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Toxicity of a particulate formulation for the intraperitoneal application of mitoxantrone

Christian P. Luftensteiner [a], Ilse Schwendenwein [b], Hans G. Eichler [c], Bettina Paul [b], Gabriele Wölfl [d], Helmut Viernstein [a,*]

[a] *Institute of Pharmaceutical Technology, University of Vienna, Althanstraße 14, A-1090, Vienna, Austria*
[b] *Center for Biomedical Research, University of Vienna, Währinger Gürtel 18–20, A-1090, Vienna, Austria*
[c] *Department of Clinical Pharmacology, University of Vienna, Währinger Gürtel 18–20, A-1090, Vienna, Austria*
[d] *Department of Medical Statistics, University of Vienna, Schwarzspanierstraße 17, A-1090, Vienna, Austria*

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Abstract

Mitoxantrone (MXN) has demonstrated therapeutic efficacy in the intraperitoneal treatment of malignancies. However, severe local toxicity is dose limiting. Therefore, a particulate formulation of MXN, the drug incorporated in albumin microspheres, was evaluated concerning tolerability. Survival rates as well as alterations in body weight, food intake, water intake, urine volume, urine specific gravity, urine protein content, and complete blood count were observed following single or multiple intraperitoneal injections of MXN solution, dispersions containing MXN-loaded microspheres or unloaded microspheres, and the injection vehicle to female and male Sprague–Dawley rats. Applied MXN dosage was equivalent to 30 mg/m^2 body surface area. Unloaded microspheres were well tolerated without signs of toxicity. Application of MXN solution or MXN-loaded microspheres resulted in similar survival rates (56% 9 weeks after single injection) and in a comparable bone marrow toxicity (mainly leucopenia). Body weight, food and water intake as well as urine volume were decreased following application of MXN solution, whereas a progressive gain in weight and no remarkable alterations in nutrition and urine excretion were noted after administration of MXN-loaded and unloaded microspheres, or of the injection vehicle. In conclusion, intraperitoneal injection of MXN incorporated in albumin microspheres exhibits in part less toxicity than conventional treatment.

Keywords: Mitoxantrone; Albumin microspheres; Intraperitoneal chemotherapy; Animal studies; Toxicity; Survival

* Corresponding author. Tel.: +43-1-31336-8470; fax: +43-1-31336-8470.
E-mail address: helmut.viernstein@univie.ac.at (H. Viernstein)

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1. Introduction

For the treatment of peritoneal carcinomatosis, a common problem in the therapy of ovarian and gastrointestinal malignancies, direct intraperitoneal (ip) administration of anticancer drugs was introduced with the intention of achieving tumoricidal drug levels locally while minimizing systemic side effects (Blöchl-Daum et al., 1988; Knüsli et al., 1989). Much has been learned about the advantages and limitations of ip chemotherapy during the past decade. For some drugs local toxicity is dose limiting, other anticancer agents are easily absorbed into the circulation, thus doses are limited by systemic toxic effects (Markman et al., 1993). Hence, new delivery strategies were recently employed to overcome these restrictions (Hagiwara et al., 1992, 1993; Lee et al., 1995; Demetrick et al., 1997).

Mitoxantrone (MXN), an anthracenedione derivative, is an interesting drug for ip chemotherapy due to its low absorption into the circulation (Blöchl-Daum et al., 1988; Markman et al., 1993) and has demonstrated therapeutic efficacy in the regional treatment of malignant ascites (Lorusso et al., 1994; Maiche, 1994). However, little work was done to reduce its local, dose limiting toxicity, including significant abdominal pain, adhesion formation, and bowel obstruction (Markman et al., 1993). The side effects within the abdominal cavity were assumed to result from toxic MXN levels caused by ip short-term infusion of the drug. Therefore, we developed a new injectable sustained delivery formulation of MXN, the drug incorporated in albumin microspheres (Luftensteiner and Viernstein, 1998). In vivo, the initial high drug levels in peritoneal fluid observed after application of MXN in the solution form were prevented by administration of the drug incorporated in microspheres, whereas tumoricidal MXN levels were maintained for a comparable time span (Luftensteiner et al., 1999).

In the present study we examined, whether application of MXN incorporated in albumin microspheres reduces its local or systemic toxicity.

2. Materials and methods

2.1. Materials

Solid MXN dihydrochloride was generously supplied by Wyeth-Ayerst Lederle (Pearl River, New York) and MXN dihydrochloride concentrate for injection (Novantron®) was obtained from Wyeth-Lederle Austria (Vienna, Austria). Dextran FP40 (pyrogen-free) was purchased from Serva (Heidelberg, Germany). Heparin 1000 I.E./ml was obtained from Immuno (Vienna, Austria), isoflurane (Forane®) from Abbott (Vienna, Austria), and T61, a solution of embutramide, mebezoniumiodide, and tetracainhydrochloride, from Hoechst (Vienna, Austria). All other chemicals were of analytical grade.

2.2. Preparation and characterization of MXN-loaded and unloaded microspheres

MXN-loaded and unloaded albumin microspheres were manufactured and characterized as described previously (Luftensteiner and Viernstein, 1998; Luftensteiner et al., 1999). Briefly, an aqueous solution of human serum albumin (and MXN dihydrochloride for drug-loaded particles) was dispersed in dichloromethane containing poloxamer 188. The droplets were hardened by chemical crosslinking of the protein with glutaraldehyde and dehydrated with 1-butanol. After washing with acetone and water the microspheres were lyophilized. The products were characterized in terms of mean particle size and dispersity (span) applying a laser diffraction type particle size analyzer. MXN content was measured photometrically after enzymatic degradation of the microspheres and MXN release was studied in vitro using a continuous flow system. For the determination of residual glutaraldehyde content the particles were extracted by ultrasonication and the aldehyde concentration was measured by high-performance liquid chromatography (HPLC) after derivatization with 2,4-dinitrophenyl-hydrazine.

A gas chromatographic (GC) assay was developed for the determination of residual content of solvents (dichloromethane, 1-butanol, acetone) in the microspheres. Samples were prepared by

degradation of particles. Therefore, 5 mg microspheres were dispersed in 25 ml of a pH 2.0 buffer solution (KCl/HCl, 0.1 M) containing 50 mg pepsin (activity 3090 units/mg solid). Complete dissolution of matrix components was achieved after shaking the dispersion for 48 h in a water bath (37°C) and verified by light microscopy. 1 ml of an aqueous solution of toluene (4 μl/l) was added as internal standard. An aliquot of the mixture was incubated at 85°C for 10 min prior to injection to the GC using a head space autosampler (HS 850; Fisons Instruments, Rodano, Italy). The samples were analyzed applying a HRGC Mega 2 8560 apparatus (Fisons) equipped with a flame ionization detector and a capillary column (HP Innowax, 30 m × 0.53 mm i.d., 1.0 μm film thickness; Hewlett Packard, Vienna, Austria). The injection (170°C) and the detector temperature (270°C) were maintained constant. The oven temperature was programmed as follows: 40°C for 4 min, increase to 60°C using a 10°C/min rate, increase to 160°C using a 30°C/min rate, and finally 160°C for 3 min. Helium (7 psi) was used as the carrier gas. The chromatograms were recorded using Chrom-Card for Windows (Fisons). Controls were performed by spiking microspheres-containing or free samples with dichloromethane, 1-butanol as well as acetone and incubation with pepsin.

2.3. Animal studies

2.3.1. Animals

Forty-eight female, weighing 205–355 g, and 48 male, weighing 276–412 g, Sprague–Dawley rats were obtained from the Research Institute for Laboratory Animal Breeding of the University of Vienna (Himberg, Austria). The rats were maintained under standard conditions (standard diet, water ad libitum, and conventional housing) and received humane care according to the institutional guidelines for the use of animals in research throughout the studies. The animal studies were approved by the Austrian Federal Ministry of Science and Transport.

2.3.2. Drug preparation and dosage

Similar to clinical studies with anticancer agents, dosages in these studies were calculated from individual body surface area (SA) using the following formula (Chappell and Mordenti, 1991): SA (m^2) = 1.85 (body weight (g)/70000)$^{2/3}$. In order to achieve peritoneal fluid retention, thus simulating therapeutic conditions of a non-absorptive peritoneum, drugs were injected using a hyperosmotic dextran 40 solution (20 w/v%), which further contained 0.9 w/v% sodium chloride. The volume dosage was kept constant at 150 ml/m^2 SA. MXN was administered as solution (MXN–SOL) or in the form of a dispersion containing MXN-loaded microspheres (MXN–MSD), which were prepared by dilution of Novantron® or by dispersion of microspheres in the injection vehicle using an ultrasonic bath (60 W, 30 s), respectively. MXN dosage was 30 mg/m^2 SA. Dispersions containing unloaded (placebo) microspheres (PLA–MSD) were administered applying a dosage equivalent to MXN–MSD (384 mg microspheres/m^2 SA).

2.3.3. Design of the animal studies

In a multiple dose study two groups of 16 rats, with equal numbers of females and males, were treated ip with either MXN–SOL or MXN–MSD at day 0, 21, and 42.

A single dose study was performed applying four groups of rats, each consisting of eight females and eight males. One each of these groups was treated with injection vehicle (control), MXN–SOL, MXN–MSD, or PLA–MSD. Drugs were administered only once at day 0.

Total observation time in both studies was 9 weeks.

Body weight, food intake, water intake, urine volume, urine specific gravity, urine protein content, white blood cell count, red blood cell count, and platelet count were investigated at weekly intervals, starting at day 0 (baseline values). All 16 animals of a group were examined in terms of body weight and blood count, whereas four females and four males per group were investigated for food and water intake as well as urine volume, urine specific gravity, and urine protein content. These variables were measured after keeping the

animals in metabolic cages for 24 h. Urine specific gravity was determined using a refractometer (Krüss, Hamburg, Germany). Urine protein content was measured semiquantitatively by protein precipitation with 5-sulfosalicylic acid (Jaksch and Glawischnig, 1981). Blood was obtained from a metatarsal vein of a hind leg using a needle, which was previously rinsed with heparin solution, after anaesthetizing the rat with isoflurane. The blood samples were collected into K_3EDTA tubes (Becton Dickinson, Heidelberg, Germany) and the complete blood count was determined by an automated blood analyzer (Cell-Dyn 3500; Abbott Diagnostics, Vienna, Austria); the threshold and gain settings were adjusted to rat blood.

At day 63 the surviving animals were anaesthetized with isoflurane, euthanized by intracardiac injection of T61, and examined postmortem after laparotomy. The peritoneal cavity was examined whether tissue inflammation, ascites, or adhesions occurred or not.

2.3.4. Data analysis

A log-rank test (Kaplan Meier) was used to compare the survival of rats of different treatment groups (Kleinbaum, 1996).

An analysis of variance with repeated measurements was applied for the variables body weight, food intake, water intake, urine volume, urine specific gravity, white blood cell count, red blood cell count, and platelet count (Vonesh and Chinchilli, 1996). The factors were drug (control, MXN–SOL, MXN–MSD, and PLA–MSD), sex, and the interaction drug–sex. The covariable was the respective baseline value (day 0). A post hoc analysis (Tukey test) was performed for each observation time to localize the significant differences (Scheffé, 1959). From the analysis of variance with repeated measurements the factor time and the interaction term time–drug were considered.

The relations between urine protein content and drug as well as between urine protein content and sex were analyzed by Fisher's exact test (Sachs, 1992). Separate analyses were made for each timepoint.

In all cases, $P < 0.05$ was accepted to denote significance.

3. Results

3.1. Microsphere properties

The batches of MXN-loaded and unloaded microspheres administered in the animal studies were characterized as shown in Table 1. Residual dichloromethane content in the microspheres was lower than the limit (500 ppm) given by the US Pharmacopeia (1995) and residual content of 1-butanol as well as of acetone was under the limit (5000 ppm) proposed by the ICH (International Conference on Harmonisation, 1997). Also the extractable amount of residual glutaraldehyde in the particles was very low and therefore no restriction on the application of the formulation.

Table 1
Characteristics of MXN-loaded and unloaded microspheres administered in the animal studies (means of three measurements)

Parameter	Formulation	
	MXN-loaded microspheres	Unloaded microspheres
Mean particle size (μm)	29.1	30.8
Dispersity (span)[a]	1.14	1.24
MXN content (w/w%)	7.82	–
First order MXN release constant (h^{-1})[b]	0.0481	–
Residual glutaraldehyde content (ppm)	5.2	8.5
Residual dichloromethane content (ppm)	356	236
Residual 1-butanol content (ppm)	<10[c]	<10
Residual acetone content (ppm)	120	67

[a] Span $= (D_{90\%} - D_{10\%})/D_{50\%}$ where $D_{10\%}$, $D_{50\%}$, and $D_{90\%}$ are the 10, 50, and 90% quantiles (μm) of the size distribution (Luftensteiner et al., 1999).

[b] $A_t = A_0 e^{-kt}$ where A_t (mg) is the amount of drug remaining to be released, A_0 (mg) is the original amount of drug present in the carrier matrix, k (h^{-1}) is the first order release constant, and t (h) is the time in which the release of drug occurs (Luftensteiner and Viernstein, 1998).

[c] Detectable, but under the quantifiable limit.

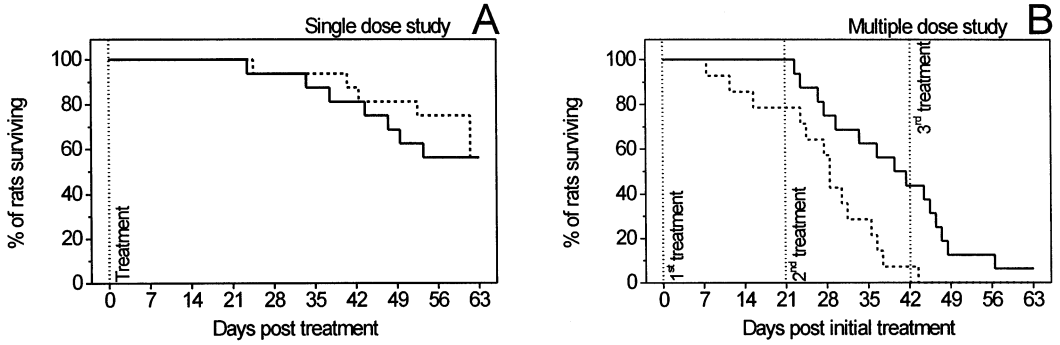

Fig. 1. Survival of rats after ip injection of MXN–SOL (– – –) or MXN–MSD (—). (A) single dose study ($n = 16$ in both groups); (B) multiple dose study ($n = 14$ in the MXN–SOL group due to application failure in two animals; $n = 16$ in the MXN–MSD group).

3.2. Animal studies

3.2.1. Animal survival

In the single dose study none of the animals receiving PLA–MSD or the injection vehicle died during observation time, whereas only nine of 16 animals of the groups treated with MXN–SOL or MXN–MSD survived for 9 weeks. An even higher mortality was observed in the multiple dose study. The respective survival curves are shown in Fig. 1. Animals treated in 3-weekly intervals with MXN–MSD survived significantly ($P < 0.005$) longer than rats receiving MXN–SOL, whereas no significant difference between these treatment groups was found in the single dose study.

3.2.2. Effects and interactions of drug, sex, and time on body weight, nutrition, urine excretion, and blood count

Due to the high mortality data analysis of the variables concerning body weight, nutrition, urine excretion, and blood count was only justifiable for as long as all animals of the study were alive. Hence, these variables were statistically analyzed entirely for the single dose study up to day 21.

A progressive increase in body weight was observable after administration of injection vehicle, MXN–MSD, and PLA–MSD, whereas the animals treated with MXN–SOL slightly lost weight up to 2 weeks after injection (effect of drug: $P < 0.0001$; Fig. 2), thus explaining the significant ($P < 0.0001$) interaction time–drug on body weight. This effect was more pronounced in males than females (effect of sex: $P < 0.05$; interaction drug–sex: $P < 0.001$). The post hoc analysis revealed a significant ($P < 0.05$) higher body weight of animals treated with MXN–MSD than rats receiving MXN–SOL.

Food and water intake was markedly reduced following administration of MXN–SOL to female rats, whereas no remarkable alterations in feeding and drinking were noted in the other treatment groups (effect of drug: $P < 0.001$) and, generally, concerning male rats (effect of sex: $P < 0.01$; Fig. 3A–D). The Tukey test showed a significant ($P < 0.05$) higher food and water intake of animals receiving MXN–MSD than of rats treated with MXN–SOL, but no significant differences between the groups, where MXN–MSD, PLA–MSD, or the injection vehicle were administered.

Urine volume decreased considerably in female animals treated with MXN–SOL. Alterations were less obvious in the other treatment groups (effect of drug: $P < 0.001$) and in males (Fig. 3E and F), even though the effect of sex and the interaction drug–sex were not significant. Differences between rats treated with MXN–SOL or MXN–MSD were also not significant. The factor drug showed no influence on urine specific gravity ($P > 0.05$). There was also no significant difference between the four drug groups concerning urine protein content, but, generally, female rats (< 10 mg/100 ml) showed a lower urine protein

content than male animals (10–100 mg/100 ml; $P < 0.0001$).

White blood cell count decreased in male and female rats treated with MXN irrespective of the administered formulation type (effect of drug: $P < 0.0001$). The nadir was observed at day 7 after injection and was followed by a slow, progressive increase (interaction time–drug: $P < 0.0001$; Fig. 4A and B). Also the red blood cell count decreased considerably after ip injection of the cytotoxic drug (effect of drug: $P < 0.0001$; interaction time–drug: $P < 0.0001$), the nadir at day 14 was less severe than the drop in the leucocyte counts and was more evident in female than male animals (effect of sex: $P < 0.0001$; interaction drug–sex: $P < 0.005$; Fig. 4C and D). As shown by the Tukey test the differences between the groups receiving MXN–SOL or MXN–MSD were not significant, but their leucocyte and erythrocyte counts were significantly ($P < 0.05$) different from animals treated with injection vehicle or PLA–MSD. Platelet counts were markedly decreased at day 7 and increased at day 14 (significantly higher in the MXN–SOL group than in rats receiving MXN–MSD, $P < 0.05$) after administration of MXN in comparison to the levels in rats treated with unloaded microspheres or dextran solution (effect of drug: $P < 0.005$; Fig. 4E and F), which is confirmed by a highly significant ($P < 0.0001$) interaction time–drug and by significant ($P < 0.05$) differences between the respective groups in the Tukey test at both time points. Platelet counts returned to baseline values by day 21 after drug administration.

Differences between rats treated with injection vehicle or PLA–MSD concerning all the observed variables were not significant at any time point.

3.2.3. Autopsy

Almost all of the animals treated with MXN developed severe chemoperitonitis, irrespective of whether the drug was applied as solution or incorporated in microspheres. Peritoneal inflammation was accompanied by peritoneal bleeding, hemorrhagic ascites, peritoneal adhesions, and, in some cases, intestinal impaction due to adhesions. Treatment with injection vehicle or PLA–MSD was well tolerated.

4. Discussion

MXN interferes with the function of human peritoneal mesothelial cells at the concentration of 10^{-4} M in vitro, which might contribute to the chemotherapy induced peritoneal toxicity (Witowski et al., 1995). Recently, we have demonstrated that in contrast to treatment with MXN–SOL ip drug levels were far below 10^{-4} M after application of MXN–MSD equivalent to 30, 60, or 120 mg MXN/m^2 SA. Hence, the incidence of acute chemoperitonitis was markedly lower after administration of MXN–MSD than following injection of the equivalent MXN dosage in the

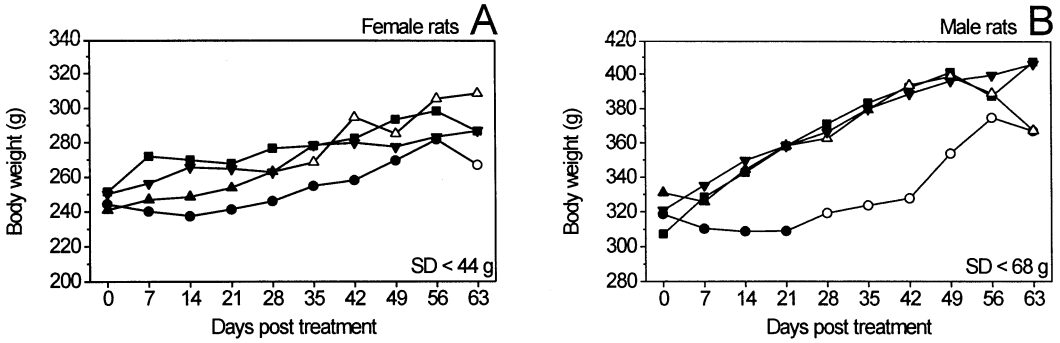

Fig. 2. Changes of body weight following ip administration of injection vehicle (■), MXN–SOL (●), MXN–MSD (▲), or PLA–MSD (▼) to female (A) or male (B) rats. Closed symbols represent means of eight, open symbols means of less than eight animals (SD: standard deviation).

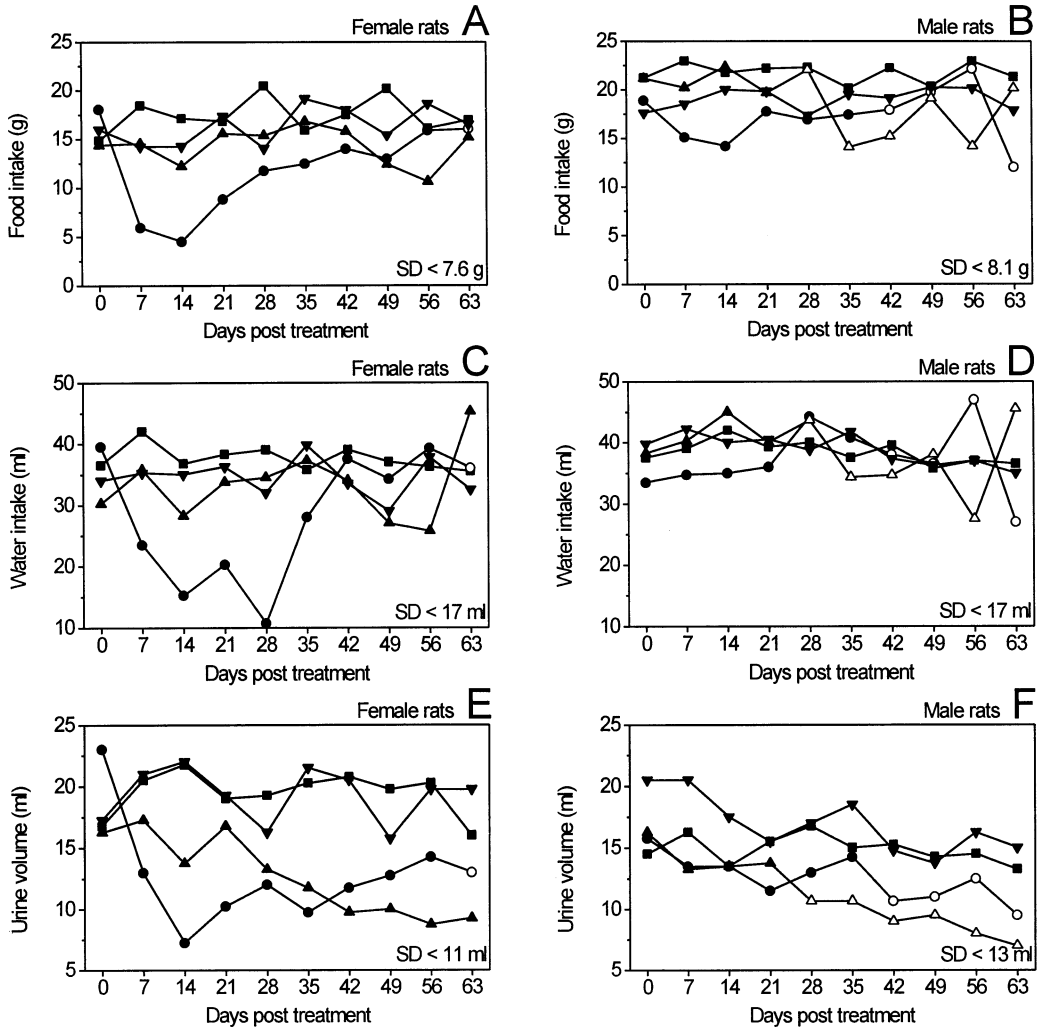

Fig. 3. Changes of food intake (A, B), water intake (C, D), or urine volume (E, F) following ip administration of injection vehicle (■), MXN–SOL (●), MXN–MSD (▲), or PLA–MSD (▼) to female (A, C, E) or male (B, D, F) rats. Closed symbols represent means of four, open symbols means of less than four animals (SD: standard deviation).

solution form. Based on the results of the previous short-term study a dosage increase from 30 to 60 mg MXN/m^2 SA was considered to be feasible in ip chemotherapy (Luftensteiner et al., 1999).

Therefore, the initial concept for the long-term toxicity studies described in this paper was to evaluate initially a MXN dosage of 30 mg/m^2 SA, which was recommended as the maximal dose in ip chemotherapy applying short-term infusion (Blöchl-Daum et al., 1988), and subsequently 60 mg/m^2 SA. The high mortality observed in the present studies was unexpected and suggests a higher toxicity of ip MXN delivery in rats in comparison to man. Higher systemic toxicity might be explained by an increased absorption of MXN to circulation in rats, indicated by a lower ratio of AUC (area under the concentration–time curve) in peritoneal fluid to AUC in plasma than in man (370 versus 1408, respectively), whereas AUC in peritoneal fluid was comparable (Alberts et al., 1988; Luftensteiner et al., 1999). Application of the hyperosmotic dextran solution as injec-

tion vehicle might have influenced absorption to circulation and local tolerability of MXN. However, the use of the hyperosmotic dextran solution was necessary in order to achieve peritoneal fluid retention, thus simulating therapeutic conditions of a non-absorptive peritoneum and enabling MXN release from the microspheres, and seemed to be ethically more justifiable than pretreatment with irritating agents, such as carrageenan (Diwan et al., 1995).

Hematological toxicity was comparable after treatment with MXN–SOL or MXN–MSD, which might be explained by similar AUC values of MXN in plasma, even though peak concentrations were lowered by a factor of 3.5 applying the sustained delivery formulation (Luftensteiner et al., 1999). Leucopenia due to bone marrow toxicity is a common side effect of MXN, whereas a decrease in red blood cell counts was unexpected, but might also be explained by a bone marrow damage as erythrocyte life span in rats is shorter than in man, about 45–50 in comparison to 120

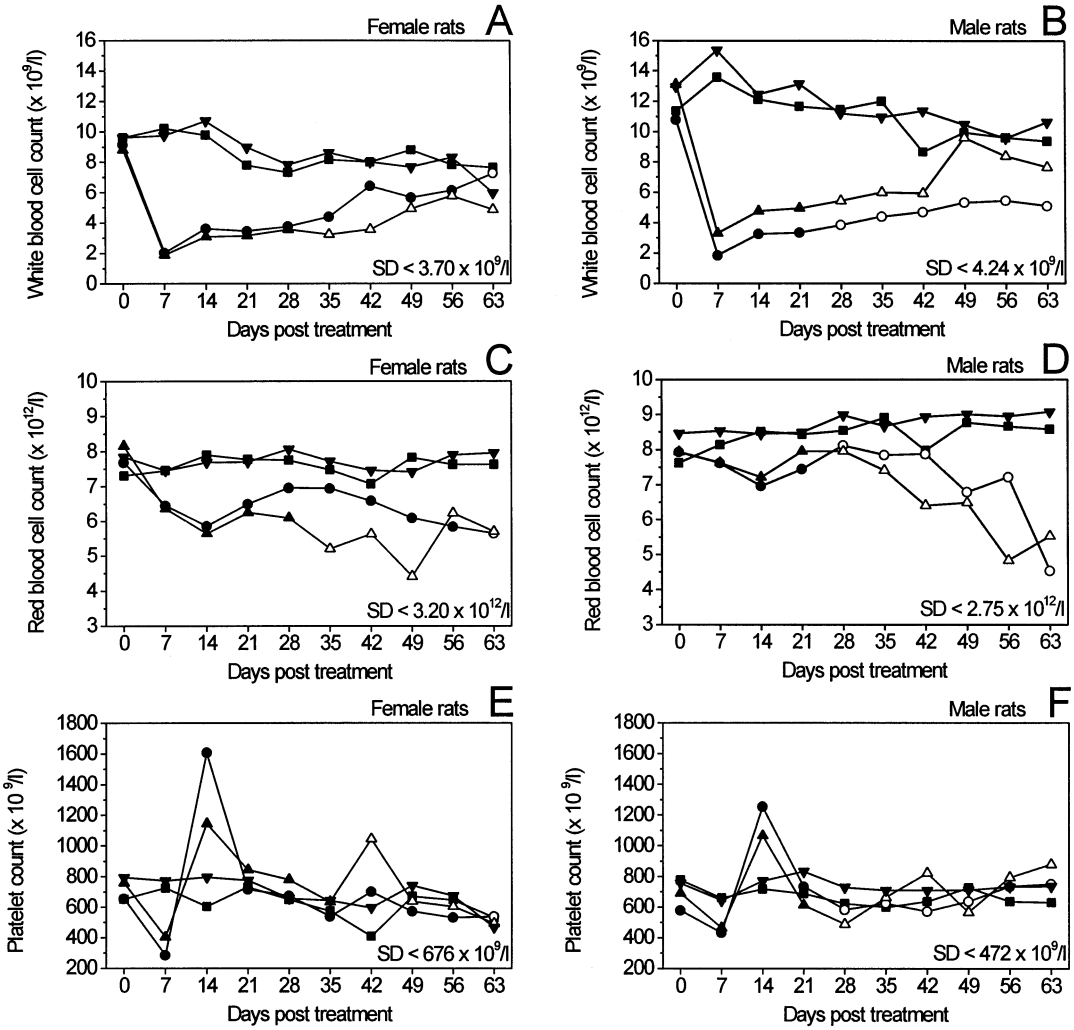

Fig. 4. Changes of white blood cell count (A, B), red blood cell count (C, D), or platelet count (E, F) following ip administration of injection vehicle (■), MXN–SOL (●), MXN–MSD (▲), or PLA–MSD (▼) to female (A, C, E) or male (B, D, F) rats. Closed symbols represent means of eight, open symbols means of less than eight animals (SD: standard deviation).

days, respectively (Jain, 1993). Progressive peritoneal bleeding might be responsible for decreased platelet counts at day 7, which were then markedly increased at day 14, suggesting a reactive platelet generation triggered by chemoperitonitis and hemorrhages.

In addition to hematological toxicity, the severe lesions within the peritoneal cavity were supposed to be further causes for animal morbidity and mortality. Neither survival analysis nor autopsy revealed marked differences between the animals treated with MXN–SOL and MXN–MSD.

However, the comprehensive analysis of body weight and nutrition revealed a superior tolerability of MXN–MSD over MXN–SOL. Decreased food and water intake as well as weight loss are related to severe morbidity of the animals. Concerning nutrition female animals seemed to be more sensitive to treatment with MXN–SOL than males.

As systemic toxicity was comparable following application of the two formulations of MXN differences in body weight, feeding as well as drinking and, consequently, in morbidity were considered to result from a different local toxicity, at least in the first 3 weeks after drug application. However, the long-term toxicity of the two MXN formulations seems to be equal, indicated by similar survival rates and a comparable grade of peritoneal lesions.

In conclusion, we have shown that ip injection of MXN incorporated in albumin microspheres exhibits in part less toxicity than treatment with MXN–SOL, even though not to the extent, which was expected from the improved MXN concentration profiles in peritoneal fluid after administration of MXN–MSD demonstrated previously (Luftensteiner et al., 1999). Unloaded albumin microspheres prepared by our manufacturing technique were well tolerated without any local or systemic side effects and are therefore proposed as potential drug carriers for intracavitary application.

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References

Alberts, D.S., Surwit, E.A., Peng, Y.M., McCloskey, T., Rivest, R., Graham, V., McDonald, L., Roe, D., 1988. Phase I clinical and pharmacokinetic study of mitoxantrone given to patients by intraperitoneal administration. Cancer Res. 48, 5874–5877.

Blöchl-Daum, B., Eichler, H.G., Rainer, H., Jakesz, R., Salzer, H., Steger, G., Schüller, J., Günther, E., Proksch, B., Ehninger, G., 1988. Escalating dose regimen of intraperitoneal mitoxantrone: phase I study—clinical and pharmacokinetic evaluation. Eur. J. Cancer Clin. Oncol. 24, 1133–1138.

Chappell, W.R., Mordenti, J., 1991. Extrapolation of toxicological and pharmacological data from animals to humans. In: Testa, B. (Ed.), Advances in Drug Research, vol. 20. Academic Press, London, pp. 1–116.

Demetrick, J.S., Liggins, R.T., Machan, L., Davis, N.L., Burt, H.M., Hunter, W.L., 1997. The development of a novel intraperitoneal tumor-seeding prophylactic. Am. J. Surg. 173, 403–406.

Diwan, P.V., Margaret, I., Ramakrishna, S., 1995. Influence of gymnema sylvestre on inflammation. Inflammopharmacology 3, 271–277.

Hagiwara, A., Takahashi, T., Kojima, O., Sawai, K., Yamaguchi, T., Yamane, T., Taniguchi, H., Kitamura, K., Noguchi, A., Seiki, K., Sakakura, C., 1992. Prophylaxis with carbon-adsorbed mitomycin against peritoneal recurrence of gastric cancer. Lancet 339, 629–631.

Hagiwara, A., Takahashi, T., Kojima, O., Yamaguchi, T., Sasabe, T., Lee, M., Sakakura, C., Shoubayashi, S., Ikada, Y., Hyon, S.H., 1993. Pharmacologic effects of cisplatin microspheres on peritoneal carcinomatosis in rodents. Cancer 71, 844–850.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1997. ICH Harmonised Tripartite Guideline, Impurities: Guideline for Residual Solvents, Step 4 (Final Draft), 17 July.

Jain, N.C., 1993. Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia, PA.

Jaksch, W., Glawischnig, E., 1981. Klinische Propädeutik der inneren Krankheiten und Hautkrankheiten der Haustiere, 2nd edn. Parey, Berlin.

Kleinbaum, D.G., 1996. Survival Analysis. Springer, Berlin.

Knüsli, C., Ruff, P., Laffer, U., Stoll, H.R., Obrist, R., Weber, W., Obrecht, J.P., 1989. Intraperitoneal chemotherapy in peritoneal malignancy: impact of intensive system care on practicability. Acta Med. Austriaca 16, 70–73.

Lee, J.S., Takahashi, T., Hagiwara, A., Yoneyama, C., Itoh, M., Sasabe, T., Muranishi, S., Tashima, S., 1995. Safety and efficacy of intraperitoneal injection of etoposide in oil suspension in mice with peritoneal carcinomatosis. Cancer Chemother. Pharmacol. 36, 211–216.

Lorusso, V., Catino, A., Gargano, G., Fioretto, A., Berardi, F., De Lena, M., 1994. Mitoxantrone in the treatment of recurrent ascites of pretreated ovarian carcinoma. Eur. J. Gynaec. Oncol. 15, 75–80.

Luftensteiner, C.P., Viernstein, H., 1998. Statistical experimental design based studies on placebo and mitoxantrone-loaded albumin microspheres. Int. J. Pharm. 171, 87–99.

Luftensteiner, C.P., Schwendenwein, I., Paul, B., Eichler, H.G., Viernstein, H., 1999. Evaluation of mitoxantrone-loaded albumin microspheres following intraperitoneal administration to rats, J. Control. Release, 57, 35–44.

Maiche, A.G., 1994. Management of peritoneal effusions with intracavitary mitoxantrone or bleomycin. Anti-Cancer Drugs 5, 305–308.

Markman, M., Reichman, B., Hakes, T., Curtin, J., Jones, W., Lewis, J.L. Jr., Barakat, R., Rubin, S., Mychalczak, B., Saigo, P., Almadrones, L., Hoskins, W., 1993. Intraperitoneal chemotherapy in the management of ovarian cancer. Cancer 71, 1565–1570.

Sachs, L., 1992. Angewandte Statistik, 7th edn. Springer, Berlin.

Scheffé, H., 1959. The Analysis of Variance. Wiley, New York.

US Pharmacopeia XXIII, 1995. US Pharmacopeial Convention, Rockville, MD, pp. 1746–1748.

Vonesh, E.F., Chinchilli, V.M., 1996. Linear and Nonlinear Models for the Analysis of Repeated Measurements. Dekker, New York.

Witowski, J., Breborowicz, A., Knapowski, J., 1995. Effect of methotrexate, doxorubicin and mitoxantrone on human peritoneal mesothelial cell function in vitro. Oncology 52, 60–65.