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**Abstract.** *Because of the observed immunostimulatory actions of a new fermented wheat germ extract - with standardized benzoquinone composition - we have investigated the eventual tumor growth- and metastasis-inhibiting effects of this preparation (Avemar) applied alone or in combination with vitamin C. Tumor models of different origin [a highly metastatic variant of the Lewis lung carcinoma (3LL-HH), B16 melanoma, a rat nephroblastoma (RWT-M) and a human colon carcinoma xenograft (HCR25)] - kept in artificially immunosuppressed mice were applied. The metastasis-inhibiting effects of the treatments have been studied both in the presence and in the absence (following surgical removal) of the transplanted primary tumors. Combined treatments with Avemar and vitamin C - administered synchronously - profoundly inhibited the metastasis formation in all the applied tumor models while, treatments with vitamin C alone did not exert such an inhibiting effect on the metastasizing process. The degree of the observed metastasis inhibition in certain models was significant, while in others - although it was meaningful - did not prove to be significant. It is noteworthy that treatment with Avemar alone in certain models exerted a more pronounced inhibiting effect on metastasis formation than the synchronous combined treatment with Avemar and vitamin C. Furthermore, if the time schedule of the combined treatment was changed (vitamin C - instead of being administered synchronously - was given one hour after the treatments with Avemar), the vitamin C rather decreased the metastasis inhibiting effect of Avemar. It should be mentioned however, that in the case of rat nephroblastoma, a different response was observed: while, in the case of synchronous combination significant inhibition of metastasis formation was observed, treatment with Avemar alone did not produce metastasis-inhibition. It is noteworthy that in this model the metastasis-inhibiting effect of the synchronous combination treatment proved to be even more pronounced if Avemar was administered in a 100 times smaller dose than its regularly applied dosage. Treatment with Avemar and vitamin C - administered in combination or separately - in the majority of experimental models (with the exception of rat nephroblastoma) did not inhibit the growth of the primary tumors. It is reasonable, therefore, to suppose that in the observed metastasis-inhibiting effect the eventual proliferation inhibiting effect of these remedies does not play an important role. According to the results of other experiments - carried out in our laboratory in parallel with those described here - Avemar proved to have a meaningful immunostimulatory effect. It might therefore be suggested that the observed metastasis-inhibiting effect of this preparation may be mainly due to its immunostimulatory properties. The possible therapeutic benefits of Avemar and Avemar plus vitamin C are also discussed.*

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*Key Words:* Avemar, fermented wheat germ extract, benzoquinones, vitamin C, anticancer effect, metastasis inhibition, cancer biotherapy, Lewis lung carcinoma, B16 melanoma, rat nephroblastoma, Wilms' tumor, human colon carcinoma.

The possible immunostimulatory effect of wheat germ fermented by yeast has been proposed by Szent-Györgyi (1). According to his theory, the two quinones, 2-methoxy-*p*-benzoquinone (2-MBQ) and 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ), present in wheat germ as glucosides and liberated by the yeast's glucosidase, are likely to be responsible for the supposed biological properties of fermented wheat germ, because similar quinones, together with ascorbic acid, are involved in a series of metabolic reactions of vital importance, in which molecular oxygen reduces to water (2).

According to Pethig and co-workers' observations (3) growth inhibition of Ehrlich ascites tumor can be achieved by

*Table I. Effect of Avemar on weight of primary splenic tumor and liver metastasis formation 14 days after inoculation of 3LL-HH tumor line. Spleen-liver model.*

| Group | Number of injected cells | Weight of primary splenic tumor (g, mean ± SD) | Number of liver metastases (mean ± SD) |
|---|---|---|---|
| Continuous treatment | | | |
| Control | $3 \times 10^2$ | 0.34 ± 0.18 | 68.1 ± 15.2 |
| Avemar+vitamin C | $3 \times 10^2$ | 0.46 ± 0.37 | 56 ± 14.1 |
| Control | $3 \times 10^3$ | 0.32 ± 0.24 | 104 ± 28.2 |
| Avemar+vitamin C | $3 \times 10^2$ | 0.73 ± 0.29 | 29.8 ± 16.4* |
| Pretrestment only | | | |
| Control | $3 \times 10^2$ | 0.67 ± 0.33 | 41.9 ± 19.1 |
| Avemar+vitamin C | $3 \times 10^2$ | 0.52 ± 0.32 | 33.2 ± 20.0 |

* $p < 0.01$

treatment of the tumor bearing mice with a mixture of 2,6-DMBQ and ascorbic acid. This mixture produces long lived semiquinone and ascorbic free radicals. It has been shown that quenching of quinone and ascorbic radicals depends on an NAD(P)H dependent enzyme containing an active sulfhydryl group (4). The cytotoxicity of the radical mixture has been supposed to be associated with the decreased NAD(P) reducing power of tumor cells (5).

Several quinones occur in the nature, such as ubiquinones, plastoquinones, menaquinones *etc.* They play an important role in the photosynthesis of some bacteria, in the human respiratory chain, in blood coagulation, *etc.* Quinone compounds may be formed *in vivo* from phenolic compounds *e.g.,* dopaquinone is a derivative of tyrosine. Several quinones are also used for therapeutic purposes. Adriamycin, daunorubicin, mitomycin C, *etc.* have cytostatic effects based on the production of the free radical form of these compounds. DT-diaphorase plays a role in the reduction of quinones to less toxic hydroquinones, but also in the activation of the above cytostatic agents. The expression level of DT-diaphorase is thought to correlate with chemosensitivity to mitomycin C through elevated bioactivation of the compound. The inducing effect of vitamin C on detoxifying enzymes including DT-diaphorase has also been shown. Other benżo- and hydroquinones have antimicrobial effects and are active components of antibiotics, like Tetran-B, Metacyclin, Doxycyclin, *etc.* The biological activity of quinones is connected with their participation in redoxy-cycles in the form of free reactive radicals. Their ability to produce aryl-nucleophil compounds, particularly by reaction with thiol- and amino groups may explain the extreme activity of these compounds. In the skin or in other tissues and organs the nucleophiles are probably the functional groups of amino acids in proteins, *e.g.* thiol group of cysteine or amino group of lysine. Quinones react with amines and amino acids *via* Michael-addition, and they inhibit in some cases the activity of enzymes.

Table IIa. *Effect of Avemar on spleen primary tumor and liver colonization potential of HCR25 human colorectal adenocarcinoma tumor line in immunosuppressed CBA/CA mice. Evaluation was performed 51 days after tumor inoculation.*

| Group | Weight of primary spleen tumor (g, mean ± SD) | Number of liver colonies (mear ± SD) |
|---|---|---|
| Control - nonsplenectomized | 1.02 ± 0.59 | 42.0 ± 25.8 |
| Avemar - nonsplenectomized | 0.62 ± 0.47 | 19.5 ± 19.0 |
| Control - splenectomized* | 0.10 ± 0.02 | 19.1 ± 13.5 |
| Avemar - splenectomized* | 0.08 ± 0.02 | 10.6 ± 11.6 |

* splenectomia was performed 21 days after tumor inoculation

2,6-DMBQ and 2-MBQ have been isolated from a variety of plants (6). 2,6-DMBQ in particular, is present in many different species. One of the largest known natural source for these two compounds is wheat (*Triticum vulgaris*), where these are present in the form of glucosides, and are located in the embryo.

Based on Szent-Györgyi's studies, a dried, standardized extract of wheat germ fermented by *Saccharomyces cerevisiae* was produced. The fermentation process had previously been optimized (7) to yield 0.4 mg/g (on dry matter basis) concentration of 2,6-DMBQ in the extract. The dried extract, named as Avemar, was administered alone or combined with vitamin C to tumor bearing mice and rats. The aim of our experiments was to examine the eventual tumor-growth and metastasis-inhibiting effects of these agents.

## Materials and Methods

*Preparation of Avemar.* 70 kg Saccharomyces cerevisiae, obtained from a local grain distillers company, was suspended into a 3 $m^3$ isothermic (30 ± 1°C) fermentor containing 2 $m^3$ of tap water. After mixing, 210 kg of freshly grinded wheat germ, obtained from a local grain milling company, was added into the yeast suspension. The mixture was then fermented, filtered, concentrated and spray-dried. The resulted powder was homogenized and kept in sealed containers.

*Animals.* In all experiments inbred mice and rats from our Institute were used. The animals were 8-10 weeks old, the mice and the rats weighed 20-22 g and 160-180 g, respectively. The mice and rats were kept in plastic cages (5 per cage) and were fed with rodent food pellets (LATI, Gödöllő, Hungary) and tap water *ad libitum*. The room temperature was 20-22°C, relative humidity was 50±5 %.

*Treatments.* Animals were treated every day from the 1st day after implantation of tumor till the day of evaluation. Treatments were carried out orally, via gastric tube, as aliquots of 0.1 ml, as follows (see Tables I-V): Control = tap water; Avemar = 3 g, Avemar / kg, body weight; Vitamin C = 0.9 g, vitamin C / kg, body weight; Avemar + vitamin C = 3g, Avemar / kg, body weight, supplemented with 0.9 g, vitamin C / kg, body weight.

*Tumor models. Lewis lung carcinoma (3LL-HH) - spleen-liver model:* The high metastatic variant of Lewis lung carcinoma (3LL-HH) was maintained in C57Bl/6 mice by serial intrasplenic transplantations of tumor cells obtained from liver metastases ($3 \times 10^3$ cells). Single-cell

Table IIb. *Size distribution of HCR25 liver colonies as percentages of total tumor colonie numbers.*

| Group | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 | 81-90 | 81- |
|---|---|---|---|---|---|---|---|---|---|---|
| Contr.spl.- | 1.7 | 9.4 | 11.3 | 16.3 | 17.0 | 16.3 | 12.8 | 6.6 | 5.4 | 3.2 |
| Avemar spl.- | 2.5 | 22.4 | 18.5 | 13.4 | 20.3 | 14.7 | 5.7 | 1.2 | 0 | 1.3 |
| Contr.spl.+ | 3.7 | 17.3 | 12.6 | 12.6 | 22.0 | 18.8 | 7.3 | 3.7 | 1.0 | 1.0 |
| Avemar spl.+ | 0 | 18.9 | 10.6 | 30.7 | 17.7 | 8.2 | 9.4 | 1.1 | 2.3 | 1.1 |

spl.-, nonsplenectomized groups
spl.+, splenectomia was performed 21 days after tumor inoculation

Table III. *Effect of Avemar on primary muscle tumor volume and lung metastasis formation of the B16 melanoma tumor line 21 days after tumor inoculation.*

| Group | Weight of primary tumor (g, mean ± SEM) | Number of metastases (mean ± SEM) |
|---|---|---|
| Control | 7.6 ± 0.43 | 42.4 ± 10.2 |
| Avemar+vit.C | 6.7 ± 0.79 | 19.3 ± 10.6 |
| Avemar | 7.2 ± 0 38 | 6.2 ± 3.7 # |
| vit. C | 7.2 ± 0.37 | 39.3 ± 8.8 |
| Avemar+vit.C* | 6.6 ± 0.57 | 34.8 ± 11.4 |

* vit.C was added lh after Avemar treatment
# $p < 0.01$

suspensions were obtained from 14-day-old 3LL-HH liver metastases. Tumor tissues were cut by crossed scalpels and filtered through fourfold gauze. After centrifugation (800 rpm) and washing (Medium 199) the viability of the cells was determined with trypan blue solution. $3 \times 10^2$ or $3 \times 10^3$ cells were injected (30-50 µl) into the spleen of adult mice. Animals were sacrificed after 14 days of tumor inoculation. Primary tumor weight was determined by subtracting the average weight of spleens of ten normal healthy mice of similar age from the weight of the tumor-bearing spleens. The number of liver metastases were determined by examining liver surfaces and counting the tumor colonies visible under stereomicroscope. Avemar plus vitamin C were administered either from two weeks of tumor transplantation till sacrifice ("continuous treatment"), or for only two weeks till tumor transplantation ("pretreatment only").

*B16 melanoma - muscle-lung model.* B16 tumor line was maintained in C57Bl/6 mice by serial intramuscular transplantation ($5 \times 10^5$ cells, hind leg muscle). Single-cell suspensions (prepared as described above) were obtained from 14-day-old primary tumors. $5 \times 10^5$ viable tumor cells were injected into the hind leg muscle of the animals. Primary tumor weights and number of lung metastases were determined after 21 days of tumor inoculation.

In appropriate experiments primary tumor was removed by amputation of the tumor-bearing leg after 10 days of tumor inoculation.

*Rat nephroblastoma (RWT-M) - kidney-lung model.* Metastasizing rat nephroblastoma (RWT-M) was maintained in F344 rats (8). Subcutaneous tumors were cut into 0.5 × 0.5 × 0.5 mm pieces. The left kidney of each recipient adult 150-180 g F344 rats was exposed through a flank incision, a small opening was created in the capsule on the inferior pole and a tumor fragment was inserted under the capsule. Primary tumor weights were determined on the 31st day after tumor inoculation by calculating the difference between the tumor bearing and the contralateral tumor-free kidneys. The number of lung metastases was determined by examining the surface of the lungs using a low-power stereomicroscope.

Table IV. *Effect of Avemar on metastatization of B16 melanoma in muscle-lung amputation model.*

| Group | Weight of primary tumor removed on the 10th day (g, mean+SD) | Number of metastases on the 21 st day (mean ± SD) |
|---|---|---|
| Control | 1.31 ± 0.31 | 15.6 ± 5.6 |
| Avemar | 1.07 ± 0.62 | 15. 9 ± 13.8 |

Table V. *Effect of Avemar on primary tumor and metastasis formation of the rat neproblastoma (RWT-M) in subrenal capsule assay. Evaluation was performed 31 days after tumor transplantation.*

| Group | Weight of primary tumor (g, mean ± SEM) | Number of metastases (mean ± SEM) |
|---|---|---|
| Control | 3.11 ± 1.97 | 52.1 ± 43.2 |
| Avemar+vit.C | 1.89 ± 1.60 | 23.9 ± 24.4 |
| 0.01 [Avemar+vit.C] | 0.72 ± 0.96 | 8.5 ± 13.5 * |
| Avemar | 2.71 ± 1.85 | 55.7 ± 62.4 |
| vit.C | 0.76 ± 0.96 | 15.9 ± 26.3 |

* $p < 0.01$

*Human colon carcinoma (HCR25) - spleen-liver model.* The HCR25 human colon carcinoma xenograft was established from a moderately differentiated primary human colon carcinoma in our Institute (9). The tumor was maintained in immunosuppressed CBA/CA mice by serial subcutaneous transplantation of small tumor fragments.

Single-cell suspensions were prepared from subcutaneously growing HCR25 human colon adenocarcinoma xenograft. The tumor tissue was cut into small (0.5 × 0.5 × 0.5 mm) pieces in Medium 199, followed by enzymatic digestion at 36°C for 30 minutes under mild stirring in 10 ml Medium 199 containing a mixture of the following enzymes: collagenase I (Sigma-Aldrich) 0.0071 g, DNase (Sigma-Aldrich) 0.005 g and hyaluronidase (Sigma-Aldrich) 0.00044 g. Following digestion, the number of viable cells were determined and adjusted to $5 \times 10^7$/ml. 106 cells were injected intrasplenically, and number of tumor colonies (metastases) in the liver were determined 51 days after tumor inoculation. In appropriate experiments, animals were splenectomized on the 21st day following tumor inoculation.

## Results

We have investigated the effect of Avemar on liver colony

formation from 3LL-HH cells implanted into the spleen of immunocompetent C57Bl/6 mice and from human colon carcinoma xenograft (HCR 25) implanted into the spleen of immunosuppressed CBA/CA mice.

In the 3LL-HH spleen-liver model Avemar + vitamin C significantly reduced the number of metastases in the group treated continuously after inoculation of $3 \times 10^3$ cells. A slight decrease in the metastasis number was also observed in the groups inoculated with lower number of tumor cells or treated with Avemar + vitamin C prior to tumor inoculation. Interestingly, where the inhibition of metastasis formation was the highest the primary tumor weight increased (Table I).

To test the effect of Avemar on the growth and metastatization of human cancer, human colon carcinoma tumor line (HCR25) established in our laboratory (9) was used. Growth of the primary splenic tumor was markedly inhibited, that was parallelled by significant reduction of the number of liver metastases (Table IIa). Compared to the control group the treatment also caused reduction of the size of liver colonies (Table IIb). Removal of the primary tumor resulted in lower number of metastases in the control group, that was further reduced by treating the mice with Avemar.

Using the B16 melanoma tumor line (muscle-lung model), in the first experiment the mice were treated after tumor inoculation in the presence of primary tumor (Table III). Marked inhibition of metastasis formation in the lung was observed in the group treated with Avemar alone. The inhibition was 85% in the Avemar treated group, whereas vitamin C alone had no effect. Avemar + vitamin C treatment also caused a slight reduction in the primary tumor weight. Interestingly, no inhibition of metastasis formation was observed at an other model where the primary tumor was removed 10 days after tumor inoculation (Table IV).

Wilms' tumor is one of the most frequent childhood tumors. It's transplantable rat analog, developed by some of us (8), is able to produce lung metastases when transplanted under the renal capsule. Avemar + vitamin C showed a strong inhibitory effect on the primary tumor growth as well as on the formation of lung metastases (Table V). Primary tumor weight and the number of metastases decreased even more significantly when Avemar + vitamin C was administered in a diluted form. Interestingly, Avemar alone was ineffective in this model, whereas vitamin C reduced both primary tumor weight and the number of metastases.

## Discussion

In the present study we monitored the antitumoral and antimetastatic effect of Avemar and vitamin C on relevant rodent tumor models and on a human colon cancer xenograft kept in artificially immunodeprived mice.

The synchronous combined treatment with Avemar and vitamin C profoundly inhibited both the experimental and the spontaneous (true) metastasis formation in all the applied tumor models, while - with the exception of a single model (RWT-M) - it did not inhibit the growth of the parent or "primary" tumors.

The degree of the observed metastasis inhibition in some of the models was significant, while in others - although it was meaningful - statistically it did not prove to be significant.

Earlier, Pethig and coworkers (3) described that treatment with a mixture of 2,6-DMBQ and vitamin C inhibited the growth of Ehrlich ascites tumor in mice. Since Avemar used by us is characterized by standardized 2,6-DMBQ content, it was expected that the treatment with this preparation will produce similar results: *i.e.* an inhibition of the growth of the "primary" (parent) tumors. The growth of the "primary" tumors, however, in our tumor models - with the exception of one model (RWT-M) - was not inhibited by the applied treatments. We are convinced, therefore, that in the observed profound metastasis inhibiting effect of the combined Avemar + vitamin C treatment the eventual antiproliferative action of these compounds has no major part.

In addition, two further observations led us to believe that in the metastasis inhibition observed at synchronous combined treatment with Avemar and vitamin C, Avemar - standardized to 2,6-DMBQ content - must have played a major role. One of the observation, supporting this hypothesis is that the treatment with Avemar alone in our B16 melanoma muscle-lung model elicited an even more pronounced inhibition of metastasis formation than the synchronous combined treatment with Avemar + vitamin C. The other observation supporting our above conclusion was that in the very same experiment the treatment with vitamin C alone did not inhibit the formation of metastasis at all, and the growth of the parent or "primary" tumor was not inhibited either.

By enumerating these two observations we do not intend to overshadow or deny the fact that in most of the tumor models applied the strongest metastasis inhibiting effect was produced always by the synchronous combined treatment with Avemar and vitamin C. The beneficial contribution of vitamin C to the effect of this combined treatment therefore seems to be unquestionable. Furthermore, in some of the models [(RWT-M; (Table V)] the co-administration of vitamin C to Avemar proved indispensable for obtaining a meaningful inhibition of metastasis formation. Interestingly, in the case of the RWT-M tumor model, the response to treatment with Avemar and vitamin C respectively (when each of them was administrated alone) was rather different from that seen in another tumor model (B16 melanoma muscle-lung model).

Surprisingly, in the RTW-M model the vitamin C treatment alone proved able to elicit a profound inhibition of metastasis formation, while the treatment with Avemar alone was completely ineffective in this regard. However, when the two agents were applied together in the form of the regular synchronous combined treatment, a meaningful inhibition of metastasis formation was obtained, just as in other tumor models. It is also noteworthy that in this model - unlike to that seen at other models - both the combined treatment and

the treatment with vitamin C alone, besides inhibiting the metastasis formation, inhibited the growth of the parent or "primary" tumor, as well.

These observations suggest that the response to these compounds might be somewhat different depending on the type of the tumor model, particularly if each of these compounds is applied alone. However, when they were administered together in the form of synchronous combined treatment, a similar effect-a strong inhibition of metastasis formation - was consistently seen in all the applied tumor models, *i.e.* the effect was not dependent any more on the tumor model applied.

It seems, therefore, that when administered together (in the form of the synchronous combined treatment) the two compounds (Avemar and vitamin C) are mutually and favourably influence each other's effect. The mechanism of this reciprocally favourable influence on each other's effect is unknown at the present.

With regard the vitamin C [ascorbic acid (AA)], it is well known that despite its role in the prevention and suppression of carcinogenesis, which has been a subject of longstanding interest, some controversies still continue to exist (10,11). It was also found *in vitro* that high dosages elicited the opposite effect (12). The mode of administration (13), the diet (14) and its administration in a mixture with another compound (15) were all reported to influence the antitumoral effect of the AA.

The eventual antimetastatic effect of AA was rarely studied. Concerning the B16 melanoma, however, it was reported (14) that spontaneous metastasis was inhibited by ascorbate in mice fed restricted (low in tyrosine and phenylalanine) diet.

It is not the place here to discuss the mechanism of effect possibly involved in the tumor-suppressive effect of AA, particularly because it is still far from clear (10,11,15,16).

The mechanism of the antimetastatic action of the synchronous combined treatment with Avemar and vitamin C is not clarified yet either. There are, however, a few observations or facts which can provide some clues in this regard. One of these facts is that Avemar proved to be contribute largely to this metastasis inhibiting effect of the synchronous combined treatment since - at least in some of the tumor-models (B-16 melanoma muscle-lung metastasis model) - the treatment with Avemar alone exerted a similar or even more pronounced metastasis inhibiting effect than that was displayed following the synchronous combined treatment. The other observation relevant in this regard, was derived from another series of experiments carried out separately by us with Avemar and vitamin C (20). In *in vitro* experiments it was shown that the treatment with Avemar alone significantly enhanced the blastic transformation of mouse peripheral blood lymphocytes while, vitamin C - applied in very high doses - prevented this effect. On the other hand, in *in vivo* experiments it was found that both Avemar alone and the combined (Avemar + vitamin C) treatment acted for the restoration of immune function, the combined treatment being more active (20). This restorative effect on the immune functions was observed in thymectomized mice using the B10 LP to C57 B1 skin graft system and it manifested itself in a significantly faster rejection of the skin grafts in the treated than in the untreated thymectomized mice. In the light of these observations and, being aware of the fact that the immune system of a tumor-bearing organism is more or less always impaired, we assume that in the metastasis inhibiting effect of Avemar and in that of the combined treatment seen in several tumor models the observed restoring action of these treatments on the immune functions might play a major role.

Metastasis formation is a multistep process involving tumor/host-interaction in which immune processes are also involved. It is known that immunocompetence can influence the overall load of metastases and it is believed that even in situations where a tumor has been able to become established, the immune system could still play a role in preventing or delaying its further spread (17). There are observations indicating that if the immune system has adequately stimulated the activated lymphocytes, they may even obtain the capacity to cross the blood-brain barrier and could infiltrate and destroy even an intracerebral tumorous lesion (18). Just how strong the immune functions restoring action of the Avemar and the combined treatment might be, is well reflected in the fact that the treatment with these compounds proved to be able to decrease the formation of experimental metastasis, even in severly immune deprived (thymectomized, lethaly irradiated and bone marrow reconstituted) mice - regularly used by us for human tumor xenograft transplantation experiments (19) - bearing the HCR-25 human colorectal cancer xenograft.

This observation also confirms the finding concerning the immunorestorative effect seen in the skin grafting experiments (20) and indicates again that the applied treatments do not require an intact host immune system to work and could therefore find an application and be beneficial in the advanced stages of cancer when the host's immune functions are usually severly impaired.

During the last 50 years tremendous progress has been made and impressive results have been achieved in the field of cancer therapy. About 50% of the cancer patients, however, is still incurable by the established and widely used conventional treatment modalities (surgical-, radio- and chemotherapy). Furthermore, the overwhelming majority of cancer patients, in the hope of remission, cure and prolonged survival are constrained to face and endure all the severe side effects inseparably associated with these modalities, which have aptly been characterized by "heavy handedness" such as amputations radiation, and highly toxic doses of chemo-therapeuticals (21).

It is natural, therefore, that researchers questioned whether more biological ways could be found for the treatment of cancer. In recent years an intensive research has

resulted in a significant change in the general approach to the study of cancer treatment. Besides searching for better cytotoxic chemotherapeutic agents, much attention has been focused on more natural modalities (17,21,22). One of the most fruitful branches of such research has been the study of the diverse effects of cytokines. This branch of research has, on the one hand, provided improved insight into the regulation of immune response, and on the other hand, led to the discovery that these same molecules may have direct effects upon neoplastic cells giving rise to the concept of cancer biotherapy (22). Advances in recombinant DNA technology have facilitated the economic production of rare lymphokines and cytokines and made possible the clinical trials of factors such as lone agents, and chemotherapeutic protocols (20). Many other distinct strategies of cancer biotherapy have been formulated over the past two decades (17, 18, 21, 22, 23, 24). The results achieved by this diverse area of research collectively have led to the development of a fourth modality for the treatment of human cancers - cancer biotherapy-as an addition to surgery, radiation and chemotherapy modalities (24). One of the most characteristic features of the various treatment strategies of cancer biotherapy is that they try to stimulate rather than suppress antitumor immune mechanisms, while the physically and chemically based conventional therapies, such as surgery-, radio- and chemotherapy have a suppressive effect on the immune system (17). It is certainly not accidental that Szent-Györgyi (1) had long ago shown great concern about this problem and initiated research aiming to find a more biological approach to cancer treatment.

Thus, the possible immunostimulatory effect of wheat germ, fermented by yeast, was proposed by Szent-Györgyi (1). According to his theory, the two quinones, 2-methoxy-*p*-benzoquinone (2-MBQ) and 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ), present in wheat germ as glucosides and liberated by the yeast's glucosidase, are responsible for the immunostimulatory properties of wheat germ, because similar quinones are involved with ascorbic acid in a series of metabolic reactions of vital importance, in which molecular oxygen reduces to water (2).

The new fermented wheat germ extract, with standardized benzoquinone composition and named as Avemar, which is the subject of the present study, has been developed from Szent-Györgyi's concept and studies (1,2).

Our studies have demonstrated that this extract (Avemar) lived up to our expectations, has indeed shown powerful biological activity, and proved able to stimulate the immune functions in hosts with severly impaired immune system. Last but not least, it has shown a powerful metastasis inhibiting effect in several tumor models without eliciting toxic side effects. It is our hope that this preparation will find a place in the biotherapeutical treatment of human cancer. In this hope various studies are in progress to learn more about the mechanism of the effect of this agent.

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