B-cell line (BL-41). MSC did not induce a similar degree of apoptosis and MHC down regulation in healthy, resting peripheral blood mononuclear cells. By these mechanisms the fermented wheat germ extract could inhibit the initiation phase of colonic carcinogenesis. The clear molecular mechanism of this latter effect is yet to be established.

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