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Age-dependent stimulatory effect of desipramine and fluoxetine pretreatment on metastasis formation by B16F10 melanoma in male C57BL/6 mice

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Abstract:
Although recent data may provide theoretical support for the preventive use of antidepressants in cancer patients, so far no study has demonstrated the clinical benefits of such strategies in the general population of cancer patients [39, 41]. Moreover, an association between antidepressant use and the risk of tumor promotion could neither be excluded nor established.
The aim of this study was to compare the effect of desipramine (a tricyclic antidepressant, TCA) and fluoxetine (a selective serotonin reuptake inhibitor, SSRI) on tumor growth of the mouse B16F10 transplanted melanoma in "young" 6–9 month old and "aged" 18–23 month old male C57BL/6 mice. Drugs were administered daily at a dose of 10 mg/kg, *ip*, for two weeks and tumor cells were inoculated 2 h after the last antidepressant administration. Control animals were treated with saline. Tumor growth was significantly slower in aged than in young saline-treated control animals. Pretreatment with desipramine dramatically promoted metastasis formation and increased mortality rate but inhibited primary tumor growth in young males. On the other hand, both antidepressants increased primary tumor growth in aged animals, whereas metastasis was only moderately promoted. To determine the effect of antidepressant drug pretreatment and tumor progress on some parameters of cell-mediated immunity (proliferative activity and cytokine production by splenocytes) and angiogenesis, vascular endothelial growth factor (VEGF) and metalloproteinase (MMP)-9 plasma levels were established. The prometastatic effect of desipramine in young animals was connected with an increase of VEGF and MMP-9 plasma levels.

Key words:
desipramine, fluoxetine, melanoma, metastasis

Introduction

Episodes of major depression affect more than 6% of the general population. Tricyclic antidepressant drugs and selective serotonin reuptake inhibitors (SSRI) like fluoxetine, ameliorate the symptoms of depression in the majority of patients. Antidepressants are prescribed not only to treat depression but also for anxiety disorders, attention deficit-hyperactivity disorder, obsessive-compulsive disorder, panic disorder, bulimia nervosa and chronic pain [39, 41]. One in 10 women and 4 in 100 men are currently taking antidepressant medications [47]. Antidepressants have potent analgesic properties when used alone or as potentiators of narcotics. Some of them enhance sleep, appetite and energy [24].

Some reports in the oncological literature have suggested that antidepressants may be linked with tumor induction and growth, raising concern about prescribing such medication on a regular basis. It remains to be determined whether antidepressants alone can promote or reverse primary tumor growth and the formation of metastases.

Tumor metastasis (word "metastasis" from the Greek for "change in position") is the leading cause of mortality in melanoma, as is the case for most malignant tumors. Metastasis occurs in certain organs more frequently than in others depending on the origin of the primary tumor. B16F10 melanoma tumor cells inoculated subcutaneously (*sc*) show preferential metastasis to the secondary lymphatic organs, liver and skin.

The invasion and metastasis of many tumor types, including melanoma, have been connected with the release of metalloproteinases (MMPs) by both tumor and stromal cells (for review see Hofmann et al. [17]). MMPs are Zn^{2+}-endopeptidases that degrade basement membrane and extracellular matrix (ECM) components, which enables them to facilitate the migration, invasion and metastasis formation of tumor cells [21, 30]. Two particular MMPs, MMP-2 and MMP-9, were shown to be involved in melanoma progression and metastasis, and their presence correlates with poor prognosis [16, 26, 45].

In the present study, two well-known antidepressant drugs, fluoxetine and desipramine, were used. Fluoxetine hydrochloride (Prozac) belongs to the class of SSRI and mediates its antidepressant action through inhibiting serotonin reuptake transporters in neurons. Fluoxetine is among the most commonly used antidepressants due to its efficacy, safety and tolerability and is the first choice for the treatment of depression, bulimia nervosa and obsessive-compulsive disorder. Desipramine is a well-known tricyclic antidepressant (TCA) that has been used for the treatment of clinical depression for almost five decades. This drug inhibits the reuptake of noradrenaline much stronger than that of serotonin.

Neoplastic disease is largely a disease of old age and because of a constantly rising percentage of aged individuals in the population, a study of the effect of antidepressant drugs on cancer progression in aging subjects seems to be particularly important. Although aging constitutes the major cause for the development of most neoplastic diseases, tumors in aged people are characterized by a lower degree of aggressiveness than in young patients. It seems, therefore, that the age of the host may influence both tumorigenesis (enhancing effect) and tumor progression (inhibitory influence) [12, 13]. Since most of experimental cancer research has been performed on young animals, it may not faithfully reflect human disease. The studies performed by Doin et al. [12] showed an important role of experimental splenectomy in the modulation of cancer progress, although spleen-produced cancer modulators were not studied.

In Europe, the incidence of melanoma malignum is about 1% in men and 1.8% in women. The frequency of its occurrence has increased much faster than other cancers [10]. The aim of this work was to evaluate the effect of chronic antidepressant treatment on neoplastic pathology in young and aged C57BL/6 males. For that purpose, we evaluated the effect of a two-week treatment with desipramine or fluoxetine before the inoculation of tumor cells on B16F10 melanoma growth in young and aged C57BL/6 male mice. In addition, since an involvement of the spleen in the modulation of tumor progress has been postulated, we also analyzed the effect of antidepressant pretreatment on cell mediated immunity in these animals. Specifically, we examined the macrophage and B-cell or T-cell proliferative responses to the selective mitogens, lipopolysaccharide (LPS) or concanavalin (Con A), respectively, and the splenocytic production of the following anti- and pro-tumoral cytokines: interleukin (IL)-4, IL-6, IL-12p40, interferon (IFN)-γ, tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF). The serum levels of VEGF and MMPs were also established.

Materials and Methods

Cell culture

The B16F10 mouse melanoma cell line was obtained from ATCC (Manassas, VA, USA). Cells were cultured in the Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland. B16F10 cells grew in monolayers, adherent to the bottom of culture flasks filled with RPMI 1640 supplemented with 10% of fetal calf serum, penicillin and streptomycin. The cultures were maintained at 37°C in 5% CO_2. Cells were harvested in the exponential growth phase at 80% confluence, briefly exposed to 0.05% trypsin solution and mixed by pipetting to obtain a homogeneous cell suspension. The cell suspension was centrifuged, washed once with phosphate buffered saline (PBS) and resuspended in PBS at a concentration of 2×10^6 cells per ml. Cell viability was determined by trypan blue exclusion. Only single-cell suspensions with 95% viability were used for inoculation.

Animals

The experiment was performed on 6–8-month-old and 19–23-month-old male C57BL/6 mice, hereafter referred to as "young" or "aged", respectively, that were bred in the Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland. Animals were housed up to five per cage, at 22°C and 40% humidity under a 12 h light-dark cycle, with free access to water and standard food.

Six- to nine-month-old animals were called "young" by analogy with human population, since people around 30 years old are still considered "young".

Experiment

The experiment was performed on 30 "young" and 30 "aged" animals. "Young" and "aged" animals were divided into three groups of ten animals per group, and the mice received daily intraperitoneal injections of desipramine (10 mg/kg, Research Biochemicals International, USA), fluoxetine (Lilly Laboratories, USA) or saline (Polpharma S.A., Poland) for 14 days. Three hours after the last injection of saline or an antidepressant drug, mice were inoculated *sc* with 3×10^5 B16F10 cells suspended in 0.15 ml of PBS into the left flank region. Tumor growth was monitored for 23 days in "young" and 35 days in "aged" males. Tumor growth was assessed every two or three days by measuring the average perpendicular tumor diameter using the formula $(\text{width})^2 \times \text{length} \times 0.52$.

At each time point, mean tumor volume was compared between the treatment groups and the control group using a two-tailed Student's *t*-test. The level of statistical significance was set at $p < 0.05$. The survival rate was assessed by counting the surviving mice every day.

Young animals and aged animals were killed by cervical dislocation 23 and 35 days after tumor cells inoculation, respectively. The spleen, thymus, lungs, heart, liver, kidneys, adrenals, testes, gastrointestinal tract, peritoneal cave, skeletal muscles and skin were removed from each animal, and the number of melanotic nodules was counted under a dissecting microscope. On the basis of this inspection, each organ was scored as either containing a metastatic nodule or being free of microscopic tumors. The percentage of organs positive for metastasis was calculated for the total number of surviving mice in each group (metastasis incidence). The metastasis development index was determined for each organ as follows: the number of recorded metastases per organ was multiplied by the average surface percentage occupied by metastatic tissue per organ. The metastatic development index was based on a metastatic diameter measurement using an eye reticle calibrated with a stage micrometer. To avoid subjective influences on the microscopic study of metastases, the recordings were made in a blinded fashion. Paired and multiple organs were considered as single organ sites, so that the calculated incidence values and the metastasis development indices included both or all of the organs, respectively, within an animal.

Proliferative response of splenocytes to mitogen stimulation *in vitro*

The measurement of the proliferative response of spleen cells was previously described by Kubera et al. [23]. Briefly, 4×10^6 splenocytes per ml were stimulated with 2.5 μg/ml of Con A or 5 μg/ml of LPS. The cells were incubated in 96-well plates at 37°C in a final volume of 0.2 ml for 72 h. Cell proliferation was determined by adding 10 μl (0.5 μCi) of ^{3}H-thymidine per well (ICN Pharmaceuticals, USA; SpA 6.7 Ci/mmol)

16 h before the end of the incubation. The cultures were harvested with an automatic cell harvester (Scatron, Norway), and ^{3}H-thymidine incorporation was estimated with a liquid scintillation counter (Beckman LS 6500).

Determination of cytokines

Mouse splenocytes were tested for their ability to produce IL-4, IL-6, IL-10, IL-12p40, TNF-α, IFN-γ and VEGF. Splenocyte suspensions were seeded at a concentration of 4×10^6 cells/ml in 24-well Corning tissue culture plates, and were then stimulated with a Con A solution (2.5 μg/ml) or remained unstimulated. Cell-free supernatants were collected 48 h later and stored at –20°C. VEGF levels were also estimated in plasma. All of the enzyme-linked immunosorbent assays (ELISA) were based on monoclonal-monoclonal antibody pairs and were performed using DuoSet ELISA Development Kits (R&D Systems, Inc, Minneapolis, USA). The viability of cells was checked with trypan blue.

Gelatin zymography

Zymography was performed as described previously [22]. Briefly, plasma samples were first normalized for protein concentration. Then, the samples were electrophoresed in 10% SDS-polyacrylamide gels containing 1% porcine gelatin, (Sigma-Aldrich) under non-reducing conditions. The gels were washed twice in 2.5% Triton X-100 (15 min each) and developed overnight at 37°C in an incubation buffer (50 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$, 0.02% NaN_3, 1 μM $ZnCl_2$). The gels were fixed and stained with 0.5% Coomassie brilliant blue (Sigma-Aldrich) in acetic acid/isopropanol/distilled water (1:3:6) and then washed in equilibrating solution with 40% methanol, 10% acetic acid and 3% glycerol (all from Sigma-Aldrich). Protein bands with gelatinolytic activity appeared as clear lysis zones within the blue background of the gelatin gel. The degradation of gelatin was visualized under long wave UV light. A pre-stained broad range molecular weight standard (Bio-Rad) was used for gelatinase identification. Densitometric analysis of protein bands was performed with the UVISoft-UVIMap program (UVItec, Ltd.). The data from the densitometric analyses are presented in graphs in terms of relative intensity of gelatin lysis zones.

Statistics

The results were statistically assessed by analysis of variance (ANOVA). Multiple *post-hoc* differences were checked by means of Fisher's least significant difference test ($p = 0.05$).

Results

Tumor development in young and aged animals pretreated with antidepressants

To assess the effect of antidepressant drug pretreatment on the growth rate of B16F10 melanoma, solid tumor volumes were measured every two or three days over 23 days in young animals and over 35 days in old animals (Fig. 1A, B). The wet weights of these tumors were estimated after sacrifice of the animals on days 23 and 35 in young and old animals, respectively (Fig. 1C, D). Experiments were first performed on old animals, in which tumor growth did not induce death of any of the control saline pretreated animals. In contrast, 10% and 20% of the desipramine and fluoxetine pretreated animals died during the 35 days after tumor cell inoculation, respectively (Fig. 2B). Inoculation of tumor cells into young animals induced much more rapid primary tumor growth in vehicle-treated control animals (Fig. 1A, C) threatening the breakage of skin continuity in some animals on 23rd observation day. Moreover, 60% of desipramine-pretreated young animals died during the first 20 days after *sc* tumor cell inoculation (Fig. 2A), which is why we decided to sacrifice all surviving young animals on day 23 after tumor cell inoculation.

The primary solid tumor growth in all six groups increased progressively with time ($r > 0.63$; $p < 0.001$), reaching its maximum mean size on day 23 in young animals and on day 35 in aged animals (Fig. 1A, B). Primary solid tumors grew faster in young compared with aged saline-treated control animals. Tumor volume and weight were almost three times lower in old control males than in young control males despite the fact that the tumors grew 12 days longer in old animals than in the young (Fig. 1A–D).

Desipramine and fluoxetine pretreatment inhibited the growth of primary solid B16F10 melanoma tumors in young animals by 66% and 43%, respectively, (although the effect was statistically significant only

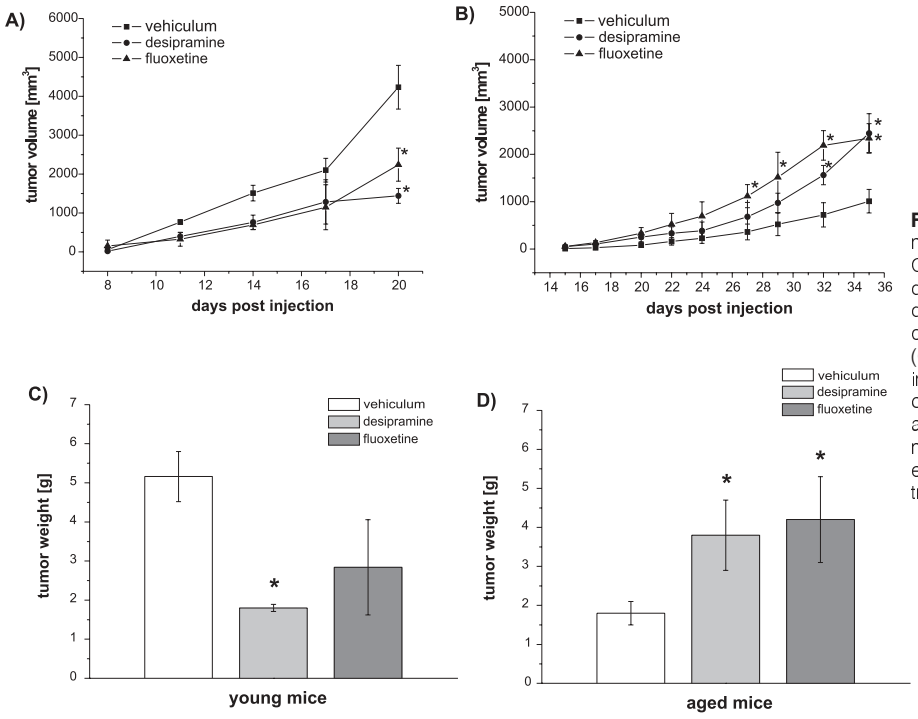

Fig. 1. Growth curve for B16F10 melanoma in young (**A**) and aged (**B**) C57BL/6 male mice pretreated with desipramine or fluoxetine. The effect of desipramine or fluoxetine pretreatment on primary tumor wet weight in young (**C**) and aged (**D**) C57BL/6 male mice inoculated with B16F10 melanoma cells estimated on day 23 for young animals and on day 35 for aged animals. Ten animals were included in each group. * $p < 0.05$ *vs.* vehicle-treated respective control group

for desipramine) and induced an opposite effect in aged animals in comparison to vehicle-treated age-matched controls. In desipramine-pretreated animals, primary solid tumors were two times bigger in aged than in young animals, and a similar effect was observed for fluoxetine-treated animals, although comparing the weights and volumes of tumors can be only an estimate because the experiment was considerably shorter for young than for old animals (Fig. 1A–D).

We observed significant differences between young and old animals in the development of metastases. Metastases developed in 100% of the desipramine-pretreated young mice, in more than 70% of fluoxetine-pretreated young mice and 12.5% of saline-treated young mice. In contrast, in old animals metastases were observed only in about 20% of desipramine- or fluoxetine-pretreated animals and in none of the control animals, despite the fact that the animals were examined for metastases 12 days later than the young animals so the time of tumor development in these animals was 35% longer than in young animals. The metastases were found in six of 11 different organs. Data regarding the organ distribution and growth of metastases after the injection of murine B16F10 melanoma cells into antidepressant-pretreated animals are presented in Table 1 for young animals and in Table 2 for old animals. Fluoxetine pretreatment induced metastases mainly in the skin and spleen of young animals, whereas desipramine pretreatment induced metastases mainly in the gastrointestinal tract and peritoneal cavity.

Tab. 1. Organ distribution and growth of metastases after injection of murine B16F10 melanoma cells into antidepressant drug-pretreated young male C57BL/6 mice

	Vehiculum		Desipramine		Fluoxetine	
	MI*	AMDI**	MI*	AMDI**	MI*	AMDI**
Spleen	25	0.8	100	80	42.9	7
Liver	0	0	50	16	28.6	5
Skeletal muscle	0	0	50	10	0	0
Skin	0	0	50	10	71.4	35
Gastrointestinal tract	0	0	50	15	28.6	7
Peritoneal cavity	0	0	50	70	28.6	7

* MI – metastasis incidence (each organ was scored either as containing a metastatic nodule or as free of metastasis) is presented as the percentage of animals with metastases sacrificed on day 35 (number of animals with metastases divided by the total number of mice per group and multiplied by 100). ** AMDI – average metastasis development index, the number of recorded metastases per organ was multiplied by the percentage of surface occupied by metastasis and divided by the number of animals with metastases in this particular organ [46]

Tab. 2. Organ distribution and growth of metastases after injection of murine B16F10 melanoma cells to antidepressant drug-pretreated aged male C57BL/6 mice

	Vehiculum		Desipramine		Fluoxetine	
	MI*	AMDI**	MI*	AMDI**	MI*	AMDI**
Spleen	0	0	11	3	25	9
Liver	0	0	0	0	0	0
Skeletal muscle	0	0	0	0	0	0
Skin	0	0	11	3	12.5	2
Gastrointestinal tract	0	0	22	3	25	9
Peritoneal cavity	0	0	22	3	25	9

* MI – metastasis incidence (each organ was scored either as containing a metastatic nodule or as free of metastasis) is presented as the percentage of animals with metastases sacrificed on day 35 (number of animals with metastases divided by the total number of mice per group and multiplied by 100). ** AMDI – average metastasis development index, the number of recorded metastases per organ was multiplied by the percentage of surface occupied by metastasis and divided by the number of animals with metastases in this particular organ [46]

The survival rate (Fig. 2A) showed that desipramine pretreatment of young animals significantly shortened their lifespan after tumor cell inoculation, which supports the observation concerning the prometastatic effect of desipramine in young animals.

Relative spleen and thymus weights

The mean relative spleen weights were significantly lower in fluoxetine- but not in desipramine-pretreated young mice (Fig. 3A), whereas in aged mice, both fluoxetine and desipramine pretreatment decreased relative spleen weight (Fig. 3B). Relative thymus weight was not affected by repeated pretreatment with desipramine and fluoxetine in either young or aged animals in comparison to age-matched controls. On the other hand, the mean thymus weights were more than two times lower in aged than in young animals (Fig. 3C, D).

Proliferative activity of splenocytes

There was no difference between desipramine-, fluoxetine- and saline-treated mice or between young and aged animals in terms of the proliferative activity of splenocytes in response to Con A stimulation. The proliferative activity of splenocytes in response to LPS was significantly lower in young and aged antidepressant-pretreated mice compared to control mice (respectively, $F(1,16) = 8.3$, $p < 0.05$; $F(1,33) = 6.66$, $p < 0.05$). *Post-hoc* comparison revealed that desipramine pretreatment of young mice and desipramine and fluoxetine pretreatment of aged mice decreased the proliferative response to LPS in comparison to the appropriate controls by 23%, 38% and 22%, respectively. There was also no difference in the proliferative activity of non-stimulated splenocytes.

Lymphokine production by splenic lymphocytes

There were no differences between antidepressant- and saline-pretreated aged mice in Con A-induced and/or unstimulated IL-4, IL-6, IL-10, IFN-γ, IL-12p40 and TNF-α production by splenocytes *ex vivo*. The production of IL-6, IL-10 and IL12p40 was decreased in Con A-stimulated splenocytes obtained from desipramine-pretreated young animals by 34%, 52% and 28%, respectively, in comparison to saline-treated young control mice (Fig. 4A, B, C). Fluoxetine pretreatment decreased IL-12p40 production by

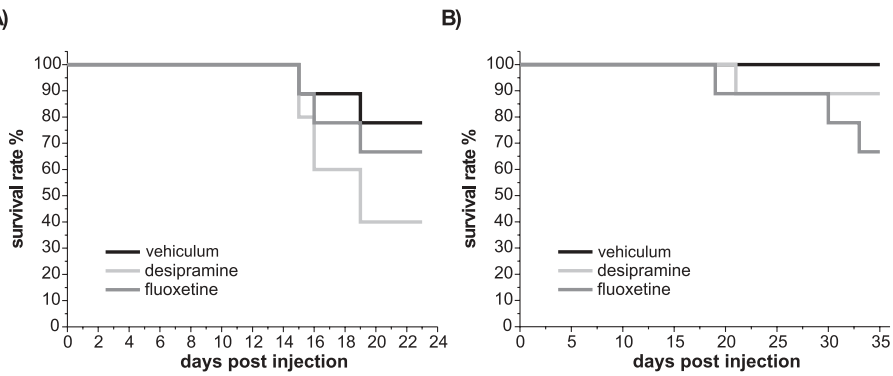

Fig. 2. Survival curves for young (**A**) and aged (**B**) C57BL/6 mice pretreated with desipramine or fluoxetine at a dose of 10 mg/kg, daily for two weeks before inoculation of B16F10 melanoma cells. The young mice were monitored for 23 days, whereas aged mice were monitored for 35 days. Ten animals were included in each group

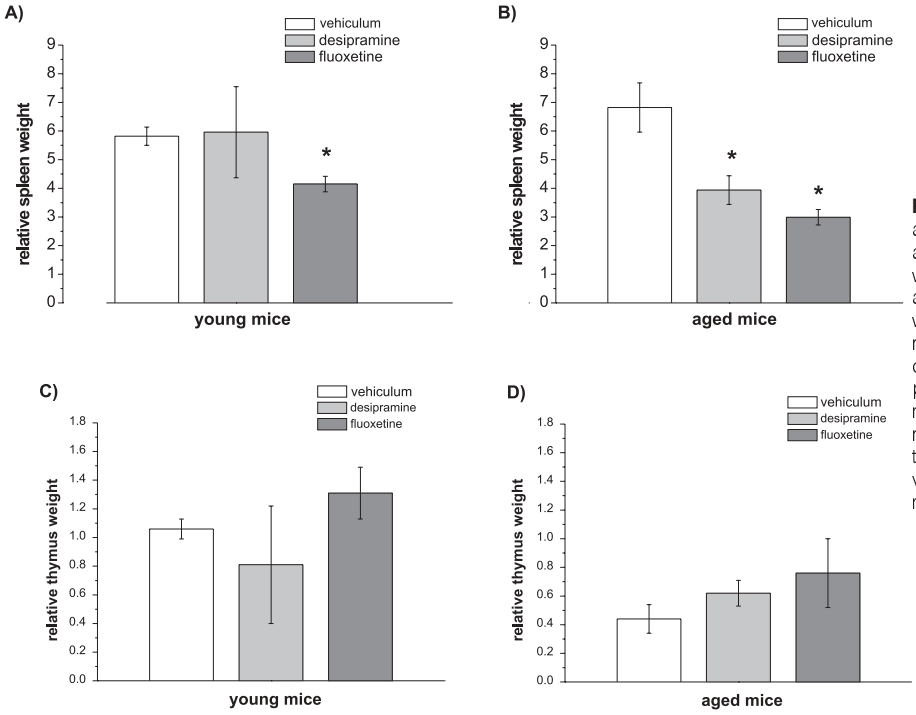

Fig. 3. Relative spleen weight (**A**, **B**) and thymus weight (**C**, **D**) in young and aged C57BL/6 male mice pretreated with desipramine or fluoxetine at a dose of 10 mg/kg, daily for two weeks before inoculation of B16F10 melanoma cells. Ten animals were included in each group. The data are presented as the mean ± SD. The significance of differences between the means was calculated by Duncan's test following a two-way analysis of variance. * $p < 0.05$ *vs.* vehicle-treated respective control group

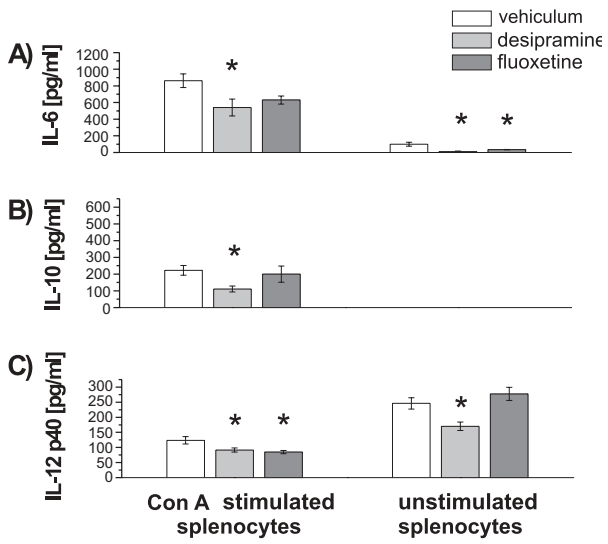

Fig. 4. Production of IL-6 (**A**), IL-10 (**B**) and IL-12p40 (**C**), by "unstimulated" or concanavalin A (Con A)-"stimulated" splenocytes, obtained from young C57BL/6 male mice 23 days after inoculation of B16F10 melanoma cells. Young mice were pretreated with desipramine or fluoxetine at a dose of 10 mg/kg, daily for two weeks before inoculation of B16F10 melanoma cells. Ten animals were included in each group. The data are presented as the mean ± SD. The significance of differences between the means was calculated by Duncan's test following a two-way analysis of variance. * $p < 0.05$ *vs.* saline-treated control young animals

34% in Con A-stimulated splenocytes. Moreover, *post-hoc* comparisons revealed a higher IFN- γ/IL-10 ratio in young desipramine-treated animals.

Desipramine pretreatment decreased production of IL-6 by 51% and IL12p40 by 29%, whereas fluoxetine pretreatment decreased IL-6 production by 49% in unstimulated splenocytes obtained from young animals (Fig. 4A, C).

VEGF plasma levels and production by splenic lymphocytes

Figure 5 shows that desipramine and fluoxetine pretreatment significantly increased plasma levels of VEGF in young animals by 57% and by 37%, respectively. In aged saline-treated animals, the VEGF plasma level was two times lower than that in saline-treated young animals, and antidepressant pretreatment did not change this level in a significant manner (data not shown).

There were no differences between saline, desipramine and fluoxetine in terms of Con A-stimulated and unstimulated VEGF production by splenocytes obtained from young and aged males.

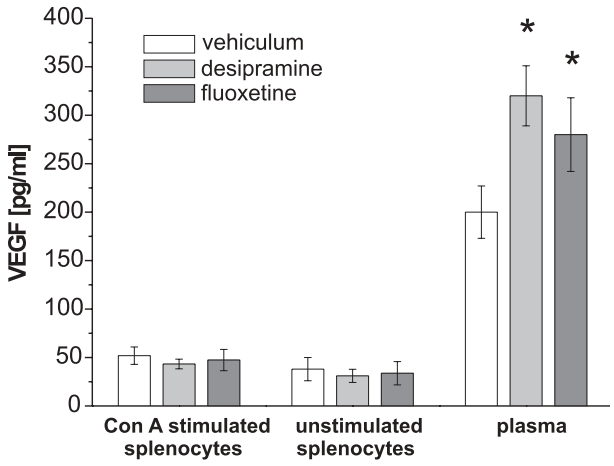

Fig. 5. VEGF levels in plasma obtained from blood and supernatants from concanavalin A (Con A)-stimulated and unstimulated splenocytes obtained from young mice. For details see Materials and Methods

MMP-9 and MMP-2 activity in plasma

In young C57BL/6 mice, both antidepressants significantly increased the plasma levels of the zymogen and active forms of MMP-9 induced by melanoma inoculation (Fig. 6A). Desipramine and fluoxetine enhanced the release of pro-MMP-9 by 17% and 40% and increased active MMP-9 levels by 65% and 80%, respectively. The release of pro-MMP-2 was enhanced only upon fluoxetine pretreatment, whereas higher levels of active MMP-2 were detected after desipramine administration in young mice (in both cases by approx. 25%) (Fig. 6B). No significant differences in the melanoma-induced plasma MMP-9 levels were detected between control and antidepressant-treated aged mice (Fig. 6C). Neither amount of pro-MMP-2 was altered in those animals by the drug pretreatment,

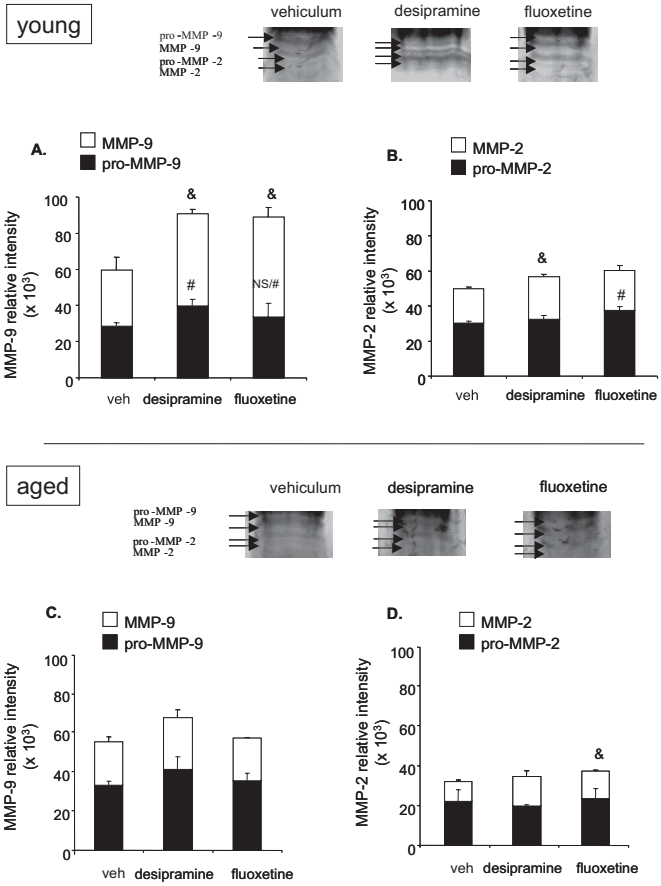

Fig. 6. Gelatinase activity in plasma samples collected from young (**A** and **B**) and aged (**C** and **D**) C57Bl/6 male mice inoculated with B16F10 melanoma cells. Some animals were additionally pretreated with either desipramine or fluoxetine. The levels of (**A** and **C**) pro-MMP-9 (zymogen) and active MMP-9 and (**B** and **D**) pro-MMP-2 and active MMP-2 are shown as the relative intensity of gelatin lysis zones in gels according to densitometric analyses. # (zymogens) and & (active forms) at $p < 0.05$ *vs.* vehicle-treated control young animals. NS (not significant)/# at $p = 0.56$. Representative gels for each treatment are shown at the top of the figure

while the levels of MMP-2 were increased by fluoxetine (by approx. 35%) but not by desipramine administration (Fig. 6D). Thus, when comparing both groups of mice, we observed no significant effects of antidepressants on melanoma-induced MMP-9 synthesis in aged mice, which is in contrast to young mice that produced significantly more MMP-9 after desipramine and fluoxetine pretreatment (Fig. 6A *vs*. 6C). Moreover, while the plasma MMP-2 levels were similarly increased by the drugs, in general the young mice produced considerably more MMP-2 than the aged animals (Fig. 6B *vs.* 6D).

Discussion

The main findings of the present paper are as follows:

1) There was a significant increase in metastasis formation in young and aged animals chronically pretreated with desipramine and to lesser extent with fluoxetine before induction of cancer process in comparison to vehicle-treated control mice; 2) the mortality rate was markedly increased in desipramine-pretreated young but not aged animals with B16F10 melanoma; 3) solid primary tumor growth was significantly decreased in aged saline-pretreated controls in comparison to young saline-pretreated controls; 4) solid primary tumor growth was significantly increased in desipramine- and fluoxetine-pretreated aged animals in comparison to saline-treated control animals; 5) plasma levels of VEGF and the active form of MMP-9 (by 65–80%) were significantly elevated in desipramine- or fluoxetine-pretreated young animals.

Metastasis results from several sequential steps including: a) release of tumor cells from the primary tumor; b) invasion into vascular or lymphatic vessels; c) adherence of the metastatic cells to the endothelial cells in affected organs; d) extravasation of tumor cells out of the blood vessels; e) survival in the new tissue environment; f) ultimately, the proliferation of tumor cells following variable periods of tumor latency in order to form deleterious secondary tumors. Metastasis is the culmination of discrete sequential steps – each of variable efficiency – leading to formation of secondary tumors [27].

It is not unlikely that antidepressant drug pretreatment increases the number of metastases by increasing implantation efficiency of melanoma cells and inhibiting the early host immune response against tumor cells [34]. In experimental models of metastasis and in drug-pretreated recipients of tumor cells, the first few hours after the injection of tumor cells seem to be critical for metastasis formation [5].

In the present study, desipramine specifically increased the number of metastases in the gastrointestinal tract. Although no other data on the influence of TCAs on metastasis of the gastrointestinal tract have been reported, it has been suggested that long-term use of tricyclic antidepressants might be associated with the initiation of tumors in this system. TCAs have been shown to stimulate of cell proliferation in the rat intestinal crypt epithelium and promote experimental carcinogenesis in the rat colon [18, 44].

In the present study, fluoxetine, a serotonin reuptake inhibitor, specifically increased the number of metastases in the skin. The immune and vascular systems in the skin are traditional targets for bioregulation by serotonin (5-HT) [31]. The mammalian skin is both a site for the production of and a target for bioregulation by 5-HT [29, 35, 36, 38]. The major source of 5-HT in the skin is platelets, which release biogenic amines upon aggregation. In addition, rodent mast cells produce 5-HT as well as proteins that transport 5-HT. Moreover, epidermal and dermal skin cells express the enzymes required for the transformation of tryptophan to 5-HT, and certain skin cells, such as melanocytes, have been demonstrated to produce 5-HT. The rate of the synthesis of 5-HT from tryptophan can be enhanced by brain-derived neuronal growth factor, cytokines and steroids. We may speculate that antidepressants indirectly, by stimulating brain derived neurotrophic factor (BDNF) production not only in the central nervous system but also in blood cells, may increase skin serotonin synthesis. Skin cells express functionally active, membrane-bound receptors for 5-HT and proteins that transport 5-HT. For example, the presence of 5-HT_{2B} receptors was confirmed in the skin of C57BL/6 mice and in mouse Cloudman S91melanoma cells [28, 37]. We may speculate that fluoxetine as a SSRI increases the level of 5-HT in skin blood vessels and in the extracellular matrix of the skin by blocking the 5-HT by platelets, mast cells or skin cells. On the other hand, 5-HT may enhance the permeability of skin vessels to melanoma cells and may stimulate tumor cell proliferation.

It is not unlikely that the mechanism for the antidepressant-induced facilitation of metastasis in-

volves the chemokine system. While leukocytes have long been known to express specific sets of chemokine receptors that govern localization and tissue specific migration, cancer cells have only recently been shown to express these proteins. It has been established that B16 melanoma cells may express such chemokine receptors as CCR7, CXCR4 or CCR10. On the other hand, lymphatic endothelial cells and secondary lymphoid organs are both rich sources of the chemokine CCL21 (a ligand for CCR7), lungs are rich source of CXCL12 (a ligand for CXCR4), whereas keratinocytes in the skin constitutively produce CCL27 (a ligand for CCR10) [28]. We may speculate that drug pretreatment increases CCL21 expression in lymphoid organs and CCL27 in the skin; however, this assumption requires further study.

The present paper confirms the observation in humans and experimental animals that tumor growth is slower in aging organisms than in young ones. With regard to tumor progression, several factors may be responsible for the slower growth rate and reduced aggressiveness of neoplasms in the old, including: a decreased proliferative capacity of senescent cells, decreased activity of endocrine factors and growth factors with age and reduced microvessel counts. These factors lead to diminished tumor cell proliferation and increased tumor cell apoptosis [14], a decrease in DNA methylation and repair and a decrease in immune system-dependent tumor growth enhancement. In the present study, young and aged animals were injected with the same tumor cells, so the effects of the age of tumor cells should be excluded. Consequently, the study should focus on the age-dependent changes in immunity and plasma growth factor levels, particularly those engaged in carcinogenesis.

In the present paper, antidepressant drug pretreatment inhibited primary tumor growth in young animals but increased primary tumor growth in aged animals in comparison to age-matched controls. Most other studies have focused on the effect of antidepressants given after tumor cell inoculation in young animals. Only one study published recently by Frick et al. [15] showed that chronic fluoxetine pretreatment reduced solid tumor growth in young animals that were subcutaneously injected with aggressive T-cell lymphoma. However, contrary to the present study, fluoxetine increased the survival of the mice [15]. Other studies performed in young animals have shown that antidepressant treatment after the injection of tumor cells either inhibited jejunal and colonic tumors in rats and xenografts of human colorectal and prostate cancers in fluoxetine-treated athymic nude mice [1, 44] or enhanced melanoma growth in mice [7]. Studies involving the prolonged, two-year administration of fluoxetine in the diets of mice and rats also did not provide an unequivocal answer about the pro- or antitumorigenic activity of antidepressants [4, 8, 41].

The promotion of carcinogenesis in animals receiving the drugs for two weeks prior to cancer cell inoculation, characterized by aggravated metastasis formation, the drastically decreased survival rate of young males and increased solid tumor size in aged animals, can be attributed to an immunomodulatory effect of the drugs and age-related immunity changes.

In the present study, the T cell-mediated immune response, as measured by splenocyte proliferation and Con A-stimulated cytokine synthetic capacity, did not differ between young and aged animals despite a greater than 50% drop in the thymus weight in the latter. The decrease in thymus weight that occurs with age usually results in a reduction of T cell-mediated reactivity, which, on the one hand, should decrease the ability to eliminate cancer cells, and on the other, should lower the production of pro-carcinogenic factors, like cytokines, and proinflammatory and/or protective antibodies. In the present study, besides a marked reduction in thymus weight, we observed no differences in the immune parameters between young and aged animals, which indicated that peripheral immunity was still efficient in aged animals (possibly resulting from a constant stimulation with tumor antigens). We also found that there was no effect of these factors on tumor growth or the effect was opposite to that expected. The lack of a correlation between immune system efficacy and cancer cell elimination has also been observed in humans where a high T cell-mediated antitumor immunity was preserved in the periphery even during metastatic disease. The finding points toward the model of concurrent evolution of immunity and tumor escape and may indicate a high level of activation of the mechanisms responsible for cancer escape from immune system control [3].

We can speculate that the decreased tumor growth in aged control animals results from a lowered release of tumor growth-promoting agents from the amorphic thymus, such as thymosin [9].

The LPS-induced proliferative response of B cells was markedly impaired after antidepressant drug administration in both young and aged animals, which

could result, at least in young animals, from the reduced production of B cell-stimulating cytokines, like IL-6 or IL-10.

Treatment of young animals with the drugs did not influence spleen weight, while in aged animals tumor growth stimulation by the drugs was connected with a decrease in relative spleen weight. This finding may suggest the reduced secretion of factors protecting the organism from tumor growth.

Donin et al. [12] showed a protective role of the spleen against B16 melanoma at all ages, and specifically a protective role of this organ for highly metastatic melanoma in aged C57BL/6 mice, although the authors did not identify the protective factors secreted by this organ.

Regarding the possible mechanism of the antidepressant effects on immune system, it should be mentioned that desipramine and fluoxetine induce adaptive changes in central monoaminergic neurotransmission, which itself might modulate immune reactivity. Lymphocytes and macrophages are innervated by the sympathetic and parasympathetic nervous system, and this innervation modulates immune reactivity. Immune cells are known to have receptors for neurotransmitters and antidepressants. Therefore, the drugs may modulate the activity of immune cells by acting on these receptors. The effect of antidepressants on the hypothalamic-pituitary adrenal (HPA) axis is also noteworthy, since they promote the negative feedback of cortisone and incapacitate the immunosuppressive effect of the HPA axis. Antidepressants may also have a direct effect on immune cells. Several *in vitro* studies have shown that antidepressants reduce the stimulated production of pro-inflammatory cytokines (IFN-γ and TNF-α) and increase the production of the anti-inflammatory cytokine IL-10. It has been established that antidepressant drugs are effective in decreasing natural killer (NK) cell activity. Moreover, fluoxetine has been associated with several reported cases of the reactivation of herpes simplex or zoster infection suggesting that it can impair the function of the NK cells *in vivo* [32]. We may speculate that in the present study, chronic administration of antidepressant drugs induced a significant impairment of NK cell activity, which are then not able to eliminate melanoma cells. For this reason, metastasis was increased in young and aged mice and tumor weight was elevated in aged mice compared to aged control mice.

It has been shown that NK cells are mainly important in the elimination of tumor cells during the initial phase after *iv* injection (first 24 h) [33, 34].

We cannot exclude the possibility that antidepressant drugs, acting *via* a decrease in NK activity, may increase the retention of tumor cells in several organs during the first 24 h after injection. The β-adrenergic agonists epinephrine and norepinephrine are the best characterized factors known to modulate the activity of NK cells *in vitro* and *in vivo*. NK cells express β2-adrenergic receptors and the stimulation of these receptors results in inhibition of NK cell cytotoxicity *in vitro* and *in vivo* [33, 48]. Moreover, it was shown that chronic antidepressant administration induced a Th2 biased immune response and there was evidence that a decrease of Th1/Th2 cytokine balance can reduce NK cell activity [43].

In the present paper, Con A-stimulated splenocytic production of IL-6, IL-10 and IL-12p40 was decreased and plasma levels of VEGF were increased 23 days after tumor cell inoculation in antidepressant drug-pretreated young but not aged animals.

Interleukin 6 is a bifunctional cytokine that can differentially affect the growth properties of melanoma cells. Growth of early stage melanomas is inhibited by endogenous IL-6. Transfection of IL-6 into B16 melanoma cells causes growth retardation by arresting the cell cycle at the G1/G0 phase [42]. On the other hand, advanced stage melanomas are not only resistant to the antiproliferative effect of IL-6, but endogenous IL-6 may behave as a growth stimulator for the cells by acting on a specific receptor [42]. We may speculate that the decreased growth of primary solid tumors in young antidepressant-treated animals is the result of the lack of the stimulatory effect of IL-6 in advanced-stage melanoma cells present in this tumor. Although we do not know the level of IL-6 at the time of tumor cell inoculation in the animals treated with the drugs for two weeks, if levels had been decreased at that time we can assume that the inhibitory effect of IL-6 on early stages of tumor growth was lacking. Hence, we can speculate that the observed increase in the number of metastases after antidepressant drug treatment with concomitant suppression of primary tumor growth in young animals may result from the lowered IL-6 levels.

In our studies, we observed a decrease in IL-12p40 production by unstimulated splenocytes obtained from desipramine-pretreated young animals and a decrease in IL-12p40 production by Con A-stimulated sple-

nocytes obtained from desipramine- and fluoxetine-pretreated young animals. IL-12p40 is known to be a component of the bioactive cytokines IL-12 and IL-23, but it is not widely recognized as having intrinsic functional activity. When it is considered, the cytokine is an antagonist because the recombinant murine IL-12p40 homodimer (IL-12p80) competitively binds to the common receptor component IL-12Rb1 and prevents IL-12-mediated inflammation from occurring. In contrast to this negative regulatory role, it has been suggested that IL-12p40 might have a pivotal and independent early agonistic role in initiating the immune response [11]. We may speculate that the inhibitory effect of desipramine and fluoxetine on IL-12p40 production leads to the enhancement of the pro-inflammatory and prometastatic effects of IL-12Rb1-bound cytokines in comparison to the saline-treated animals.

In the present paper, we documented the increase in the IFN-γ/IL-10 ratio in desipramine-treated young animals. The IFN-γ/IL-10 ratio in culture supernatants is of critical importance for determining their pro- or anti-inflammatory capacity, i.e., either activation (IFN- γ) or inhibition (IL-10) of monocytic and lymphocytic function [19, 20]. This observation suggests a high activation of the pro-inflammatory response in desipramine-treated young animals.

The basis of the relationship between the systemic inflammatory response and metastasis is not clear, although some studies have shown significantly enhanced serum levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α in metastatic patients [2, 25]. In the present study, the *ex vivo* ability of splenocytes to produce cytokines was only established in animals that survived for at least three weeks from tumor cell inoculation.

We can assume that these animals were characterized by a much lower cytokine level than animals that died due to metastases or that an intensive production of proinflammatory cytokines triggered an immunosuppressive mechanisms in the spleens of the survivors. We can also assume that the drugs induce prometastatic actions *via* a route that is independent of the synthesis of proinflammatory cytokines.

The significant stimulatory effect of desipramine and fluoxetine on serum VEGF levels in young animals can produce a significant pro-metastatic effect, despite the fact that splenocytes are not a source of serum VEGF and that splenocytes were observed to show a tendency towards decreasing synthesis of this cytokine in young animals. The inhibitory effect on VEGF production was established for extracts from St. Johns wort (*Hypericum perforatum*), which have antidepressant activity [6].

In vitro studies demonstrated a dose-dependent cytotoxic effect of antidepressant drugs on B16F10 cells [40]. In the present study, however, the B16F10 tumor growth was stimulated *in vivo*. The drug can accumulate in the mice over a two-week treatment period, but its destructive pro-angiogenic effect and destructive influence on antitumor immunity likely prevails over a potential cytotoxic effect of the drug on tumor cells. We observed an entirely different effect of desipramine and fluoxetine when the drugs were administered after melanoma cell inoculation, with fluoxetine showing a particularly strong anticancer action (paper in preparation).

In summary, tumor growth was significantly slower in saline-treated aged mice in comparison to young saline-treated animals. Treatment with antidepressant drugs increased metastasis formation and shortened survival in young and aged animals, although significant results were obtained only for desipramine-treated young animals. In young animals pretreated with antidepressants before the induction of cancer, some lymphocyte-dependent immunological parameters were attenuated (e.g., proliferation of B-cells, and production of IL-6, IL-10 and IL-12p40 by splenic T-cells), while the synthesis of angiogenesis/metastasis-related factors VEGF and MMP-9 was increased. In aged animals, antidepressants increased primary tumor growth and reduced the proliferative activity of splenocytes in response to LPS to the same degree as was observed for young animals but had no effect on cytokine production.

In conclusion, the present study demonstrates the prometastatic effects of both desipramine and fluoxetine. The effect was age dependent and associated with high VEGF and MMP-9 plasma levels in young antidepressant drug-pretreated animals. This study indicated that antidepressants may have opposite effects on solid tumor growth in young and aged animals and may strongly promote this process in the latter group. No correlation of these changes with immune system activity in aged animals was observed. Although the above results point to prometastatic effects of both desipramine and fluoxetine, one should be very cautious about applying these data to human clinical studies. This study was performed on a specific strain of mice using a particular melanoma cell line. Recently, we

observed that desipramine pretreatment protected Wistar rats from the metastasis of MADB 106 cells. The mechanism of metastatic promotion by pretreatment with antidepressants before tumor cell inoculation is unknown, and this problem requires further examination of immunity and endothelial permeability during antidepressant drug administration. This suggestion may be particularly important for cancer patients taking antidepressants before surgical manipulation because of an increased risk of cancer cell dissemination during surgery.

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References:

1. Abdul M, Logothethetis CJ, Hoosein NM: Growth-inhibitory effects of serotonin uptake inhibitors on human prostate carcinoma cell lines. J Urol, 1995, 154, 247–250.
2. Ahmed OI, Adel AM, Diab DR, Gobran NS: Prognostic value of serum level of interleukin-6 and interleukin-8 in metastatic breast cancer patients. Egypt J Immunol, 2006, 13, 61–68.
3. Anichini A, Vegetti C, Mortarini R: The paradox of T-cell-mediated immunity in spite of poor clinical outcome in human melanoma. Cancer Immunol Immunother, 2004, 53, 855–864.
4. Bendele RA, Adams ER, Hoffman WP Gries CL, Morton DM: Carcinogenicity studies of fluoxetine in rats and mice. Cancer Res, 1992, 52, 6931–6935.
5. Ben-Eliyahu S, Page GG: In vivo assessment of natural killer cell activity in rats. Prog Neuroendocrinimmuol, 1992, 5, 199–214.
6. Bhuvaneswari R, Yuen GY, Chee SK, Olivo M: Hypericin-mediated photodynamic therapy in combination with avastin (bevacizumab) improves tumor response by downregulating angiogenic proteins. Phytochem Photobiol Sci, 2007, 6, 1275–1283.
7. Brandes LJ, Arron RJ, Bogdonavic RP Tong J, Zaborniak CL, Hogg GR, Warrington RC et al.: Stimulation of malignant growth in rodents by antidepressant drugs at clinically relevant doses. Cancer Res, 1992, 52, 3796–3800.
8. Brandes LJ, Cheang M: Letter regarding „Response to antidepressants and cancer: cause for concern?". J Clin Psychopharmacol, 1995, 15, 84–85.
9. Cha HJ, Jeong MJ, Kleinman HK: Role of thymosin β_4 in tumor metastasis and angiogenesis. J Natl Cancer Inst, 2003, 95, 1674–1680.
10. Chlebda E, Merwid-Ląd A, Szumny D, Trocha M, Fereniec-Gołębiewska L, Gebarowska E, Kowalski P, Szeląg A: Antitumor effect of macrolides – erythromycin and roxithromycin in B16 melanoma-transplanted mice. Pharmacol Rep, 2007, 59, Suppl, 269–274.
11. Coopre AM, Khader SA: IL-12p40: an inherently agonistic cytokine, Trends Immunol, 2007, 28, 33–38.
12. Donin N, Sinai J, Michowitz M, Hiss J, Nordenberg J, Leibovici J: Role of immune response as determinant of tumor progression in function of host age in the B16 melanoma. Mech Ageing Dev, 1995, 80, 121–137.
13. Donin N, Sinai J, Staroselsky A, Mahlin T, Nordenberg J, Leibovici J: Comparison of growth rate of two B16 melanomas differing in metastatic potential in young versus middle-aged mice. Cancer Invest, 1997, 15, 416–421.
14. Franco S, Segura I, Riese HH, Blasco MA: Decreased B16F10 melanoma growth and impaired vascularization in telomerase-deficient mice with critically short telomeres. Cancer Research, 2002, 62, 552–559.
15. Frick LR, Palumbo ML, Zappia MP, Brocco MA, Cremaschi GA, Genaro AM: Inhibitory effect of fluoxetine on lymphoma growth through the modulation of antitumor T-cell response by serotonin-dependent and independent mechanisms. Biochem Pharmacol, 2008, 75, 1817–1826.
16. Guruvayoorappan C, Kuttan G: Amentoflavone inhibits experimental tumor metastasis through a regulatory mechanism involving MMP-2, MMP-9, prolyl hydroxylase, lysyl oxidase, VEGF, ERK-1, ERK-2, STAT-1, nm23 and cytokines in lung tissues of C57BL/6 mice. Immunopharmacol Immunotoxicol, 2008, 30, 711–727.
17. Hofmann UB, Westphal JR, Van Muijen GN, Ruiter DJ: Matrix metalloproteinases in human melanoma. J Invest Dermatol, 2000, 115, 337–344.
18. Iishi H, Tatsuta M, Baba M, Taniguchi H: Enhancement by the tricyclic antideprssant, desipramine, of experimental carcinogenesis in rat colon induced by azoxymethane. Carcinogenesis, 1993, 1837–1840.
19. Kaczmarek P, Pokoca L, Niemirowicz J, Majewska E, Baj Z: Effect of luteinizing hormone-releasing hormone (LHRH) analogue treatment on a cytokine profile in prostate cancer patients. Pharmacol Rep, 2008, 60, 399–403.
20. Katsikis PD, Cohen SB, LondeiM, Feldman M: Are $CD4^+$ T_h1 cells pro-inflammatory or anti-inflammatory? The ratio of IL-10 to IFN-γ or IL-2 determines their function. Int Immunol, 1995, 7, 1287–1294.
21. Klein G, Vellenga E, Fraaije MW, Kamps WA, de Bont ES: The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. Crit Rev Oncol Hematol, 2004, 50, 87–100.
22. Kołaczkowska E, Chadzinska M, Ścislowska-Czarnecka A, Płytycz B, Opdenakker G, Arnold B: Gelatinase B/matrix metalloproteinase-9 contributes to cellular infiltration in a murine model of zymosan peritonitis. Immunobiology, 2006, 211, 137–148.
23. Kubera M, Symbirtsev A, Basta-Kaim A, Borycz J, Roman A, Papp M, Claesson M: Effect of chronic treatment with imipramine on interleukin 1 and interleukin 2 production by splenocytes obtained from rats subjected to a chronic mild stress model of depression. Pol J Pharmacol, 1996, 48, 503–506.

24. Lieb J: Antidepressants, eicosanoids and the prevention and treatment of cancer. A review. Prostaglandins Leukot Essent Fatty Acids, 2002, 65, 233–239.
25. Manu KA, Kuttan G: Boerhaavia diffusa stimulates cell-mediated immune response by upregulating IL-2 and downregulating the pro-inflammatory cytokines and GM-CSF in B16F-10 metastatic melanoma bearing mice. J Exp Ther Oncol, 2008, 7, 17–29.
26. Marconi C, Bianchini F, Mannini A, Mugnai G, Ruggieri S, Calorini L: Tumoral and macrophage uPAR and MMP-9 contribute to the invasiveness of B16 murine melanoma cells. Clin Exp Metastasis, 2008, 25, 225–231.
27. Murakami T, Cardones AR, Hwang ST: Chemokine receptors and melanoma metastasis. J Dermatol Sci, 2004, 36, 71–78.
28. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T et al.: Involvement of chemokine receptors in breast cancer metastasis. Nature, 2001, 410, 50–56.
29. Nordlind K, Azmitia EC, Slomiński A: The skin as a mirror of the soul: exploring the possible roles of serotonin. Exp Dermatol, 2008, 17, 301–311.
30. Opdenakker G, Van den Steen PE, Dubois B, Nelissen I, Van Coillie E, Masure S, Proost P, Van Damme J: Gelatinase B functions as regulator and effector in leukocyte biology. J Leukoc Biol, 2001, 69, 851–859.
31. Ptak W, Geba GP, Askenase PW: Initiation of delayed-type hypersensitivity by low doses of monoclonal IgE antibody. Mediation by serotonin and inhibition by histamine. J Immunol, 1991, 146, 3929–3936.
32. Reed SM, Glick JN: Fluoxetine and reactivation of the herpes simplex virus. Am J Psychiatry, 1991, 49, 949–950.
33. Shakhar G, Ben Eliyahu S: In vivo β-adrenergic stimulation suppresses natural killer cell activity and compromises resistance to tumor metastasis in rats. J Immunol, 1998, 160, 3251–3258.
34. Shingu K, Helfritz A, Kuhlmann S, Zielinska-Skowronek M, Jacobs R, Schmidt RE, Pabst R, von Hörsten S: Kinetics of the early recruitment of leukocyte subsets at the sites of tumor cells in the lungs: natural killer (NK) cells rapidly attract monocytes but not lymphocytes in the surveillance of micrometastasis. Int J Cancer, 2002, 99, 74–81.
35. Słomiński A, Pisarchik A, Semak I, Sweatman T, Wortsman J: Characterization of the serotoninergic system in the C57BL/6 mouse skin. Eur J Biochem, 2003, 270, 3335–3344.
36. Słomiński A, Pisarchik A, Semak I, Sweatman T, Wortsman J, Szczęśniewski A, Sługocki G et al.: Serotoninergic and melatoninergic systems are fully expressed in human skin. FASEB J, 2002, 16,896–898.
37. Słomiński A, Pisarchik A, Wortsman J: Expression of genes coding melatonin and serotonin receptors in rodent skin, Biochim Biophys Acta, 2004, 1680, 67–70.
38. Słomiński A, Wortsman J, Tobin DJ: The cutaneous serotoninergic/melatoninergic system: securing a place under the sun. FASEB J, 2005, 19, 176–94.
39. Steingart AB, Cotterchio M: Do antidepressants cause, promote, or inhibit cancers? J Clin Epidemiol, 1995, 48, 1407–1412.
40. Stepulak A, Rzeski W, Sifringer M, Brocke K, Gratopp A, Kupisz K, Turski L, Ikonomidou C: Fluoxetine inhibits the extracellular signal regulated kinase pathway and suppresses growth of cancer cells. Cancer Biol Ther, 2008, 7, 1685–1693.
41. Sternbach H: Are antidepressants carcinogenic? A review of preclinical and clinical studies. J Clin Psychiatry, 2003, 64, 1153–1162.
42. Sun WH, Kreisle RA, Philips AW, Ershler WB: In vivo and in vitro characteristics of interleukin-6-transfected B16 melanoma cells. Cancer Res, 1992, 52, 5412–5415.
43. Takeda K, Tsutsui H, Yoshimoto T, Adachi O, Yoshida N, Kishimoto T, Okamura H et al: Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity, 1998, 8, 383–390.
44. Tutton PJ, Barkla DH: Effect of an inhibitor of noradrenaline uptake, desipramine, on cell proliferation in the intestinal crypt epithelium. Virchows Arch B Cell Pathol, 1989, 57, 349–352.
45. Väisänen AH, Kallioinen M, Turpeenniemi-Hujanen T: Comparison of the prognostic value of matrix metalloproteinases 2 and 9 in cutaneous melanoma. Hum Pathol. 2008, 39, 377–85.
46. Vidal-Vanaclocha F, Alvarez A, Asumendi A, Urcelay B, Tonino P, Dinarello ChA: Interleukin 1 (IL-1)-dependent melanoma hepatic metastasis in vivo; increased endothelial adherence by IL-1-induced mannose receptors and growth factor production in vitro. J Natl Cancer Inst, 1996, 88, 198–205.
47. Warner-Schmidt JL, Duman RS: VEGF as a potential target for therapeutic intervention in depression. Curr Opin Pharmacol, 2008, 8, 14–19.
48. Whalen MM, Bankhurst AD: Effects of β-adrenergic receptor activation, cholera toxin and forskolin on human natural cell function. Biochem J, 1990, 272, 327–331.

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