Fermented Wheat Germ Extract (Avemar) in the Treatment of Cancer and Autoimmune Diseases

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ABSTRACT: Avemar, the product of industrial fermentation of wheat germ, possesses unique cancer-fighting characteristics. Taken orally, Avemar can inhibit metastatic tumor dissemination and proliferation during and after chemotherapy, surgery, or radiation. Benefits of Avemar treatment have been shown in various human cancers, in cultures of *in vitro* grown cancer cells, in the prevention of chemical carcinogenesis, and also in some autoimmune conditions. This document reviews the clinical and experimental results obtained with this extract so far. Special references are made for its safety, including its coadministration with anticancer drugs, as well as for its immunomodulatory activity, its molecular targets, and its use in cancer clinical trials.

KEYWORDS: fermented wheat germ extract; Avemar; cancer; autoimmune diseases

INTRODUCTION

Wheat germ, if left in flour, has an adverse effect on the functional properties of dough and therefore on breadmaking quality. Therefore, most germ is milled as part of mill feed, and a smaller portion is separated during the milling process. Separated wheat germ is traditionally included in healthy foods, is consumed as is, or serves as raw material for extracts rich in vitamin E.

During the 1990s, a new, fermented wheat germ extract for human consumption was invented by Professor Máté Hidvégi in Hungary.[1] The standardized manufacturing technology included the extraction of wheat germ, the fermentation of the extract, followed by separation of the fermentation liquid, microencapsulation, drying, and granulation. The resulting powder was named Avemar pulvis (or simply Avemar), and the granulate is also known as Avemar. For a 70-kg weight adult, the

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single daily dosage of Avemar contains 8.5 g of Avemar pulvis plus flavoring ingredients, such as fructose and arome. After being dissolved in 150 ml of cold water, Avemar should be drunk preferably before a meal. The product has been approved as a dietary food for special medical purposes in cancer patients by the National Institute of Food Safety and Nutrition of Hungary. Avemar has been consumed by cancer patients for more than 6 years.

Since its invention, a series of *in vitro* and *in vivo* studies and clinical trials have been carried out to determine whether Avemar could help cancer patients struggling with both the effects of their disease and the side effects of standard anticancer therapy (SAT). Subsequently, evidence of the efficacy of the fermented wheat germ extract in some autoimmune diseases has also been found. At this time, sufficient study of this compound has been done and enough data have emerged that some useful and valid conclusions can be made regarding the value of Avemar as a supportive tool in therapy. The benefits observed, mechanisms of action known, and study results are summarized in this review. (In the following text and references, the terms "fermented wheat germ extract," "Avemar," and its code name "MSC" denote the same preparation.)

COMPOSITION

The original composition of wheat germ is substantially modified due to extraction followed by fermentation; therefore, Avemar cannot be replaced by wheat germ, germinated wheat, or any extract or derivative of these. Methoxy-substituted benzoquinones, present originally in the crude wheat germ as glycosides and liberated as aglycones by glycosidases during fermentation, are the indicator compounds for quantitative standardization.[2] Avemar is also characterized by its specific high performance liquid chromatography fingerprint spectra. Avemar is currently manufactured by Biromedicina in Hungary in a Good Manufacturing Practice (GMP)-certified pharmaceutical plant in the Kunfeherto-Kiskunhalas region.

SAFETY

Much evidence is available to demonstrate the safety of Avemar under the conditions of its intended use.[3] Avemar has been investigated in numerous animal and human studies of its efficacy; in none of these studies has any indication of adverse effects been identified. Avemar has been sold in numerous countries for many years with no reports of adverse effects. Finally, Avemar has been subjected to acute toxicity studies in the rat and mouse, a subacute toxicity study in the rat, and subchronic toxicity studies in the rat and mouse in addition to genotoxicity, mutagenicity, and carcinogenicity screening tests, and it has been evaluated for hematologic effects in multi-year studies in human cancer patients.

Based on the absence of adverse effects, the acute oral LD_{50} of Avemar in male and female mice and rats was >2,000 mg/kg, and the no-observable adverse effect level (NOAEL) of the extract in a subacute study with rats was determined to be the tested dose of 2,000 mg/kg/day. The NOAEL of Avemar in a subchronic study with mice and rats was determined also to be the tested dose of 3,000 mg/kg/day.

The effect of long-term administration of Avemar on the hematologic status of carcinoma patients was examined in two hospital centers in Hungary. Hematologic data included white blood cell count, red blood cell count, hemoglobin level, hematocrit, platelet count, erythrocyte sedimentation rate, lymphocyte count, neutrophil granulocyte count, monocyte count, eosinophil granulocyte count, and prothrombin level. After 1, 3, and 5 years of Avemar treatment, all values remained within normal limits.

DRUG INTERACTIONS

Vitamin C

In an early animal study, the effects of Avemar alone and Avemar plus vitamin C on tumor growth and metastasis in laboratory mice and rats were studied.[4] Involved were an aggressive variant of the Lewis lung carcinoma (3LL-HH), B16 melanoma, a rat nephroblastoma (RWT-M), and a human colon carcinoma xenograft (HCR25) in immunosuppressed mice. Effects on metastases were studied both with the primary tumors intact and after their surgical removal. Vitamin C alone had a significant inhibitory effect on metastases in some of these tumor models but not in others. However, combined treatment with Avemar plus vitamin C administered simultaneously profoundly inhibited metastases in all tumor models. Interestingly, in some tumor models, treatment with Avemar alone had a greater inhibitory effect on metastasis formation than did Avemar plus vitamin C. It was therefore recommended that if vitamin C is being administered, Avemar should be consumed at least 2 hours before or after treatment with vitamin C-containing preparations.

Cytostatic Drugs

To determine whether Avemar's beneficial effects might or might not compromise the efficacy of a variety of cytostatic drugs commonly used in cancer treatment, researchers tested Avemar alone and in combination with those drugs in malignant cell lines and in animals with cancer.[5] *In vitro*, Avemar neither increased nor decreased the effect on viability of MCF-7, HepG2, or Vero cells resulting from treatment with dacarbazine (DTIC), 5-fluorouracil (5-FU), or doxorubicin. In mice with transplanted 3LL-HH tumors, the combination of Avemar with cyclophosphamide, vinorelbine, and doxorubicin did not lessen those drugs' inhibition of tumor growth. Avemar produced no toxic effects in the mice, and its addition to the treatment regimen did not increase the toxicity of the drug treatment. Strong synergism in antimetastatic activities was seen with the combined use of Avemar and cytostatic drugs: DTIC plus Avemar in B16 mouse melanoma, muscle/lung metastasis model and 5-FU plus Avemar in C38 mouse colorectal carcinoma, spleen/liver metastasis model resulted in statistically complete eradication of lung and liver metastases, respectively.[6] These results make us confident that Avemar may be administered along with these conventional chemotherapy drugs with little risk of negatively affecting the cytostatic drugs' efficacy, or increasing their undesirable side effects.

Cytokines

Avemar may safely be administered together with the cytokine preparations used in clinical practice. The antineutropenic efficacy of the hematopoietic cytokines plus Avemar in combination is better than that of the cytokines alone.[3]

Tamoxifen

Researchers at the National Institute of Chemical Safety in Budapest conducted an *in vitro* study of the effects of a tamoxifen plus Avemar combination administered to cultures of the MCF-7 (ER+) breast cell line as a preclinical model of human breast cancer.[7] MCF-7 cells were treated with tamoxifen and/or Avemar for 24, 48, and 72 h. Cytotoxicity was measured by MTT assay; the percentages of mitosis and apoptosis were determined by hematoxylin and eosin staining and by immunochemistry, and estrogen receptor activation was studied by semiquantitative determination of the estrogen-responsive pS2 gene mRNA production. The percentage of apoptotic and proliferating cell fraction (S-phase) was determined by flow cytometry. Tamoxifen had no effect on the percentage of apoptotic cell fraction, while significantly reducing the ratio of S-phase cells. After an exposure time of 48 h, Avemar increased apoptosis significantly. Tamoxifen + Avemar increased apoptosis significantly after 24 h, with a negligible effect on mitosis and S phase. Estrogen receptor activity of MCF-7 cells treated for 24, 48, and 72 h was enhanced by Avemar and decreased by tamoxifen as well as by tamoxifen + Avemar. The increase in apoptosis by the combined use of tamoxifen + Avemar suggests that the addition of Avemar to tamoxifen may enhance the efficacy of tamoxifen in ER+ breast cancer. There is no contraindication to their combination in clinical practice.

AVEMAR AND IMMUNITY

Evidence of the immunomodulatory effects of Avemar was first obtained in a study on the effect of the compound on immune function in mice.[8] Results in this study showed that Avemar significantly increased the degree of blastic transformation of peripheral blood T lymphocytes stimulated by concanavalin A.

In other experiments, $C_{57}B1$ mice were given skin transplants from the coisogenic mice strain $B_{10}LP$, which normally could be expected to be tolerated for 16–25 days before rejection. Thymectomized control (untreated) mice rejected the transplants at a gender mean of 52 (male) or 41 (female) days. Thymectomized mice treated with Avemar rejected the grafts at a mean of 29 days (male) or 33 days (female). Control (untreated) mice not thymectomized rejected the transplants at a gender mean of 21 or 29 days. These results, with immune function of mice seriously immunocompromised by thymectomy restored to near that of nonthymectomized mice (untreated), demonstrate the very significant immune restorative effects of Avemar treatment in these animals.[8] Interestingly, other experiments done as part of this group, aimed at determining whether Avemar's immunostimulatory effects could be ascribed to one active molecule, 2,6-dimethoxy-*p*-benzoquinone, showed they could not, as this substance administered alone did not shorten graft rejection time. From a therapeutic point of view, the immunomodulatory and immunorestor-

ing effects of Avemar may be exploited in various clinical manifestations of impaired immune response.

The potential of Avemar treatment on features of experimental systemic lupus erythematosus (SLE) in naive mice, induced by idiotypic manipulation, was also studied.[9] When the product was given in the preimmunization period, downregulation of autoantibody production (anti-dsDNA, mouse 16/6 Id, and antihistones) after treatment with Avemar was noted (e.g., anti-dsDNA decreased from 0.898 OD at 405 nm to 0.519 after treatment). This effect was sustained for at least 4 weeks after discontinuation of therapy. Serologic manifestations were associated with a delay in the Th2 (interleukin [IL]-4 and IL-10) response (e.g., IL-4 decreased from 92 to 60 ng/ml in splenocyte condition media). The mice showed normal erythrocyte sedimentation rate and WBC, and less than 100 mg/dl of protein in the urine in comparison to >300 mg/dl protein in the SLE nontreated mice. It was concluded that oral intake of Avemar could ameliorate the clinical manifestations of experimental SLE by affecting the Th1/Th2 network inhibiting the Th2 response. Based on these results, a double-blind clinical study with Avemar in lupus patients was recently initiated.[10]

In mice, Avemar proved effective in the restoration of hemopoiesis in bone marrow impairment induced by sublethal irradiation and/or cyclophosphamide therapy.[11] Elevation of the platelet count started on postirradiation day 7, and the baseline level was achieved on day 21. At the same time, no substantial increase was detected in the WBC count. As regards cyclophosphamide therapy, restoration of thrombopoiesis as well as of erythropoiesis could be observed as a result of Avemar treatment. These results are in consonance with the 5-year long clinical observation that Avemar has no hematotoxic side effect. A decrease in febrile neutropenia episodes during intensive chemotherapy of Avemar-treated pediatric cancer may help confirm the clinical relevance of bone marrow protection assessed in the experimental setting.

MOLECULAR TARGETS OF AVEMAR

Although the one (or more) molecule of fermented wheat germ extract responsible for the wide variety of biologic effects of this medical food has not yet been identified, molecular targets of Avemar, which could explain the effects, are (at least, partially) known.

PARP

Proliferation, differentiation, and cell death are under similar molecular control in all mammalian cells. Cancer cells develop severe defects in the regulation of homeostasis and cell proliferation, including resistance to apoptosis. Avemar inhibited the growth of leukemia cells in a dose-dependent manner. Laser scanning cytometry and gel electrophoresis with Western immunoblotting of stained cells indicated that the growth-inhibiting effect was consistent with a strong induction of apoptosis by activating the caspase-3–catalyzed cleavage of the poly(ADP-ribose) polymerase (PARP) enzyme.[12] PARP is a key player in DNA repair. The activity of this enzyme is extremely high in cancer cells.[13] Cleavage of PARP results in genom-

ic instability, leading to DNA fragmentation and thus to apoptosis in tumor cells. As the activity of PARP is accelerated in cancer cells, these cells can be selectively sensitized by PARP inhibitors (such as Avemar) to agents (such as 5-fluorouracil [FU] or DTIC), inducing base excisions or lesions in DNA. It has also been indicated that besides apoptosis induction, the mechanism through which Avemar mitigates metastasis involves decreasing cell motility. It was further demonstrated that although Avemar induced apoptosis in different leukemic human cells, it did not trigger programmed cell death in their healthy, resting counterpart, peripheral blood mononuclear cells.

MHC-I

Avemar treatment resulted in a decrease in the MHC class I (MHC-I) protein level on the surface of tumor cells, and hence it may expose them to natural killer (NK) cell activity.[14] As inhibition of tyrosine phosphatase activity also resulted in elevated downregulation of MHC-I molecules, control of protein tyrosine phosphorylation in this process was indicated. Involvement of lymphocyte-specific signaling molecules, the nonreceptor tyrosine kinase $p56^{lck}$, and the receptor tyrosine phosphatase CD45 in the Avemar-triggered cell response has been excluded.

A way for tumors to survive in the host environment is to evade the defense control of the host by mimicking themselves as normal cells for the survey of the immune system. Natural killer cells, which play an important role in antitumor defense, recognize and are blocked by the expression of MHC-I molecules on their target cells.[15] Consequently, tumor cells develop an effective camouflage by expressing high levels of MHC-I to avoid recognition by NK cells. This is a common characteristic of metastatic tumor cells to avoid NK surveillance.[16] As Avemar reduces the MHC-I level on human tumor cells, it may sensitize them against NK killing, thus reducing their metastatic activity.

ICAM-1

Endothelial cells of the vasculature of human solid tumors are known to have decreased expression of ICAM-1 compared to normal endothelial cell tissue, and this phenomenon can be considered a tumor-derived escape mechanism because the development of an efficient leukocyte infiltrate of the tumor is impaired.[17] It has been shown that Avemar upregulates the expression of intercellular adhesion molecule-1 (ICAM-1) on tumor-derived endothelial cells and also potentiates the similar effect of the primary anticancer cytokine, tumor necrosis factor-alpha (TNF-α).[18]

Pentose Phosphate Pathway

Avemar regulates tumor cell proliferation also by altering the rate of glucose intake and the synthesis of nucleic acid ribose through the nonoxidative steps of the pentose phosphate pathway (PPP).[19] This effect of Avemar is most efficiently present in the ribosomal RNA fraction of cancer cells. As ribose is a close metabolite of glucose and ribosomal RNA is essential for *de novo* enzyme protein synthesis and cell proliferation, it is evident that inhibiting the formation of ribose from glucose to build ribosomal structures is one of the important underlying mechanisms by which Avemar regulates tumor cell growth. Avemar also has remarkable effects on lipid

synthesis and the oxidation of the first carbon of glucose through the oxidative steps of the PPP. Avemar increases glucose oxidation in the PPP in a dose-dependent fashion and therefore acts as an important agent in controlling oxidative stress and damage to the cells. The oxidative steps of the PPP play a very limited role in the synthesis of ribose to build nucleic acids in tumor cells. Tumor cells broadly use nonoxidative reactions, whereas normal cells heavily depend on oxidative synthesis, and then recycling of ribose back to glycolysis through the nonoxidative steps. The selectivity of Avemar in inhibiting tumor cell proliferation but promoting normal immune cell expansion can be explained by its selective inhibitory action on unique metabolic steps only observed in cancer. The metabolic changes observed in Avemar-treated cancer cells provide explanations for the clinically detected weight gain and slow disease progression in Avemar-treated cancer patients. Increased PPP activity (glucose oxidation and pentose recycling) increases *de novo* fatty acid synthesis, chain elongation, and desaturation. It is also likely that decreased oxidative ribose synthesis is unable to supply tumor cells' metabolic needs for reducing equivalents that would intensively be used for the reduction of ribonucleotides to deoxyribonucleotides during DNA replication. The simultaneous decrease in nucleic acid synthesis from glucose leads to a decrease in cell proliferation, which explains the slow disease progression and the increased survival rate of the patients. Decreased glucose consumption of the tumors also leads to a metabolic harmony with the host and weight gain in patients with even advanced cancers. As a result, Avemar-treated patients also have improved tolerance for surgeries, chemotherapy, or radiation therapy. This effect of redistributing glucose carbon use from nonoxidative nucleic acid ribose synthesis to direct glucose oxidation and lipid synthesis is a novel mechanism of antiproliferative action only described in connection with Avemar treatment.[20] Avemar treatment is likely associated with the phosphorylation or transcriptional regulation of metabolic enzymes that are involved in reverting glucose carbons from cell proliferation-related structural and functional macromolecules (RNA, DNA) to direct oxidative degradation of glucose. It was demonstrated that Avemar treatment was about 50 times less effective in peripheral blood lymphocytes in inducing the aforementioned effects than in cancer (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.[12]

Ribonucleotide Reductase

Ribonucleotide reductase (RR) is responsible for the conversion of ribonucleotides to deoxyribonucleoside triphosphates, which are precursors of DNA synthesis. Ribonucleotide reductase was demonstrated to be significantly upregulated in tumor cells in order to meet the increased need for dNTPs of these rapidly proliferating cells for DNA synthesis.[21] 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 the free radical scavenging of a free tyrosine radical, which is needed for iron stabilization. To determine whether Avemar's action in HT-29 human colon carcinoma cell line involves such RR inhibition, first an *in situ* enzyme assay was employed. Radiolabeled

cytidine had to be reduced by RR in order to be incorporated into DNA. The *in situ* RR activity of HT-29 cells were inhibited by Avemar in a concentration-dependent manner. These results were then confirmed by the determination of dNTP pool sizes after the incubation of HT-29 cells with Avemar. The compound did inhibit RR, the key enzyme of *de novo* DNA synthesis, which might also explain its effect on tumor cells, in particular its antitumor effects in patients with colorectal cancer.[22]

Cyclooxygenases

Cyclooxygenases (COX-1 and COX-2 enzymes) were incubated with increasing concentrations of Avemar. 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, whereas a concentration of 300 μg/ml inhibited COX-2 activity to 50% of the control.[22] These significant results demonstrate the COX-inhibiting capacity of Avemar, and no selectivity towards one of the COX enzymes could be observed.

The nonselective inhibition of COX enzymes by Avemar may partly explain this extract's anti-inflammatory activities against adjuvant arthritis in rats and rheumatoid arthritis in humans, the results of which are currently in publication.

As inhibition of COX enzymes is also generally considered a preventive tool in colorectal cancer, these results may also shed light on the mechanism of the chemopreventive activity of Avemar against carcinogenic chemically induced experimental colon cancer. It was examined whether Avemar might inhibit colon carcinogenesis in mammals, using as a model F-344 rats.[23] One hundred 4-week-old rats were divided into four groups. *Group 1*: untreated controls; *Group 2*: rats given the carcinogen azoxymethane (AOM) in three subcutaneous injections 1 week apart; *Group 3*: animals that started to receive Avemar via gastric tube 2 weeks prior to the first injection of AOM, daily and continuously thereafter until all animals were killed 32 weeks later; *Group 4*: animals that received the basal diet and Avemar only. At autopsy, no tumors were found in the untreated controls and in *Group 4* (Avemar only) animals. In *Group 2* (AOM only) 83.0% developed colon tumors with a mean of 2.3 tumors per animal; in *Group 3* (Avemar and AOM) 44.8% ($P < .001$) developed tumors with 1.3 ($P < .004$) tumors per animal. All the tumors were neoplastic. There were 4.85 aberrent crypt foci per cm^2 in *Group 2* (AOM only) compared to 2.03 in *Group 3* (AOM plus Avemar) ($P < .0001$). These results showed a powerful anticarcinogenic effect associated with the prophylactic use of Avemar as a cancer preventative in animals.

AVEMAR IN CLINICAL CANCER TRIALS

Oral Cavity Cancer

An open-label, nonrandomized, controlled, phase-II clinical study was performed in the Semmelweis University Clinic of Oral and Maxillofacial Surgery, Budapest. Forty-three patients with a definitive diagnosis of less than 3 months of either locally advanced oral cavity squamous cell carcinoma (OCC) (UICC stage II-III) or locally advanced stage IV (i.e., T4a N0-N1M0) were enrolled. Twenty-one consecutive patients received SAT, consisting of radical surgery + postoperative irradiation and/

or adjuvant chemotherapy, and 22 consecutive patients received SAT + 12 months of Avemar treatment. The objective was to assess whether Avemar has any influence on the outcome of locally advanced OCC when applied concomitantly with SAT. The end-point was disease progression. As regards baseline characteristics (age, clinical stage, chemotherapy, and site of primary tumor), there was no significant difference between the two groups apart from the previous treatment with radiotherapy drawback to the Avemar group (3 of 22 in the Avemar group vs 9 of 21 in the control group) ($P < .05$). At end-point, incidences of local recurrences and disease progression differed significantly between the two groups: 4.5% and 9.09% in the SAT + Avemar, 57.1% and 61.9% in the control group (SAT alone), respectively ($P < .001$). Risk analysis revealed that the 12 months of Avemar treatment significantly reduced the risk of overall progression (death, new loco-regional recurrences, new distant metastases) by 85% (Mantel-Haenszel test, $P < .001$).[24]

Based on these results and taking into consideration the results obtained in a non-comparative quality of life (QOL) study (QLQ-C30 questionnaire of the European Organization for Research and Treatment of Cancer [EORTC], Brussels) of 50 patients with head and neck cancer treated at the Oto-rhino-laryngology Clinic of Semmelweis University, in which patients experienced substantial improvement in cachectic symptoms, and long-term delay of progression in five of six advanced-stage salivary gland tumor patients, supportive use of Avemar in this manner may improve QOL and enhance the antitumor efficacy of SAT.[25]

Colorectal Cancer

Between 1998 and 1999, an open-label, pilot-scale, nonrandomized, controlled, phase II clinical study was carried out in the Uzsoki Hospital of Budapest to document whether or not supportive Avemar treatment adds any benefit to SAT in colorectal cancer.[26] Altogether 30 patients with advanced colorectal cancer (CRC) had been enrolled. All patients underwent curative surgery. The control group (18 patients) received SAT, whereas the Avemar group (12 patients) received SAT plus continuous and uninterrupted Avemar treatment. The end-point was progressive disease. Although, at baseline, patients of the Avemar group had more advanced disease stages, after an average observation period of 9 months, no disease progression had developed in the Avemar group, whereas three patients died and one patient had developed metastatic disease in the control group. Due to the small sample size, this difference had no statistical significance.

Between 1998 and 2003 also an open-label, pilot-scale, nonrandomized, controlled, phase II clinical study was done in the Berettyoujfalu Regional Hospital, including 34 patients with advanced adenocarcinoma of the rectum or the sigma.[27] Following radical surgery, 17 patients received SAT and 17 SAT plus continuous Avemar. The end-point was overall survival. After an average observation period of 46 months, significantly longer survival was found in the Avemar group.

A multicentric, open-label, cohort, phase III clinical study with the participation of 170 CRC patients enrolled from three onco-surgical centers in Hungary (Uzsoki Hospital of Budapest, University Clinics of Szeged and Debrecen) compared SAT vs. SAT + Avemar therapy.[28] Cohort allocation was based on the patient's choice. Sixty-six patients received Avemar as a supportive agent in addition to SAT (radical surgery plus radiotherapy and/or chemotherapy), whereas 104 patients were enrolled

as controls without Avemar. The Mayo Clinic chemotherapy regimen and/or postoperative irradiation were regarded as SAT. Major eligibility criteria were: histologically or cytologically confirmed adenocarcinoma, curative surgery (at the time of diagnosis) with complete removal of primary tumor (completed with removal of metastases in selected cases of solitary or localized multifocal liver metastases) + removal of an adequate number of regional lymph nodes, a World Health Organization (WHO) performance status of 0–2, and life expectancy of at least of 6 months. Clinicopathologic stage, date of diagnosis, prior chemotherapy-/radiotherapy were disregarded as selection criteria. The primary end-point for both cohorts was progression-free survival. The mean age of control patients was higher, while distribution according to clinical stages was significantly less favourable in the Avemar group, which had a substantially (and significantly) higher percentage of International Union Against Cancer (UICC) stage IV (Dukes D) patients. There was a substantial difference between cohorts in average time elapsed from diagnosis to onset of therapy (drawback to the prognosis of Avemar cohort), while the interval from diagnosis to evaluation was similar. There was a significant difference between the two groups in number of patients previously treated with radiotherapy ($P < .001$) also drawback to the Avemar cohort. In summary: apart from mean age alone, prognostic variables in Avemar-treated cohort were much less favourable than in the control one.

Results: Progression-related events (new recurrences, new distant metastases, death) occurred with a substantially (and significantly) higher frequency in the control group. The cumulative probabilities of both disease-free (DFS) and overall survival (OS) proved to be more favorable in the Avemar group (DFS: $P = .0184$, OS: $P = .0278$). Cox regression analysis identified UICC staging ($P = .0004$) and Avemar treatment of ≥ 6 months ($P = .0045$) as independent predictors of survival. Side effects were extremely rare, mild and transient: diarrhea (4), nausea/vomiting (2), flatulence, repletion, soft stool and constipation: one instance of each. No serious adverse event (SAE) occurred either related or non-related to the test product.

Although other clinical observations regarding the efficacy of supportive Avemar therapy in CRC have been made, this study is currently the most complete source of data sufficient for drawing conclusions about the role of Avemar in this malignancy. Although there was not optimal homogeneity of previous treatment criteria among patients, it seems clear from this study, run in a more or less adjuvant setting, that Avemar has substantial potential benefit in improving the efficacy of adjuvant therapy (or in lengthening the tumor-free interval following radical surgery) in CRC. Specifically, Avemar may reduce likelihood of CRC recurrence; it may reduce new metastatic disease occurrence; and it may increase chances of survival after diagnosis with CRC.

Malignant Melanoma of the Skin

Being aware that most patients with UICC stage III (high-risk) malignant melanoma of the skin treated with SATs will eventually develop progressive disease, and because delay of progression in this condition is specially of high clinical importance, an open-label, pilot-scale, randomized, controlled, phase II clinical study was carried out in the N.N. Blokhin Cancer Center of the Russian Academy of Sciences in Moscow to test the possible value of supportive therapy with Avemar in this respect.[29] This year-long study compared, in the postsurgical adjuvant setting, DTIC

plus 1 year of continuous Avemar administration (Avemar group, 22 patients) to treatment with dacarbazine alone (control group, 24 patients) in stage III melanoma of the skin. These patients were at high risk of recurrence and death from their disease. Interferon-alpha treatment is sometimes given to such patients, but its efficacy is controversial, undesirable side effects can be extreme, so DTIC remains an option. Chemotherapy naive, postoperative patients were randomized to either DTIC plus Avemar or to DTIC on its own (control) groups. In addition to cytostatic monotherapy, patients of the Avemar group took Avemar uninterruptedly up to 12 months. All patients were evaluated at baseline, at the end of each DTIC cycle, and 1, 5, and 9 months after completion of chemotherapy. The end-point was progression-free survival. Primary and/or nodal disease recurrence and new lymphatic and/or distant metastatic disease occurrence were regarded as progression-related events. There was no statistical difference in baseline parameters of the two groups. Eligibility criteria were: histologically confirmed malignant melanoma of the skin, with regional lymph node metastases and without evidence of distant metastases (stage III disease: pT1a-4bN1-3M0); WHO performance status: 0, 1, or 2; life expectation of $\geq$12 months; radical surgery including complete removal of the primary tumor followed by complete lymph node resection, achieving a macroscopically disease-free state. At end-point analysis, there were significantly more control patients with progressive disease (Avemar: 36% vs control: 75%; $P < .01$). Log-rank analysis (Kaplan-Meier estimate) showed a significant difference in time-to-progression (median, days) in favor of the Avemar group (Avemar: 366 vs control: 231, $P = .0042$). Continuous supplementation of dacarbazine treatment with Avemar is beneficial to stage III (high-risk) melanoma patients in terms of progression-free survival.

Side effects were related to DTIC treatment in both groups. Note that there were generally fewer toxic side effects in patients receiving the combined treatment than in those of the DTIC only group.

QUALITY OF LIFE

Lung Cancer

Downturn of quality of life (QOL) of advanced-stage cancer patients is a significant clinical issue, potentially influencing even patient survival probabilities. Consequently, improvement of QOL is of great importance among these patients. A pilot study was performed with enrolment of 16 lung cancer patients (8 males, 8 females, 7 small-cell and 9 adenocarcinoma cases) in the Korányi National Institute of Pulmonology, Budapest.[30] The patients had been treated with chemotherapy and/or radiotherapy. Avemar was administered for 8 months; the EORTC QLQ-C30 questionnaire was self-administered and the resulting data used for assessing the change in QOL. After 12 weeks of Avemar administration, significant improvement was reported in global state of health ($P < .01$) and in fatigue ($P < .05$). This improvement was maintained throughout the observation period. A modest improvement developed in pain, loss of appetite, and mood parameters. No adverse side effects were reported. Improvement in fatigue syndrome is of special importance.

Breast Cancer

A multicentric clinical study of Avemar in breast cancer is still ongoing. However, a QOL study involving Avemar use in breast cancer patients has been completed.[31] A total of 55 patients were enrolled in the study at Szeged Unversity Clinic of Surgery, and gauging of QOL and changes in it were based on the EORTC QLQ-C30 questionnaire. Main baseline characteristics included: mean age: 55 years; UICC stage: I: 8, II: 19, III: 15, and IV: 13; concurrent therapies: chemoradiotherapy: 10, chemotherapy only: 9, radiotherapy only: 2, and none in 34 cases. The mean observation period was 32 months. Several components of QOL showed significant improvement due to supportive therapy with Avemar. Significant improvements were achieved in physical functions ($P < .05$), emotional functions ($P < .001$), global state of health ($P < .01$), fatigue ($P < .01$), nausea, vomiting ($P < .01$), insomnia ($P < .01$), and constipation ($P < .01$). Effects were manifested after 3 months of treatment and remained stable throughout the entire length of the study.

FEBRILE NEUTROPENIA

There is a very attractive clinical observation concerning the indirect beneficial effect of Avemar—in malignant tumors—in connection with immune-stimulatory and bone marrow protective properties. An open-label, matched pair-based, controlled clinical study was performed in the 2nd Clinic of Pediatrics of the Semmelweis University in Budapest, with the enrolment of 22 (2×11) children with various types of pediatric solid cancers.[32] The matched pair setting was based on diagnosis, histopathology, clinical stage, gender, treatment schedule, and age. Avemar treatment was started simultaneously with cytostatic therapy and continued until the end of treatment and later on. Eleven children were given Avemar, and eleven served as controls. The end points of the study were the number and frequency of febrile neutropenia episodes accompanying intensive chemotherapy in the two groups. In the course of 121 cycles of chemotherapy, altogether 30 episodes of febrile neutropenia (24.8%) were observed in the Avemar group, 106 cycles were completed in the control group, and 46 episodes of febrile neutropenia (43.3%) were registered. The difference is significant in favor of the Avemar group ($P < .01$). It should be emphasized that principles in prophylaxis and therapy of febrile neutropenia were identical in the two groups. The results are convincing in the successful control of a life-threatening complication; the study can be regarded as clinical evidence of the immunostimulatory and bone marrow-protecting effect of Avemar.

OTHER CANCERS

Clinical studies with Avemar in cancer of the urinary tract and in chronic myelogenous leukemia are still ongoing in Europe.[33] In Israel, a multicentric, double-blind clinical trial with Avemar in metastatic CRC is currently under an interim analysis. There is much observational data about the favorable use of Avemar in a variety of other human malignancies, including ovarian cancer, gastric cancer, thyroid cancer, non-Hodgkin's lymphoma, and multiple myeloma.[3] Regression in patients with

advanced hepatocellular carcinoma who were taking Avemar on a continuous basis has been observed.[3] Interestingly, regression in skeletal metastatic lesions has also been reported in last-stage breast, prostatic, and non-small-cell lung cancer patients after Avemar administration.[3] The therapeutic spectrum of Avemar is much wider than it had been thought some years ago.

REFERENCES

1. HIDVÉGI, M. 1998. Current results of Avemar research. (In Hungarian). Nogyogyaszati Onkol. **3:** 241–243.
2. TOMOSKOZI-FARKAS, R. & H.G. DAOOD. 2004. Modification of chromatographic method for the determination of benzoquinones in cereal products. Chromatographia **60:** S227–S230.
3. AVEMAR. (In Hungarian). 2004. Pharmindex Handbook of Oncology 2004/2005. :611–617. CMP Medica. Budapest.
4. HIDVEGI, M. *et al.* 1998. Effect of Avemar and Avemar + vitamin C on tumor growth and metastasis in experimental animals. Anticancer Res. **18:** 2353–2358.
5. SZENDE, B. *et al.* 2004. 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.
6. HIDVEGI, M. *et al.* 1999. MSC, a new benzoquinone-containing natural product with antimetastatic effect. Cancer Biother. Radiopharm. **14:** 277–289.
7. MARCSEK, Z. *et al.* 2004. The efficacy of tamoxifen in estrogen receptor-positive breast cancer cells is enhanced by a medical nutriment. Cancer Biother. Radiopharm. **19:** 746–753.
8. HIDVEGI, M. *et al.* 1999. Effect of MSC on the immune response of mice. Immunopharmacology **41:** 183–186.
9. EHRENFELD, M. *et al.* 2001. 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.
10. SUKKAR, S.G. & E. ROSSI. 2004. Oxidative stress and nutritional prevention in autoimmune rheumatic diseases. Autoimmunity Rev. **3:** 199–206.
11. GIDALI, J. *et al.* 2000. The effect of Avemar treatment on the regeneration of leukocytes, thrombocytes and reticulocytes in sublethally irradiated or cyclophosphamide treated mice. 1st Congress of the Hungarian Society of Clinical Oncology. Budapest, Hungary, November 10–11.
12. COMIN-ANDUIX, B. *et al.* 2002. 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.
13. VIRAG, L. & CS. SZABO. 2002. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol. Rev. **54:** 375–429.
14. FAJKA-BOJA, R. *et al.* 2002. 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.
15. LOPEZ-BOTET, M. & T. BELLON. 1999. Natural killer cell activation and inhibition by receptors for MHC class I. Curr. Opin. Immunol. **11:** 301–307.
16. KUNDU, N. & A.M. FULTON. 1997. Interleukin-10 inhibits tumor metastasis, downregulates MHC class I, and enhances NK lysis. Cell Immunol. **180:** 55–61.
17. GRIFFIOEN, A.W. *et al.* 1996. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. Cancer Res. **56:** 1111–1117.
18. TELEKES, A. *et al.* 2005. Synergistic effect of Avemar on proinflammatory cytokine production and Ras-mediated cell activation. Ann. N.Y. Acad. Sci. **1051:** 515–528.
19. BOROS, L.G. *et al.* 2001. Wheat germ extract decreases glucose uptake and RNA ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells. Pancreas **23:** 141–147.

20. CASCANTE, M. *et al.* 2002. Metabolic control analysis in drug discovery and disease. Nat. Biotechnol. **20:** 243–249.
21. TAKEDA, E. & G. WEBER. 1981. Role of ribonucleotide reductase in expression in the neoplastic program. Life Sci. **28:** 1007–1014.
22. ILLMER, C. *et al.* 2005. Immunologic and biochemical effects of the fermented wheat germ extract Avemar. Exp. Biol. Med. **230:** 144–149.
23. ZALATNAI, A. *et al.* 2001. Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats. Carcinogenesis **22:** 1649–1652.
24. FULOP, E. *et al*. 2004. Results of the administration of Avemar in oral cavity cancer patients. 7th Congress of the Hungarian Society of Oral and Maxillofacial Surgery. Pecs, Hungary, October 16–18.
25. RIBARI, O. *et al.* 2000. Early results on the supportive treatment of head and neck cancer patients with Avemar. 1st Congress of the Hungarian Society of Clinical Oncology. Budapest, Hungary, November 10–11.
26. JAKAB, F. *et al.* 2000. First clinical data of a natural immunomodulator in colorectal cancer. Hepatogastroenterology **47:** 393–395.
27. KOTI, CS. & L. LENGYEL. 2004. Tumours of the sigma and rectum: the completion of postoperative chemotherapy with Avemar. (In Hungarian). Magy Seb **57:** 168.
28. JAKAB, F. *et al.* 2003. A medical nutriment has supportive value in the treatment of colorectal cancer. Br. J. Cancer **89:** 465–469.
29. DEMIDOV, L.V. *et al.* 2002. Antimetastatic effect of Avemar in high-risk melanoma patients. 18th UICC International Cancer Congress. Oslo, Norway, June 30–July 5.
30. HIDVEGI, M. *et al.* 2003. Fermented wheat germ extract improves quality of life in lung cancer patients. (In Hungarian.) Medicus Anonymus/Pulmono **11:** 13–14.
31. BALOGH, A. 2001. The supportive value of AVEMAR during chemotherapy. New Results of Avemar Research. Symposium. 24th Congress of the Hungarian Cancer Society. Budapest, Hungary, November 22–24.
32. GARAMI, M. *et al.* 2004. Fermented wheat germ extract reduces chemotherapy induced febrile neutropenia in pediatric cancer patients. J. Pediatr. Hematol. Oncol. **26:** 631–635.
33. FARKAS, E. 2004. The role of Avemar in oncology. A review. (In Hungarian). Magy Belorv. Arch. **57:** 4–9.