Immunologic and Biochemical Effects of the Fermented Wheat Germ Extract Avemar

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Avemar (MSC) is a nontoxic fermented wheat germ extract demonstrated to have antitumor effects. Avemar has the potential to significantly improve the survival rate in patients suffering from malignant colon tumors. We studied its effects in the HT-29 human colon carcinoma cell line. Avemar had an inhibiting effect on colonies of HT-29 cells with an IC_{50} value of 118 μg/ml (7 days of incubation); this value could be decreased to 100 and 75 μg/ml in the presence of vitamin C. In the cell line examined, Avemar induced both necrosis and apoptosis, as demonstrated by Hoechst/propidium iodide staining. The incubation of cells with 3200 μg/ml Avemar for 24 hrs caused necrosis in 28% and the induction of apoptosis in 22% of the cells. Avemar inhibited the cell-cycle progression of HT-29 cells in the G1 phase of the cell cycle. In addition, Avemar inhibited the activity of the key enzyme of *de novo* DNA synthesis, ribonucleotide reductase. In addition, we determined the effects of Avemar on the activity of cyclooxygenase-1 and -2. Both enzymes were significantly inhibited by Avemar with IC_{50} values of 100 and 300 μg/ml, respectively. We outline new explanations for its antitumor activity, which might serve as the basis for further studies using Avemar. Exp Biol Med 230:144–149, 2005

Key words: Avemar; HT-29; ribonucleotide reductase; apoptosis; cell cycle; cyclooxygenase

The *in vitro* and *in vivo* effects of a fermented wheat germ extract, invented by the Hungarian biochemist Mate Hidvégi, were recently described. Avemar (MSC) is an extract standardized to methoxy-substituted benzoquinones, and has been demonstrated to induce apoptosis in pancreatic carcinoma cells, T and B lymphocytic tumor cell lines, and leukemia cells *in vitro* (1–3). In lymphoid tumor cells, apoptosis was selectively induced via tyrosine phosphorylation and Ca^{2+} influx (3). In addition, Avemar was shown to have a selective inhibitory effect on glycolysis and pentose-cycle enzymes, and to cause the down-regulation of major histocompatibility complex class I proteins in tumor cells (1–3). Avemar also has metastasis-inhibiting effects and is capable of synergistically enhancing the metastasis-inhibiting effect of 5-fluorouracil (5-FU) and dacarbazine (DTIC) under experimental conditions when applied in combination with these cytostatics (4–6).

The down-regulation of auto-antibody production following treatment with Avemar was observed in a mouse model for systemic lupus erythematosus (SLE), indicating that Avemar can ameliorate the clinical manifestation of experimental SLE (7). It has also been reported that Avemar is able to inhibit experimental azoxymethane-induced colon carcinogenesis in F-344 rats. The administration of Avemar decreased the number of animals that developed experimentally induced colon tumors by 46% (8). In addition, the number of colonic tumors per animal was significantly decreased by 57%, from 2.3 to 1.3. Avemar is distributed in various countries as a dietary supplement. The effects of Avemar in patients suffering from colon carcinoma were also evaluated. The oral coadministration of Avemar with conventional treatments helped to improve the clinical outcome of colon cancer treatment when compared with treatment with conventional regimens alone (9, 10) and, at the same time, demonstrated no signs of toxicity. A multicenter study using 170 patients with colorectal cancer who received a dose of 9 g of Avemar once per day demonstrated that the coadministration of Avemar with other treatments significantly improved disease progression, incidence of metastasis, and survival rate.

These results prompted us to further investigate the biochemical effects involved in the antitumor activity of Avemar in HT-29 human colon carcinoma cells. We determined the effects of this compound on the clonogenic

efficacy of HT-29 cells. In addition, we investigated the induction of necrosis and apoptosis by Avemar, its effects on the cell-cycle phase distribution of HT-29 cells, and also whether Avemar inhibits ribonucleotide reductase (RR; EC 1.17.4.1), a key enzyme in malignant cells. Furthermore, we examined the effects of Avemar on cyclooxygenase (COX)-1 and -2 activity, because these enzymes play an important role in inflammation and the development of colon cancer. We hope that our results may help to elucidate the molecular mechanisms of Avemar's effect as a biologically active wheat germ extract on colon tumor cells.

Materials and Methods

Chemicals. Ascorbic acid (vitamin C) and radioactive [^{14}C]cytidine were purchased from Sigma (Vienna, Austria). Avemar was a gift from Fresenius-Kabi Inc., (Graz, Austria). All other reagents used were commercially available and of the highest purity.

Cell Culture. The human colon tumor cell line HT-29 was purchased from the American Type Culture Collection (Manassas, VA). Cells were grown in a RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin-streptomycin in a humidified atmosphere containing 5% CO_2. Cell counts were determined using a CC-108 micro-cell counter (Sysmex, Kobe, Japan). Cells in a logarithmic phase of growth were used for all the studies described below.

Clonogenic Assay. HT-29 cells (1×10^3 cells/well) in the logarithmic phase of growth were plated in 24-well Costar plates (Costar, Cambridge, MA) and incubated with increasing concentrations of Avemar, and also Avemar in coadministration with vitamin C (50 and 100 μM; Sigma, St. Louis, MO) for 7 days at 37°C under cell culture conditions. After crystal violet staining, colonies (>40 cells) were counted using an inverted microscope. All experiments were performed in triplicate and were repeated three times.

Determination of Necrosis and Apoptosis. HT-29 cells (0.2×10^6/ml) were incubated with Avemar (800, 1600, 2400, and 3200 μg/ml) for 24 hrs. Then, 0.5×10^6/ml cells were stained with Hoechst 33258 (HO; Sigma) and propidium iodide (PI; Sigma) stain for 1 hr at 37°C. The cells were centrifuged at 600–750 g for 5 mins. Cells were then examined using a Leica (Wetzlar, Germany) DMR XA fluorescence microscope equipped with the appropriate filters for Hoechst 33258 and PI. The cells were photographed with a COHU (San Diego, CA) high-performance CCD camera using Leica Q-fish software. This method makes it possible to distinguish between early apoptosis, late apoptosis, and necrosis. Cells were judged according to their morphology and the integrity of their cell membranes, which can easily be seen after PI staining.

Cell-Cycle Phase Distribution Analysis. HT-29 cells (0.4×10^6/ml) were incubated with various concentrations of Avemar (200, 400, and 800 μg/ml) at 37°C under cell culture conditions. After 24 hrs, the cells were harvested

and suspended in 5 ml of cold phosphate-buffered saline (PBS), centrifuged (600 g for 5 mins), resuspended, and fixed in 3 ml of cold ethanol (70%) for 30 mins at 4°C. After two washing steps in cold PBS, RNase A and PI were added to a final concentration of 50 μg/ml each and incubated at 4°C for 60 mins before measurement. Cells were analyzed using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) and cell-cycle phase distribution was calculated using ModFit LT software (Verity Software House, Topsham, ME).

Determination of Ribonucleotide Reductase (RR) In Situ Activity. Exponentially growing HT-29 cells (5×10^6) were incubated with 400, 800, and 1600 μg/ml Avemar for 24 hrs at 37°C in a humidified atmosphere containing 5% CO_2 to assess changes in RR *in situ* activity. The cells were then pulsed with [^{14}C]cytidine (Sigma, Vienna, Austria; 3.125 μl in a 5-ml cell suspension) for 30 mins at 37°C. The cells were collected by centrifugation (1200 g for 5 mins), washed twice with PBS, and processed to extract the total genomic DNA. We then measured the radioactivity of the DNA samples through the measurement of converted cytidine.

Determination of Deoxyribonucleoside Triphosphates (dNTPs). HT-29 cells were seeded for 24 hrs in 175 cm^2 flasks (4×10^7) to ensure attachment and then incubated with 400, 800, and 1600 μg/ml of Avemar for another 24 hrs. The cells were then centrifuged at 1800 g for 5 mins, resuspended in 100 μl of PBS, and extracted with 10 μl of trichloracetic acid (90%). The lysate was allowed to rest on ice for 30 mins and neutralized by the addition of 1.5 volumes of freon containing 0.5 mol/l tri-n-octylamin. Concentrations of dNTPs were then determined using the method described by Garrett and Santi (11).

Aliquots (120 μl) of the sample were analyzed using a Merck "La Chrom" high-performance liquid chromatography (HPLC) system (Merck, Darmstadt, Germany) equipped with a D-7000 interface, L-7100 pump, L-7200 autosampler, and a L-7400 UV detector. Detection time was set at 80 mins, with the detector operating on 280 nm for 40 mins and then switched to 260 nm for another 40 mins. Samples were eluted with a 3.2 M ammonium phosphate buffer (pH 3.6, adjusted by the addition of 3.2 M H_3PO_4) containing 20 M acetonitrile using a 4.6 × 250 mm PARTISIL 10 SAX column (Whatman Ltd., Kent, UK). Separation was performed at constant ambient temperature and a flow rate of 2 ml per minute. The concentration of each dNTP was calculated as a percentage of the total area under the curve for each sample. The concentrations of dCTP, dTTP, and dATP in untreated, exponentially growing HT-29 cells were 2.23, 4.71, and 0.71 μM per 10^6 cells, respectively.

COX Inhibitor Assay. An enzyme immunoassay of IBL Products (Hamburg, Germany) was used for the determination of COX-1 and COX-2 activities. The assay quantitatively determines prostaglandins F, E, and D and thromboxane B-type prostaglandins produced in COX

Table 1.   Induction of Necrosis/Apoptosis in HT-29 Human Colon Carcinoma Cells After Incubation with Avemar for 24 hrs[a]

	% viable	% necrotic	% apoptosis (early)	(late)	(total)
Control	91	8	1	0	1
800 µg/ml	86	11	2	1	3
1600 µg/ml	73	20	7	0	7
2400 µg/ml	64	30	5	1	6
3200 µg/ml	50	28	5	17	22

[a] Numbers are mean values of three determinations. Necrosis, and early and late apoptosis were determined according to the cell morphology after double-staining with Hoechst/propidium iodide.

reactions. COX-1 and COX-2 activity was determined and expressed as an IC$_{50}$ value (i.e., the concentration of Avemar that results in a 50% inhibition of the activity of each enzyme). The IC$_{50}$ values were determined after calculation of a dose-response curve by the Prism 3.03 software package (GraphPad, San Diego, CA).

Statistical Calculations. Dose-response curves were calculated using the Prism 3.03 software package, and statistical significance was determined through an unpaired t test.

Results

Clonogenic Assay. Avemar inhibited the growth of cell colonies when a clonogenic assay with an IC$_{50}$ value (i.e., a 50% inhibition of cell colonies when compared with the untreated cell colonies) of 118 µg/ml was employed.

The coadministration of vitamin C decreased this IC$_{50}$ value to 100 µg/ml when 50 µM/L vitamin C was added, and to 75 µg/ml when 100 µM/L vitamin C was added. Vitamin C administered alone inhibited the number of colonies to 87% (50 µM) and 84% (100 µM) of control cells, respectively.

Determination of Necrosis and Apoptosis. The effect of Avemar on the necrosis and apoptosis of HT-29 human colon carcinoma cells is presented in Table 1. Cells were incubated with increasing concentrations of Avemar (800–3200 µg/ml) for 24 hrs. Then cells were double-stained with a Hoechst/PI stain and judged according to

their morphology. Avemar (800–2400 µg/ml) caused the induction of necrosis, with only a few cells undergoing the changes associated with apoptosis. At a concentration of 3200 µg/ml, necrosis could be established in 28% of the cells and apoptosis in 22% of the cells.

Cell-Cycle Phase Distribution After Avemar Incubation. Figure 1 shows the effect of Avemar on the cell-cycle phase distribution in HT-29 cells. Avemar incubation in solutions up to 400 µg/ml had hardly any effect on the cell-cycle distribution of tumor cells; however, incubation in a solution of 800 µg/ml for 24 hrs led to an arrest in the G1 phase, causing a depletion of cells in the S and G2-M phases of the cell cycle.

RR *In Situ* Activity Assay. HT-29 cells were treated with Avemar (400–1600 µg/ml) for 24 hrs and then incubated with radioactive [^{14}C]cytidine for 30 mins. DNA was then extracted and the radioactivity in the DNA samples was determined as a measure of RR *in situ* activity. The incorporation of the label into the DNA decreased in a concentration-dependent manner. Incubation with 1600 µg/ml Avemar decreased the incorporation of the radiolabeled cytidine into DNA to 13.5% of control values, as depicted in Figure 2.

Determination of dNTPs. HT-29 cells were incubated with 400, 800, and 1600 µg/ml of Avemar for 24 hrs. Pool sizes of dNTPs were then determined using the HPLC method described above in the Materials and Methods
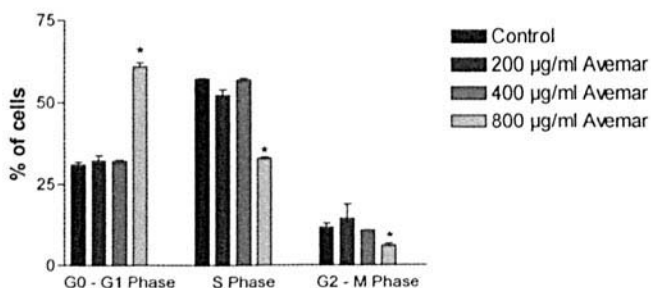
Figure 1. Effect of Avemar (24-hr incubation) on the cell-cycle phase distribution of human HT-29 colon carcinoma cells. Results are mean values ± standard errors of two determinations out of one representative experiment. *Significantly ($P < 0.05$) different from untreated controls.
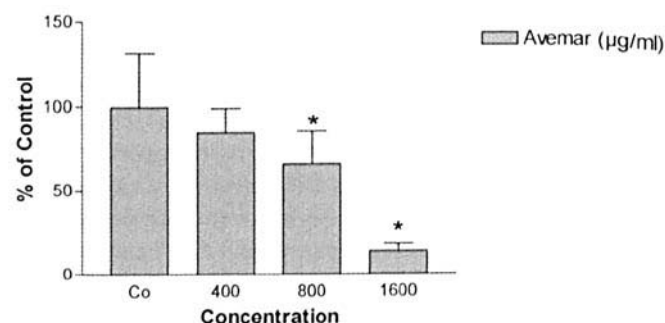
Figure 2. Effect of Avemar on the *in situ* activity of the enzyme ribonucleotide reductase in human HT-29 colon carcinoma cells. Values are mean values ± standard errors of two determinations of one representative experiment (out of three). *Significantly ($P < 0.05$) different from untreated controls.

section. The concentration of dGTP pools was below the detection limit of the method. All three other dNTP pool sizes (dATP, dCTP, and dTTP) significantly decreased after incubation with Avemar. The most pronounced effects were observed for dCTP concentrations, which decreased to 45% of control values after incubation with 400 µg/ml Avemar. Increasing Avemar concentrations further decreased dCTP pools. A significant decrease was also observed for dTTP after incubation with 800 µg/ml, and for dATP after exposing the tumor cells to 400 µg/ml Avemar. The dATP pool further decreased to 19% of control values after cells were incubated with 1600 µg/ml Avemar. Results are shown in Figure 3.

Effect of Avemar on COX Activities. COX-1 and COX-2 enzymes were incubated with increasing concentrations of Avemar for 2 mins at 37°C using a cell-free enzyme. The inhibition of COX-1 and COX-2 in the presence of Avemar was then determined, and IC_{50} values (Avemar concentration resulting in 50% enzyme inhibition) were calculated. For COX-1 activity the IC_{50} was 100 µg/ml, while a concentration of 300 µg/ml inhibited COX-2 activity to 50% of the control. These results demonstrate the COX-inhibiting capacity of Avemar; and no selectivity toward one of the COX enzymes could be observed.

Discussion

Avemar is a fermented wheat germ extract introduced as a nontoxic dietary supplement with anticarcinogenic effects. Various authors describe its beneficial *in vitro* and *in vivo* effects. In particular and most recently, Jakab and coauthors (10) conducted a clinical study using Avemar in patients with colorectal cancer. They demonstrated that the coadministration of Avemar produced a statistically significant overall survival benefit when compared with results in patients who received conventional chemotherapy alone.

As a result, we decided to investigate the biochemical mechanism of the action of Avemar in a human colon tumor cell line.

First, we determined the IC_{50} values of Avemar in HT-29 cells employing a clonogenic assay and demonstrated that the compound inhibits tumor cell colonies at an IC_{50} of 118 µg/ml.

It was previously demonstrated that the coadministration of vitamin C can influence the effects of Avemar on metastasis in experimental animals (4). We therefore tested the combined effects of Avemar and vitamin C in HT-29 cells and demonstrated their dose-dependent, synergistic clonogenic effects. The synergism observed might be the result of free radical scavenging effects or vitamin C's protection of the active ingredient of Avemar from oxidation. Because Avemar had been demonstrated to induce apoptosis in a number of cell lines, we investigated the apoptosis-inducing effects in HT-29 human colon carcinoma cells as well. Both effects—necrosis and the induction of apoptosis—were observed, depending on the

concentrations used. Morphologic analysis after double-staining with a Hoechst/PI stain revealed that lower Avemar concentrations resulted mainly in necrosis, whereas apoptotic changes could be observed with higher compound concentrations. We were also able to confirm previous findings regarding the cell-cycle phase-specific action of Avemar in colon cells. As shown by Comin-Anduix and coworkers in Jurkat cells, Avemar stopped the cell-cycle transition of HT-29 cells in the G1 phase of the cell cycle, resulting in the depletion of S and G2-M phase cells (2).

Several of Avemar's effect mechanisms have previously been described. For instance, a cancer cell–specific inhibition of glycolysis and pentose cycle enzymes was caused by Avemar in Jurkat cells, pinpointing one mechanism of action of the compound in leukemia cells. Comin-Anduix and coworkers (2) also speculated that the decreased oxidative ribose synthesis might limit the leukemia cells' metabolic needs for the reduction of ribonucleotides to dNTPs. Another mechanism could be the free radical scavenging effects of Avemar or components of Avemar. RR is responsible for the conversion of ribonucleotides to deoxyribonucleoside triphosphates, which are precursors of DNA synthesis. RR was demonstrated to be significantly up-regulated in tumor cells to meet the increased need for dNTPs of these rapidly proliferating cells for DNA synthesis (12, 13). The enzyme was therefore indicated as being an excellent target for cancer chemotherapy, and various inhibitors of RR have entered clinical practice or are under preclinical or clinical development. The enzyme consists of two subunits, the effector binding and the nonheme iron subunits. The inhibition of the nonheme iron subunit can be caused, for instance, by iron chelation or by the free radical scavenging of a free tyrosine radical, which is needed for iron stabilization. To determine whether Avemar's action in HT-29 cells involves such RR inhibition, we first employed an *in situ* enzyme assay. Radiolabeled cytidine has to be reduced by RR to be incorporated into DNA. We were able to demonstrate that the *in situ* RR activity of HT-29 cells can be inhibited by Avemar in a concentration-dependent manner. These results
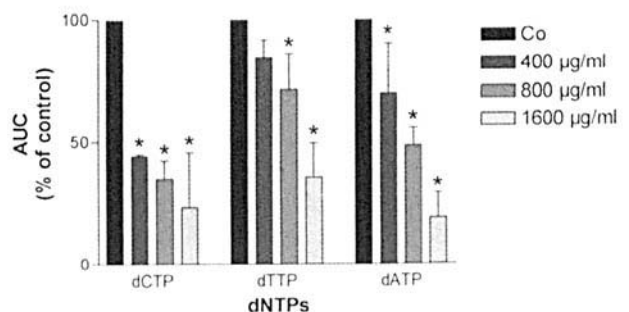
Figure 3. Effect of Avemar (incubation of cells for 24 hrs) on dNTP pool concentrations in HT-29 human colon carcinoma cells. Values are mean values ± standard errors of three determinations out of one representative experiment. Intracellular dGTP concentrations were below the detection limit of the method. *Significantly ($P < 0.05$) different from untreated controls.

were then confirmed by the determination of dNTP pool sizes after the incubation of HT-29 colon tumor cells with Avemar. The compound did inhibit RR, the key enzyme of *de novo* DNA synthesis, which might explain its effect on tumor cells, in particular the antitumor effects observed in patients with colon carcinoma described above.

Another enzyme associated with colon tumor incidence is COX, which is responsible for the production of inflammation mediators. It was demonstrated that the inhibition of COX causes a decreased incidence of colon carcinomas (14). Aspirin is the first and most widely used inhibitor of COX activity. It has been demonstrated that the regular use of aspirin can decrease the incidence of various cancers such as colon carcinoma or breast cancer. As a result, COX inhibitors are being used in the prevention and treatment of colon cancer (14, 15). The inhibition of COX-2 is responsible for the anti-inflammatory and anticancer effects of COX inhibitors, whereas the inhibition of COX-1 is responsible for the side effects of aspirin and other nonsteroidal anti-inflammatory drugs. These are mainly gastrointestinal problems such as gastric bleeding, which are observed when higher doses of COX inhibitors are being administered for longer periods of time.

We conclude that we were able to identify new biochemical targets of Avemar; notably, the enzymes RR and COX. The inhibition of these enzymes results in the impressive antitumor effects observed with Avemar in patients suffering from colon carcinoma.

There are numerous *in vitro*, experimental, and clinical studies indicating the safety of this preparation. It has been demonstrated that Avemar treatment is about 50 times less effective in peripheral blood lymphocytes in inducing biological effects than in leukemia cells, which provides a comfortable therapeutic window for Avemar to apply in patients as a supplemental treatment modality with minimal or no toxic side effects (2). It has also been shown that Avemar causes apoptosis in lymphoid tumor cells but it does not induce apoptosis in healthy resting mononuclear cells (3). Avemar has been reported to have no toxicity in acute and subacute animal studies (16–18), and no disadvantageous interactions with the cytostatic drugs widely used in clinical practice (19). It has been demonstrated that coadministration of this fermented wheat germ extract with standard chemotherapy significantly delayed disease progression in melanoma (20), and improved quality of life and alleviated fatigue in patients with advanced lung cancer (21). Recently, it has been shown that continuous Avemar administration reduced the incidence of febrile neutropenia, one of the most serious cytostatic treatment-related side effects in pediatric patients with cancer (22). It is important to note that no toxic side effects have ever been reported with the use of Avemar in humans.

For adults, the daily recommended dosage of Avemar is 9 g, which corresponds to the upper concentration ranges applied in our *in vitro* study. However, the active substance of Avemar still remains unknown. Further chemical analysis has to be performed to identify the chemical compound that is responsible for the observed biochemical effects. This would allow its clinical development as a chemopreventive and anticancer drug. Nevertheless, at the moment, administration of Avemar as a dietary supplement remains a safe and effective way to enhance the effects of conventional chemotherapy in the treatment of human cancers, especially colon carcinoma. These effects can now be explained by Avemar's biochemical activity in relation to human colon carcinoma cells.

1. Boros LG, Lapis K, Szende B, Tömösközi-Farkas R, Balogh Á, Boren J, Marin S, Cascante M, Hidvégi M. Wheat germ extract decreases glucose uptake and RNA ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells. Pancreas 23:141–147, 2001.

2. Comin-Anduix B, Boros LG, Marin S, Boren J, Callol-Massot C, Centelles JJ, Torres JL, Agell N, Bassilian S, Cascante M. Fermented wheat germ extract inhibits glycolysis/pentose cycle enzymes and induces apoptosis through poly(ADP-ribose) polymerase activation in Jurkat T-cell leukemia tumor cells. J Biol Chem 277:46408–46414, 2002.

3. Fajka-Boja R, Hidvégi M, Shoenfeld Y, Ion G, Demydenko D, Tömösközi-Farkas R, Vizler C, Telekes A, Resetár Á, Monostori É. Fermented wheat germ extract induces apoptosis and downregulation of major histocompatibility complex class I proteins in tumor T and B cell lines. Int J Oncol 20:563–570, 2002.

4. Hidvégi M, Rásó E, Tömösközi-Farkas R, Paku S, Lapis K, Szende B. Effect of Avemar and Avemar + vitamin C on tumor growth and metastasis in experimental animals. Anticancer Res 18:2353–2358, 1998.

5. Szende B, Rásó E, Hidvégi M, Tömösközi-Farkas R, Paku S, Prónai L, Bocsi J, Lapis K. A new benzoquinone-containing antimetastatic product [English translation]. Orv Hetil 139:2893–2897, 1998.

6. Hidvégi M, Rásó E, Tömösközi-Farkas R, Szende B, Paku S, Prónai L, Bocsi J, Lapis K. MSC, a new benzoquinone-containing natural product with antimetastatic effect. Cancer Biother Radiopharm 14:277–289, 1999.

7. Ehrenfeld M, Blank M, Shoenfeld Y, Hidvégi M. Avemar (a new benzoquinone-containing natural product) administration interferes with the Th2 response in experimental SLE and promotes amelioration of the disease. Lupus 10:622–627, 2003.

8. Zalatnai A, Lapis K, Szende B, Rásó E, Telekes A, Resetár Á, Hidvégi M. Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats. Carcinogenesis 22:1649–1652, 2001.

9. Jakab F, Mayer Á, Hoffmann A, Hidvégi M. First clinical data of a natural immunomodulator in colorectal cancer. Hepatogastroenterology 47:393–395, 2000.

10. Jakab F, Shoenfeld Y, Balogh Á, Nichelatti M, Hoffmann A, Kahán Z, Lapis K, Mayer Á, Sápy P, Szentpeétery F, Telekes A, Thurzó L, Vágvölgyi A, Hidvégi M. A medical nutriment has supportive value in the treatment of colorectal cancer. Br J Cancer 89:465–469, 2003.

11. Garrett C, Santi DV. A rapid and sensitive high-pressure liquid chromatography assay for deoxyribonucleoside triphosphates in cell extracts. Anal Biochem 99:268–273, 1979.

12. Elford HL, Freese M, Passamani E, Morris HP. Ribonucleotide reductase and cell proliferation. I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas. J Biol Chem 245:5228–5233, 1970.

13. Takeda E, Weber G. Role of ribonucleotide reductase in expression in the neoplastic program. Life Sci 28:1007–1014, 1981.

14. Thun MJ, Namboodiri MM, Heath CW Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 325:1593–1596, 1991.

15. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, McTiernan A, Women's Health Initiative. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res 63:6096–6101, 2003.

16. Report. Acute oral toxicity study of Avemar in mouse. Code: 9901. Department of Pharmacology and Toxicology, University of Veterinary Science, Budapest, 1999 (Biromedicina First Hungarian Corporation for Cancer Research and Oncology on file).

17. Report. Acute oral toxicity study of Avemar in rats. Code: 9902. Department of Pharmacology and Toxicology, University of Veterinary Science, Budapest, 1999 (Biromedicina First Hungarian Corporation for Cancer Research and Oncology on file).

18. Report. Subacute oral toxicity study of Avemar. Code: 0001. Department of Pharmacology and Toxicology, Szent István University, School of Veterinary Science, Budapest, 2000 (Biromedicina First Hungarian Corporation for Cancer Research and Oncology on file).

19. Szende B, Marcsek Z, Kocsis Z, Tompa A. Effect of simultaneous administration of Avemar and cytostatic drugs on viability of cell cultures, growth of experimental tumors, and survival of tumor-bearing mice. Cancer Biother Radiopharm 19:343–349, 2004.

20. Demidov LV, Manzjuk LV, Kharkevitch GY, Artamonova EV, Pirogova NA. Antimetastatic effect of Avemar in high-risk melanoma patients (abstract P868). In: Program of the 18th UICC International Cancer Congress, June 30–July 5, 2002, Oslo, Norway. Int J Cancer 100(Suppl 13):408, 2002.

21. Hidvégi M, Moldvay J, Lapis K, Ajkay Z. Fermented wheat germ extract improves quality of life in lung cancer patients [English translation]. Medicus Anonymus/Pulmono 11(Suppl 1):13–14, 2003.

22. Garami M, Schuler D, Babosa M, Borgulya G, Hauser P, Müller J, Paksy A, Szabó E, Hidvégi M, Fekete G. Fermented wheat germ extract reduces chemotherapy induced febrile neutropenia in pediatric cancer patients. J Pediatr Hematol Oncol 26:631–635, 2004.