Administration of liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE) and diclofenac in the combination attenuates their anti-tumor activities*

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The anti-tumor effects of i.p. administered cyclooxygenase inhibitor – diclofenac and i.v. administered liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE) were investigated using a s.c. growing murine fibrosarcoma tumor. Tumor growth was assessed by measuring tumor volumes and survival of the mice. Both of the drugs were administered either alone or in combination. Repeated application of diclofenac in two schedules (150 μg/mouse/day for 14 consecutive days or 2x150 μg/mouse/week for 4 weeks) or application of liposomal MTP-PE (2 x20 μg/mouse/week for 4 weeks) starting on day 5 after tumor cell transplantation significantly suppressed the tumor growth and increased the percentage of surviving mice. However, the volume of tumors and the survival time in tumor bearing mice treated with the two agents were similar to untreated counterparts. Thus, these data suggest the anti-tumor activity of either of the two drugs is lost when they are used in combination. Hematological examinations confirmed previously observed hematopoiesis-stimulating activities of the drugs when given alone. However, mutually potentiating effects after combined administration of liposomal MTP-PE and diclofenac were observed only exceptionally. Our findings corroborate the recommendation that the interactions of drugs used for the treatment of tumors must be carefully checked, if the drugs are applied in combination.

Key words: Muramyl tripeptide, diclofenac, NSAIDs, immunotherapy, tumor therapy.

The identification of immunopotentiating compounds that can perform an adjuvant function with minimal toxic side effects is of paramount importance to the development of vaccine therapies. One interesting candidate for this role is the synthetic lipophilic analogue of muramyl dipeptide, muramyl tripeptide phosphatidylethanolamine (MTP-PE; CGP 19835A), encapsulated in liposomes. Liposomes containing MTP-PE have been shown to activate the tumoricidal properties of blood monocytes and tissue macrophages *in vitro* and monocytes in situ [19, 25]. In some animal models of cancer, therapy with liposomal MTP-PE has proven effective, but not in all cases [36]. Clinical trials with anti-tumor therapy comprising liposomal MTP-PE have also been performed in humans. Liposomal MTP-PE has been used against melanoma [23], osteosarcoma [22], mammary carcinoma [13, 32], bladder carcinoma and other tumor types [8, 18, 34, 35].

Various non-steroidal anti-inflammatory drugs (NSAIDs), acting on the principle of suppressing production of prostaglandins (PGs) through inhibition of cyclooxygenase (COX), have been shown to suppress the growth of solid tumors in experiments on animals [27, 31], as well as in clinical practice [20, 29]. We have documented recently that diclofenac, one of the most widely-used NSAIDs, evokes distinct anti-tumor action on the growth of tumors arising

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from subcutaneously (s.c.) transplanted G:5:113 fibrosarcoma cells [17]. This finding may be of significance because fibrosarcoma is one of the tumors that are often resistant to non-surgical therapy.

PARANT and co-workers [26] were surprised to find that simultaneous administration to mice of muramyl dipeptide (MDP) and indomethacin, another NSAID, exerted a strong synergistic anti-infective action. Proceeding from the hypothesis that suppression of the release of the feedback inhibitor prostaglandin E_2 could intensify or prolong the activation of macrophages, BASCHANG and co-workers [2] synthesized lipophilic conjugates of COX inhibitors and MDP derivatives. The resultant compounds were very potent in activating macrophages to the tumoricidal state. Experimental evidence presented in our previous paper [11] indicated that the combination of liposomal MTP-PE with indomethacin accelerated myelopoietic regeneration in the post-irradiation period in the murine bone marrow and protected 100% of mice after lethal irradiation.

Major side effects following liposomal MTP-PE infusion are fever and chills [25]. For these reasons FUJUMAKI et al [14] recommended ibuprofen (COX inhibitor) given before liposomal MTP-PE with the aim of reducing these uncomfortable symptoms without compromising the immunostimulatory effects of liposomal MTP-PE.

Based on the proven anti-tumor properties of both liposomal MTP-PE and diclofenac, we were interested whether the combined administration of these agents could intensify their anti-tumor effects. The purpose of this study therefore was to evaluate the anti-tumor effectiveness of the combined utilisation of immunotherapy (liposomal MTP-PE) with NSAIDs (diclofenac) in comparison with the effects of the individual treatments applied separately.

Material and methods

Mice. Male C3H/DiSn mice, 9-11 weeks old (weighing 20 g in average), were obtained from Velaz, s.r.o. (Praha, Czech Republic). Animals were quarantined for a period of 2 weeks and were given Velaz/Altromin 1320 St lab chow and tap water acidified to pH 2.4 *ad libitum*. Research was conducted according to principles enunciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the State Veterinary Office of the Slovak Republic, Bratislava and NIH publication No. 85-23, revised 1985.

Reagents. Liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE, CGP l9835A) was a generous gift from Ciba-Geigy Ltd. (Basel, Switzerland). Liposomes were prepared from dry lyophilisate composed of 250 mg of phosphatidylcholine and phosphatidylserine in a molar ratio of 7:3 (with or without 4 mg of MTP-PE) and shaken with 10 ml of suspension medium (PBS, pH=7.2, without Ca^{2+} and Mg^{2+}-salts). After standing for 1 minute, the reconstituted liposomes were suspended by vortexing for 5 minutes. The liposome preparations were adjusted to 25 μmol of phospholipid/ml in PBS containing 100 μg of MTP-PE, and 0.2 ml of the preparations was injected intravenously (i.v.) into mice (20 μg of MTP-PE/5 μmol phospholipid/mouse).

Dicloreum (diclofenac sodium, injectable; henceforth referred to as diclofenac; ASW Alfa Wasermann S.p.A., Italy) was dissolved in saline and intraperitoneally (i.p.) injected in a volume of 0.2 ml (150 μg/dose/mouse).

Tumor cell line. The N-methyl-N'-nitro-N-nitrosoguanidine-induced G:5:113 fibrosarcoma cell line was kindly provided by dr. Margaret Kripke (University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA). The cells were maintained in 75 cm^2 culture flasks (Nunc, Denmark) (37 °C, 5% CO_2) in RPMI-1640 (PAN Systems GmbH, Aidenbach, Germany) supplemented with 10% heat-inactivated foetal calf serum (FCS; PAN Systems GmbH), 1 mM sodium pyruvate (ICN Biomedicals, Icn., Costa Mesa, CA, USA), 100 μg/ml streptomycin, 100 IU/ml penicillin (PAA Laboratories GmbH, Linz, Austria), 0.1 mg/ml gentamicin (PAN Systems GmbH), 8 mM L-glutamine (Gibco BRL, Paisley, Scotland), 1% nonessential amino acids 100 x (ICN Biomedicals, Inc.), 5 mM Hepes (Serva, Feinbiochemica, Heidelberg, Germany), and 50 μM 2-mercaptoethanol (Fluka, AG, Buchs SG, Switzerland). The cells used for experiments were in the exponential growth phase.

Transplantation of tumor cells. The cells were harvested by trypsinization (0.25% Trypsin/2% EDTA, Sigma, USA), washed twice with serum-free medium. Animals were anaesthetised i.p. with Narcamon/Rometar solution (5% Narcamon/2% Rometar in the ratio of 2.63:1, Spofa, Praha, Czech Republic) and injected s.c. in the flank with 10^5 viable tumor cells per mouse in a volume of 0.125 ml.

Pharmacological treatment regimen. Mice bearing G:5:113 tumors were randomised into seven groups of 6-11 animals per group. Therapeutic treatment with liposomal MTP-PE was carried out via a lateral tail vein at a dose of 20 μg MTP-PE/5 μmol phospholipid/mouse administered twice weekly for 4 weeks for a total of 8 injections starting 5 days after tumor cell transplantation (MTP-PE group); diclofenac was i.p. injected at a dose of 150 μg/mouse twice weekly for 4 weeks for a total of 8 injections (DIC 2x4 group) or was injected i.p. every day at a dose of 150 μg/mouse in a 14-day regimen (DIC 14 group). Coadministration of diclofenac and liposomal MTP-PE (twice weekly for 4 weeks) starting 5 days after tumor cell transplantation consisted in the application of diclofenac (150 μg/mouse/dose) 15 min before liposomal MTP-PE (20 μg/mouse/dose) (MTP-PE with DIC 2x4 group). The combined therapeutic regimen (MTP-PE + DIC 14 group) started with liposomal MTP-PE administered twice weekly for 4 weeks and was followed with diclofenac administered every day in a 14-day regimen. Control injections for liposomal MTP-PE con-

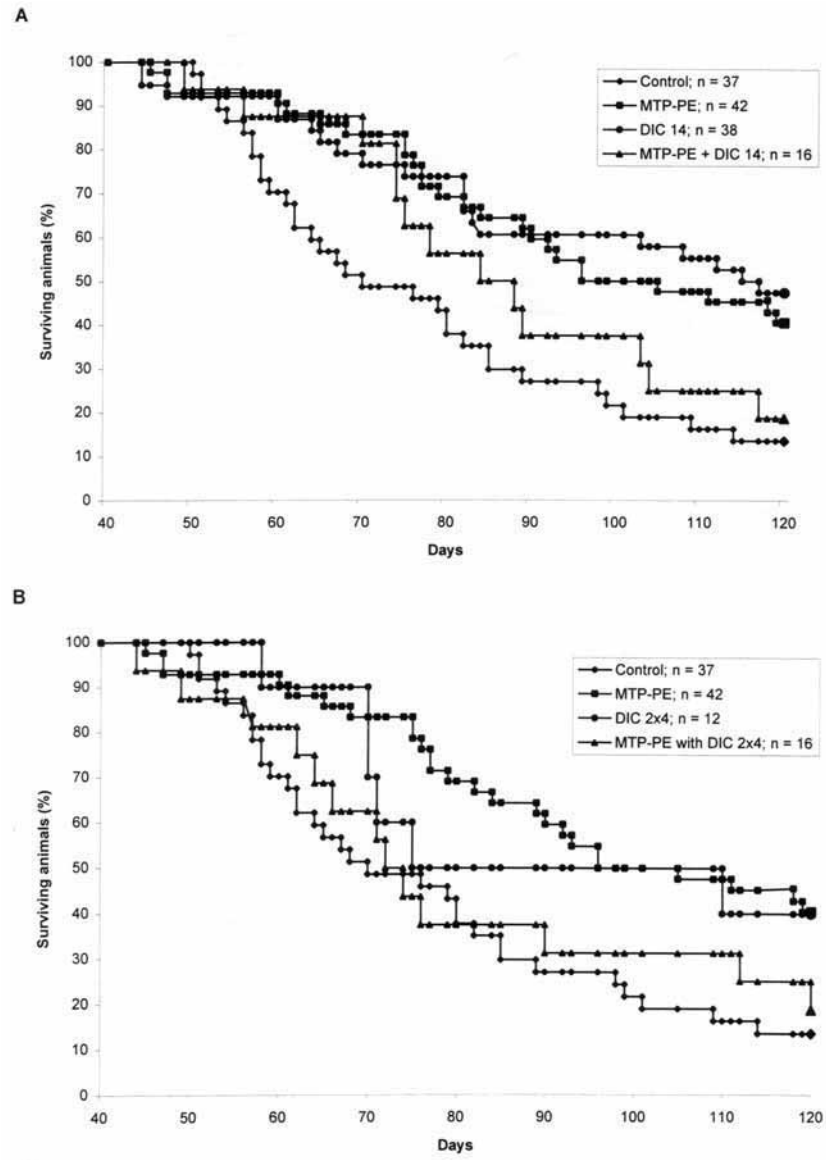

Figure 1. Survival of tumor-bearing mice after combined therapy or monotherapies. Control – non-treated tumor-bearing mice; MTP-PE – tumor-bearing mice administered liposomal MTP-PE twice weekly for 4 weeks starting on day 5 after transplantation of tumor cells; DIC 2x4 – tumor-bearing mice administered diclofenac twice weekly for 4 weeks starting on day 5; DIC 14 – tumor-bearing mice administered diclofenac daily for 14 days starting on day 5; MTP-PE with DIC 2x4 – tumor-bearing mice coadministered liposomal MTP-PE and diclofenac twice weekly for 4 weeks starting on day 5; MTP-PE + DIC 14 – tumor-bearing mice administered liposomal MTP-PE twice weekly for 4 weeks starting on day 5 and given 14 daily injections of diclofenac immediately afterwards. Survival was monitored daily for up to 120 days. n = number animals
A: Control vs. MTP-PE, $p<0.01$ (Long-rang and Wilcoxon tests); Control vs. DIC 14, $p<0.001$ (Long-rang and Wilcoxon tests); DIC 14 vs. MTP-PE + DIC 14, $p=0.06$ (Long-rank test).
B: Control vs. MTP-PE, $p<0.01$ (Long-rang and Wilcoxon tests); Control vs. DIC 2x4, $p=0.08$ (Long-rang test); MTP-PE vs. MTP-PE with DIC 2x4, $p<0.05$ (Long-rang and Wilcoxon tests)

tained a placebo (empty liposomes), while those for diclofenac contained saline. However, because no significant differences were observed in the response of mice (curves of survival and volumes of tumors) receiving the placebo or the saline, data from both control groups were pooled. Experiments were repeated two to four times.

Assessment of tumor size. Incidence and approximate tumor size were recorded weekly throughout the experimental period. Tumor location was determined by palpation and size was determined by measuring the three dimensions of each tumor using a calliper. Approximate tumor volume (cm^3) was calculated using a formula for elliptical volume ($V = \pi/6 \; x \; L \; x \; W \; x \; H$; L, W and H designate tumor diameters for length, width and height, respectively).

Hematological methods. Numbers of leukocytes per 1 μl of peripheral blood, as well as cellularity of the femoral bone marrow were determined using Coulter Counter (model ZF, Coulter Electronics, UK). Numbers of granulocytes per 1 μl of peripheral blood were assessed using blood smears. Numbers of hematopoietic progenitor cells for granulocytes and macrophages (GM-CFC) per femur were determined by the *in vitro* technique according to VACEK et al [33] using 10% lung conditioned medium as a source of colony-stimulating activity.

Survival. Survival was monitored daily and was reported as the percentage of animals surviving 120 days after tumor cell transplantation. On day 121, surviving mice were euthanized by cervical dislocation.

Statistics. The differences between survival curves were analysed by Peto's log-rank and Wilcoxon tests. Student's t-test preceded by F-test was used for evaluating the statistical significance of differences in hematological parameters. The significance level was set at $p<0.05$.

Results

From Figure 1A, B it follows that 19% of the mice treated with liposomal MTP-PE in combination with diclofenac (regardless of administration schedule) survived 120 days, whereas 41%, 47% or 40% survived after separate treatment with liposomal MTP-PE, or diclofenac in the regimens DIC 14 or DIC 2x4, respectively. Statistical analysis of survival curves shows that in both cases the combined therapies had lower efficacy than therapies with any

of the drugs given alone. The median survival time of tumor-bearing mice was significantly prolonged from 70 days (range, 62–85 days) in the control mice to 115 days (range, 83–120+ days) for the DIC 14 group and to 96 days (range, 84–120+ days) for the MTP-PE group. For the two experimental groups coadministered liposomal MTP-PE and diclofenac, i.e. the MTP-PE with DIC 2x4 group and the MTP-PE + DIC 14 group, the median survival time was 72 days (range, 64–112 days) and 84 days (range, 74–104 days), respectively.

Figure 2 A, B shows Kaplan-Meier plots of the number of mice with tumor volumes less than 400 mm^3. Experimental results demonstrated that after both combined therapies (MTP-PE with DIC 2x4 or MTP-PE + DIC 14), only about 6% of tumor-bearing mice had their tumor volumes smaller than 400 mm^3, which is comparable with the value of 8% of control mice. In all groups treated with the drugs in the form of monotherapies (MTP-PE, DIC 14 or DIC 2x4) higher proportions of animals (from 19 to 35%) with tumor volumes smaller than this upper limit were found.

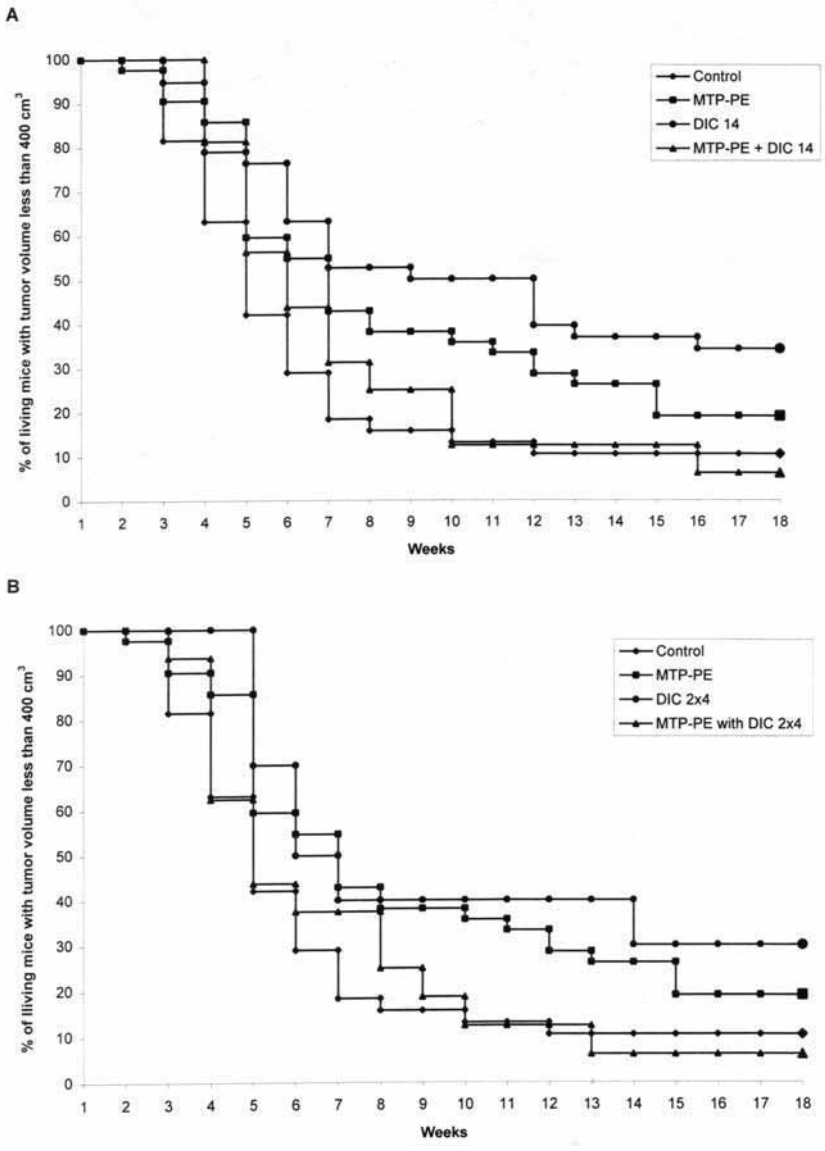

Figure 2. Kaplan-Meier plot of number of mice with tumor volume less than 400 mm^3. Tumour response was assessed for up to 120 days. For other abbreviations, symbols and details see Legend to Figure 1.

A: Control vs. MTP-PE, $p<0.05$ (Long-rang and Wilcoxon tests); Control vs. DIC 14, $p<0.001$ (Long-rang and Wilcoxon tests); DIC 14 vs. MTP-PE + DIC 14, $p<0.05$ (Long-rank test).

B: Control vs. MTP-PE, $p<0.05$ (Long-rang and Wilcoxon tests); Control vs. DIC 2x4, $p<0.05$ (Long-rang and Wilcoxon tests); MTP-PE vs. MTP-PE with DIC 2x4, $p=0.08$ (Long-rang test); DIC 2x4 vs. MTP-PE with DIC 2x4, $p=0.06$ (Long-rang and Wilcoxon tests).

The effects of the pharmacological approaches on tumor size during the first 6 weeks after tumor cell transplantation, when survival in each group was still 100%, are summarized in Figures 3 A, B. Coadministration of MTP-PE with DIC 2x4 did not suppress the growth of experimental solid tumors. It was observed that average volumes of tumors in this group were similar to those in control mice (98–103% of control values). On the other hand, average volume of tumors in this group represented 97–251% of values in comparison with monotherapy (MTP-PE or DIC 2x4). In the other case of combined therapy (MTP-PE + DIC 14) the growth curve of tumors was comparable to that of groups treated with monotherapy; the average volume of tumors in this group represented 54–96% of values in liposomal MTP-treated animals and 71–175% of values in DIC 14 group.

Several hematological parameters were determined on days 30 and 40 after tumor cell transplantation. To illustrate the hematological findings, two parameters are presented here, namely numbers of GM-CFC per femur (Fig. 4 A, B) and numbers of granulocytes per 1 μl of peripheral blood (Fig. 5 A, B). Generally, the Figures show that both the presence of tumors and the treatment with liposomal MTP-PE or diclofenac alone or in combinations contribute to some extent to hematopoietic stimulation. Fourteen-day therapy with diclofenac alone significantly increased numbers of GM-CFC per femur in comparison with both control mice without tumors and untreated tumor-bearing mice (day 40 TM group) (Fig. 4 A). The four-week treatment regimen with liposomal MTP-PE alone significantly in-

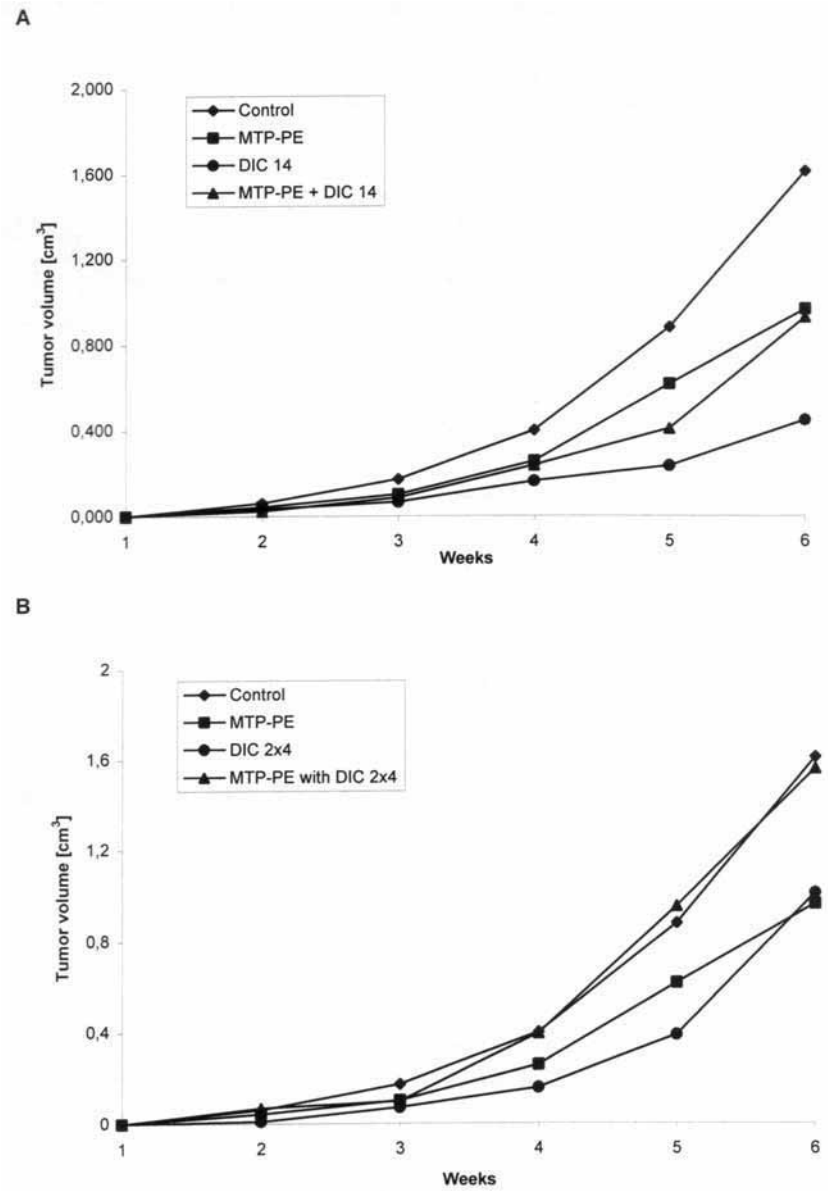

Figure 3. Effect of combined therapy or monotherapies on growth pattern of G:5:113 tumors in mice during the first 6 weeks after tumor cell transplantation, when survival in each group was still 100%. Tumour growth was monitored as a function of time by a weekly measurements of the tumor sizes (mm^3). For other abbreviations, symbols and details see Legend to Figure 1.

creased numbers of granulocytes in peripheral blood when compared with both control mice without tumors and untreated tumor-bearing mice (Fig. 5 A, B). Combined treatment with both the drugs studied was not mutually potentiating with the exception of granulocytes in peripheral blood in the group MTP-PE with DIC 2x4 (Fig. 5 B).

Discussion

Our results show that repeated administration of diclofenac alone or liposomal MTP-PE alone, in contrast to combined administration of these agents, leads to suppression of tumor growth as tested in the mouse G:5:113 fibrosarcoma model. This beneficial action of diclofenac or liposomal MTP-PE led to a significant increase in the percentage of surviving tumor-bearing animals 15 weeks after tumor cell transplantation when compared to the control mice. Combinations of both therapies were less effective than therapies with the drugs alone regardless of the administration schedule.

Recently, as well as performing *in vivo* experiments, we also studied the *in vitro* effect of three structurally different NSAIDs, including diclofenac, on the proliferation activity of G:5:113. We found only moderate suppressive effect of diclofenac on cell numbers without any changes in the cell cycle, however repeated application of diclofenac significantly suppressed the tumor growth *in vivo* and increased the percentage of surviving mice [17]. On the other hand, *in vitro* proliferation of fibrosarcoma cells depended on intact functions of lipoxygenases and cytochrome P-450-monooxygenase, however lipoxygenase inhibitors did not influence anti-fibrosarcoma activity *in vivo* (Hoferová et al., unpublished results). Since there are considerable differences between the *in vitro* and *in vivo* effects of the tested NSAIDs, the exact mechanism of their anti-carcinogenic effect is still unclear. *In vivo*, the effective antineoplastic NSAIDs dose is comparable to the amount of drug required to inhibit PGs production [37]. Thus, most of the hypotheses about the anti-cancer effects of NSAIDs have involved the common property of these drugs, that of inhibiting COX activity and thereby causing a subsequent reduction of PGs produced by both tissue stroma and tumor cells. Another mechanism of the influence of NSAIDs on tumor growth is the modulation of the immune response. Many investigators [9, 28, 31] observed that in vitro treatment with NSAIDs stimulated macrophage cytotoxicity. Braun and co-workers [3] also reported that the cytotoxicity of γ-interferon-stimulated peritoneal macrophages from ovarian cancer patients was accelerated by indomethacin but was inhibited by a lipoxygenase inhibitor. These results suggest that inhibitors of various pathways in the metabolism of arachidonic acid may produce opposing effects depending upon their inhibitory influence

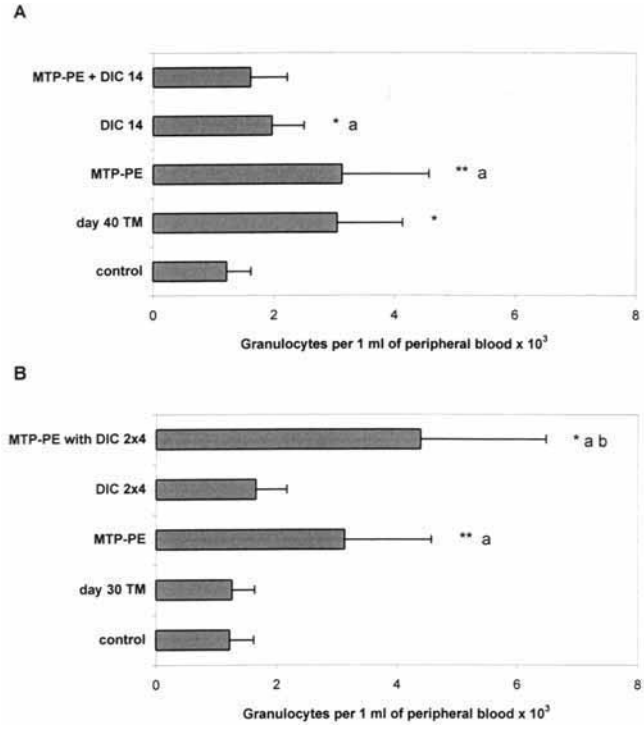

Figure 4. Numbers of granulocytes per 1 μl of peripheral blood. A, B – samplings of material performed on days 30 and 40, respectively, after transplantation of tumor cells; control – control mice without tumors; day 30 TM, day 40 TM – control tumor-bearing mice; MTP-PE – tumor-bearing mice administered liposomal MTP-PE twice weekly for 4 weeks starting on day 5 after transplantation of tumor cells; DIC 2x4 – tumor-bearing mice administered diclofenac twice weekly for 4 weeks starting on day 5; DIC 14 – tumor-bearing mice administered diclofenac daily for 14 days starting on day 5; MTP-PE with DIC 2x4 – tumor-bearing mice coadministered liposomal MTP-PE and diclofenac twice weekly for 4 weeks starting on day 5; MTP-PE + DIC 14 – tumor-bearing mice administered liposomal MTP-PE twice weekly for 4 weeks starting on day 5 and given 14 daily injections of diclofenac immediately afterwards;
***, ** – p<0.05 and p<0.01, respectively, in comparison with control mice without tumors; a – p<0.05 in comparison with day 40 TM group (part A) or day 30 TM group (part B); b – p<0.05 in comparison with DIC 2x4 group.**

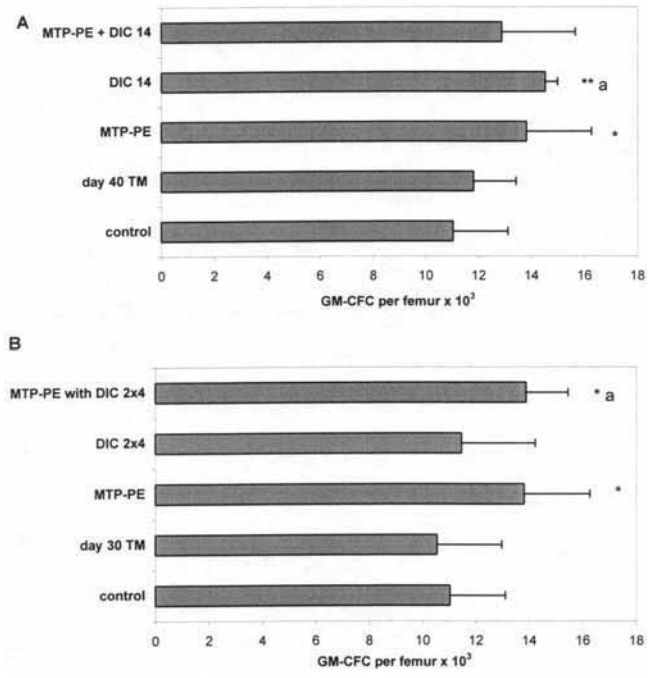

Figure 5. Numbers of GM-CFC per femur. For other abbreviations and symbols see Legend to Figure 4.

on the COX pathway or lipoxygenase pathway and that, in contrast to the immunosuppressive effects of PGE_2, lipoxygenase metabolites may be stimulatory to immune effector cells.

Muramyl tripeptides are components of the outer cell membrane of most bacteria and display most of the immunological activities associated with an infection with whole bacteria. When administered i.v., liposomal MTP-PE is delivered predominantly to macrophages. Furthermore, i.v. administered MTP-PE has been shown to induce anti-tumor reactivity, probably as a result of macrophage activation [19]. Macrophages release a number of cytokines and other products which could be involved in influencing tumor growth, including IL-1, TNF, eicosanoids, nitric oxide and oxygen radicals [25].

Very little is known about the intracellular signal transduction pathway of muramyl tripeptides. The extensive studies carried out by DIETER and his colleagues [4, 5, 6, 7] demonstrated that liposomal MTP-PE rapidly activates the map kinase isoenzymes ERK-1 and ERK-2, but not the transcription factors NF-κB and AP-1, which become detectable only after 5 hour. Activation of yet unknown transcription factors results in synthesis and release of IL-1α, IL-1β, IL-6, IL-8, TNF-α [1, 4], nitric oxide and PGE_2 [4, 5, 7]. Cellular calcium and protein kinase C isoenzymes are not involved in the signalling pathway of liposomal MTP-PE. Exogenous PGE_2 has no inhibitory effect on the map kinase isoenzymes ERK-1 and ERK-2 and TNF-α release is not suppressed [6]. Liposomal MTP-PE increases mRNA's encoding of TNF-α, iNOS and COX-2 isoenzyme, but have no effect on COX-1 levels. The data of DIETER et al [4–7] also support previous findings that the cytotoxicity of macrophages against tumor cells is mediated by TNF-α, but not by nitric oxide [12], since LPS and liposomal MTP-PE induced an identical release of nitric oxide, but differed in their cytotoxic potencies. Similarly, killing of tumor cells by cultured human monocytes was not dependent on oxygen radicals [24]. However, neither PGE_2 nor thromboxane seemed essential to the activation process, because induction of anti-tumor activity in primed macrophages by muramyl dipeptide took place and was even enhanced in the presence of the COX inhibitor – indomethacin, whereas it was decreased by exogenous PGE_2. The results of RADDASSI et al [30] suggest that COX

derivatives contribute to macrophage unresponsiveness and deactivation.

From the point of view of our results it is interesting that ibuprofen (COX inhibitor) at dose levels up to 10 μg/ml had no effect on the generation of monocyte-mediated cytotoxicity by MTP-PE and no effect on the production, secretion or mRNA expression of TNF and IL-1 [14]. By contrast, ibuprofen at dose levels of 40 μg/ml suppressed the generation of the cytotoxic phenotype but did not interfere with the killing process once the cells were activated. This dose level of ibuprofen, however, suppressed IL-1 and TNF-α production, as well as the mRNA expression of these cytokines. Since these cytokines play a crucial role in the cytotoxic function of monocytes, the findings of Fujimaki et al [14] may explain the mechanism by which ibuprofen inhibited the generation of the cytotoxic phenotype by liposomal MTP-PE. This mechanism could explain the reduced anti-tumor effect in the case of the coadministration of diclofenac with liposomal MTP-PE (MTP-PE with DIC 2x4), but cannot be valid for the combined administration of liposomal MTP-PE plus subsequent diclofenac (MTP-PE + DIC) [14]. The reasons leading to the similar results of the combined treatments which were acquired regardless of different treatment schedules, and therefore by different mechanisms, are not yet known. Our results show that combinations of two drugs which are both known to suppress the growth of an established tumor, may in fact loose their anti-tumor activities when administered in the combination and do not result in a significant prolongation of survival.

Our assessment of the hematological parameters of tumor-bearing mice with or without therapy confirmed previous findings on the hematopoiesis-stimulating effects of both liposomal MTP-PE [10, 11] and diclofenac [16, 21]. Recently we have confirmed that diclofenac also retains its hematopoiesis-stimulating action in fibrosarcoma-bearing mice [15]. Thus, our findings support the assumption that the anti-tumor effects of liposomal MTP-PE and diclofenac are mediated through stimulation of the hematopoietic and immune systems. The absence of mutually potentiating effects of liposomal MTP-PE and diclofenac found in most of our results is in general agreement with the observed undesirable action of combined administration of the drugs on the growth of tumors. The mechanisms of drug interactions leading to these undesirable effects remain still to be elucidated.

Our recent results support the following assumption: growth of fibrosarcoma G:5:113 cells *in vivo* is suppressed by various NSAIDs acting on the principle of inhibiting the production of PGs [17]. Since diclofenac does not significantly affect the proliferation of the G:5:113 cells *in vitro*, it has been concluded that its action is indirect and the cells of the granulocyte/macrophage lineage seem to be reasonable candidates for mediators of this effect. However, macrophages and other cells of the immune system are also targets for liposomal MTP-PE activating them to kill cancer cells. Undoubtedly, the functional changes in macrophages caused by diclofenac and/or liposomal MTP-PE consequently alter the activities of cytokines and other factors. Consequently, therefore, not only the direct cytotoxicity of macrophages but also the indirectly altered host defence system could be responsible for the lessened effects after combined liposomal MTP-PE and diclofenac administration. This suggests that any attempt to modulate the effects of immunotherapy using NSAIDs clinically (for example with the aim of reducing the side effects of immunotherapy) should be undertaken with great caution, until we have a better understanding of the underlying mechanisms of their action. For this reason, careful preliminary studies are recommended prior to the clinical use of NSAIDs in combination with other pharmacological approaches in cancer patients.

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