The Effect of the Substitution Level of Some Dextran-methotrexate Conjugates on their Antitumor Activity in Experimental Cancer Models

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Abstract. *Methotrexate (MTX) is widely used in the treatment of a number of oncological and hematological diseases. Due to its known limitations, MTX is often conjugated with different carriers to obtain amended forms of the drug. In this study, the potential influence of the substitution level (loading ratio) of the dextran T10- and T40-based MTX conjugates (D-MTX) on their properties were investigated in vitro and in vivo. The clear dependence of the in vitro antiproliferative effect on the substitution level was established only in the case of the dextran T10-based preparations (T10-MTX conjugates). Conjugates with the higher substitution level had the lower antiproliferative effect. For the dextran T40-based (T40-MTX conjugates) set no similar relationship was observed in the tested range of substitution levels, nor was any dependence observed between the biological properties of the D-MTX preparations in vivo and their substitution levels. However, the difference between the two conjugates was well pronounced in a multiple-dose schedule, when the advantage of T40-MTX over T10-MTX was cumulative during the prolonged course of administration.*

Methotrexate (MTX), an inhibitor of dihydrofolate reductase and thymidylate synthase, is widely used in the treatment of a number of oncological, hematological and immunological diseases (1, 2). However, MTX also has known limitations, due to its low plasma half-life, toxicity for normal proliferating cells and resistance by tumor cells. These limitations have prompted researchers to find strategies for improvement. One of the strategies frequently applied in improving drug activity is the conjugation of chemotherapeutic drugs with carriers.

Conjugation often results in a prolongation of the therapeutic effect, alteration of the toxicity profile and a reduction in the immunogenicity of the parental drugs (3). Coupling these agents with different carriers was shown to increase their plasma half-life, since macromolecules with high molecular weight (Mw) have lower clearance rates (4). Accumulation of the conjugated chemotherapeutics in the tumor was also postulated. The mechanism of this passive targeting is believed to be dependent on the increased permeability of the tumor vasculature and retention of macromolecules in the tumor site (5). We, and other authors, have published a number of reports about the conjugates of MTX with different carriers: fibrinogen (6, 7), albumin (8, 9), branched polypeptide (10), polyethylene glycol (11) and dextrans (12-15).

Recently, studies on MTX coupled with different dextrans were initiated by our group (14). These glucose polymers have been used in clinical medicine for several decades as plasma volume expanders. The structure of dextran macromolecules is suitable for conjugation due to the presence of a large number of hydroxyl groups, which can be conjugated to drugs or proteins (3). Another advantage of these macromolecules is that they are neutral in their native form (4). Altogether, these facts make dextrans good candidates for drug carriers. Comprehensive reviews, focusing on studies with dextran conjugates, have been published recently (3, 4).

There are several published reports on conjugates of MTX with dextrans, however, these mainly concentrated on dextrans with Mw 40 and 70 kDa (16-20). Our results on the influence of the Mw of the carrier on the antitumor properties and toxicity of the D-MTX conjugates were

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Key Words: Dextran, methotrexate conjugates, antitumor activity, leukemia.

recently reported (14). Several dextrans with Mw ranging from 10 to 500 kDa were tested and an increase of the toxicity with the rise of the Mw of the carrier macromolecule was observed. Due to their lower toxicity among all preparations tested, two conjugates, based on dextrans with Mw 10 and 40 kDa, were selected for further investigations.

Another possible point of concern during the synthesis of D-MTX conjugates is the level of substitution. This determines the number of MTX molecules coupled to one macromolecule of glucose polymer. The aim of this study was to investigate whether there is any dependence of the antitumor effect and toxicity of the D-MTX conjugates *in vitro* and *in vivo* on the substitution level. Two sets of conjugates were applied, based on dextrans with Mw of 10 and 40 kDa. These carriers were selected on the basis of our previous results (14). Both conjugates were also compared with the parental drug in the multiple-dose schedule in the P388 mouse leukemia *in vivo* model.

Materials and Methods

Conjugates of MTX with dextrans. All dextrans were obtained from Pharmacia (Fine Chemicals AB, Uppsala, Sweden). MTX was obtained from Lachema (Brno, Czech Republic). The method of conjugation was previously described in detail (14). Two sets of conjugates were synthesized by the reaction with the methotrexate anhydride. The first set consisted of four conjugates based on the dextran T10 (Mw 10 kDa). The conjugates were coded as T10-SP1 (0.0095; 0.6), T10-SP2 (0.0200; 1.3), T10-SP4 (0.0450; 2.8) and T10-SP8 (0.0750; 4.7). The numbers in brackets after each code represent the levels of substitution. The first number is the level of substitution expressed as number of moles of MTX per mole of the glucose monomer and the second one is the level of substitution expressed as number of moles of MTX per mole of the dextran polymer. The second set consisted of four conjugates based on the dextran T40 (Mw 40 kDa). The conjugates were coded as T40-SP2 (0.008; 2.0), T40-SP3 (0.012; 3.0), T40-SP4 (0.016; 4.0) and T40-SP6 (0.024; 6.0). For multiple-dose schedule experiments, one conjugate based on the dextran T10 and another conjugate based on the dextran T40, coded as T10-MTX and T40-MTX, respectively, were used. Both have the same levels of substitution 0.022, relative to the glucose monomer. Free MTX was used as the reference drug during all the *in vitro* and *in vivo* experiments.

Experimental animals. Male and female (C57Bl/6 x DBA/2)F1 ($B_6D_2F_1$) mice, aged 15-32 weeks, weighing 20-29 g, were applied. Each separate experiment was performed on animals of the same gender and comparable age and weight. The mice were supplied from the Animal Breeding Centre of the Medical Academy, Wroclaw, Poland, and were maintained in standard laboratory conditions. Experiments were performed according to the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing and Education issued by the New York Academy of Sciences' *Ad Hoc* Committee on Animal Research and were approved by the First Local Ethical Committee for the use of Laboratory Animals, Wroclaw, Poland.

Mice were injected with 10^6 leukemia (P388) cells *i.p.* (day–1), and 24 h later (day 0) each mouse was injected once *i.p.* with the appropriate agent. In the experiment with the multiple-dose schedule, drugs were injected on days 0, 3 and 6 of the experiment. All doses were based on the molarity of the MTX in the conjugates. Body weight and survival data were collected on a daily basis throughout the duration of the experiment. In the first and second *in vivo* experiments, all animals were randomly divided into six groups (7-8 mice). The animals in the control and the MTX groups were administered 0.9% saline solution or 40 mg/kg of free MTX, respectively. Another four groups were administered 40 mg/kg of D-MTX conjugates (based on the dextran T10 in the first experiment and on the dextran T40 in the second one). In the third experiment with a multiple-dose schedule, the animals were randomly divided into four groups. The animals in the control and MTX groups were administered 0.9% saline solution or 20 mg/kg/day of free MTX, respectively. Another two groups were administered 20 mg/kg/day of T10-MTX or T40-MTX conjugates, respectively. Two experiments with identical protocols were conducted and comparable results were obtained, so they were pooled and analyzed together.

Cell lines. A549 (human non-small cell lung carcinoma), SW707 (human colon adenocarcinoma) and P388 (murine leukemia) were obtained from the American Type Culture Collection (Rockville, MD, USA) and were maintained in culture or were frozen in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. Twenty-four hours before addition of the tested preparations, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of $0.5x10^4$ cells per well and were cultured a the mixture of RPMI 1640 and Opti-MEM (1:1). The medium was supplemented with 2 mM glutamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 100 mg/ml streptomycin (Polfa, Tarchomin, Poland) and 100 U/ml penicillin (Polfa), 5% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The cells were cultured at 37˚C in a humid atmosphere saturated with 5% CO_2. For *in vivo* experiments, passages of P388 leukemia cells in DBA/2 mice were carried out according to the NIH/NCI standard screening protocols *in vivo* (21, 22).

Antiproliferative assays in vitro. The *in vitro* cytotoxic effect of all agents was examined after 72-h exposure of the cultured cells to different concentrations of the test preparations, using the SRB assay as described by Skehan *et al.* (23). Briefly, the cells were attached to the bottom of plastic wells by fixing them with cold 50% (80% in the case of the P388 cell line) trichloroacetic acid (TCA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The plates were incubated at 4˚C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was then stained for 30 min with 0.4% sulforhodamine B (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland). Unbound dye was removed by rinsing (4x) with 1% acetic acid. The protein-bound dye was extracted with 10 mM TRIS base (POCH) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland).

Table I. *Antiproliferative in vitro activity of different D-MTX conjugates in comparison with free MTX.*

Preparation	Substitution level (relative to dextran)	IC_{50}±SD μg/ml		
		Cell line		
		A549	SW707	P388
MTX[a]		0.054±0.009	0.034±0.015	0.0046±0.0019
T10-SP1[b]	0.6	0.290±0.081	0.228±0.217	0.0191±0.0040
T10-SP2[b]	1.3	0.452±0.161	0.413±0.160	0.0326±0.0053
T10-SP4[b]	2.8	0.855±0.236	0.771±0.156	0.0578±0.0066
T10-SP8[b]	4.7	0.800±0.142	0.936±0.429	0.0524±0.0200
T40-SP2	2.0	0.512±0.206	0.366±0.122	0.0307±0.0047
T40-SP3	3.0	0.515±0.202	0.384±0.110	0.0303±0.0049
T40-SP4	4.0	0.534±0.133	0.401±0.124	0.0329±0.0051
T40-SP6	6.0	0.541±0.156	0.414±0.090	0.0364±0.0038

[a]all conjugates revealed lower *in vitro* antiproliferative activity than MTX alone ($p<0.001$).
[b]T10-SP1 *vs.* T10-SP2 ($p<0.05$), T10-SP2 *vs.* T10-SP4 ($p<0.05$), T10-SP4 *vs.* T10-SP8 (not significant). Cuzick's test showed that there was a statistically significant trend for increasing IC_{50} values with the conjugate's level of substitution in A549 ($p<0.01$), SW707 ($p<0.01$) and P388 ($p<0.01$) cell lines.

Data handling. The *in vitro* results were presented in terms of IC_{50} values. The IC_{50} is the concentration of a tested agent which inhibits the proliferation of 50% of the cancer cell population. Average IC_{50} values for each preparation were calculated using data from three independent experiments. The antitumor effect *in vivo* was evaluated as the increase in lifespan (ILS) of treated mice over control, calculated from the following formula: (MST_T/MST_C) x 100-100, where MST_T is the median survival time of treated animals and MST_C is the median survival time of untreated control mice. Overall toxicity in the experimental groups was expressed as the number of treated mice that died before the day of the first death registered in the control group.

Statistical evaluation. The analysis of *in vitro* data was performed by two-way ANOVA with post-hoc Tukey comparison after natural logarithmic data transformation to meet parametric assumptions. Cuzick's nonparametric test for trend was used to explore the relationship between the substitution level of the conjugate and the IC_{50} values (24). Survival data in experimental *in vivo* groups were compared using the Cox's F test with Bonferroni correction for multiple comparisons ($p_{adjusted} = p_{counted}$ x N, where N = number of pairwise comparisons). *P* values less than 0.05 were considered significant.

Results

In vitro antiproliferative activity of D-MTX conjugates with different levels of substitution. Two sets of D-MTX conjugates were tested in this experiment. Each set was synthesized using a different dextran carrier (T10 and T40, respectively) and conjugates in each set differed in the levels of substitution. The results of our *in vitro* experiments are summarized in Table I. Studies *in vitro* confirmed our previous report (14) that D-MTX conjugates have approximately 4- to 10-fold higher IC_{50} values in comparison with that of free MTX.

Data obtained from the first set of conjugates, synthesized using the dextran T10 carrier, revealed the dependency of IC_{50} values on the level of substitution. ANOVA analysis indicated that there were statistically significant differences between all four preparations, except the last two (Table I). This dependency was found by Cuzick's test to be statistically significant for all three lines tested: A549 ($p<0.01$), SW707 ($p<0.01$) and P388 ($p<0.01$).

Interestingly, conjugates from the second set, synthesized using dextran T40 as a carrier, did not show any dependency of *in vitro* antiproliferative activity on the level of substitution. The IC_{50} values of all four dextran T40-based preparations were approximately the same and neither ANOVA nor Cuzick's test revealed any differences between them (Table I).

Table II. *Survival data of leukemia-bearing mice treated with free MTX or dextran T10-based D-MTX conjugates, expressed as an ILS – increase in lifespan.*

Group	N[a]	ILS (%)	MST (days)[b]	L[c]
Control	8	0	11.0	
MTX, 40 mg/kg	8	50	16.5	0
T10-SP1 conjugate, 40 mg/kg	8	41	15.5	0
T10-SP2 conjugate, 40 mg/kg	8	45	16.0	0
T10-SP4 conjugate, 40 mg/kg	8	50	16.5	0
T10-SP8 conjugate, 40 mg/kg	8	36	15.0	0

[a]N, number of mice in group.
[b]Median survival time.
[c]Number of mice that died before control group.

In vivo toxicity and antitumor effect of D-MTX conjugates with different levels of substitution. We decided to check whether there was dependency of *in vivo* antitumor activity on the level of substitution of the conjugate. Briefly, $B_6D_2F_1$ mice bearing P388 leukemia were *i.p.* injected once with either free MTX or one of the four dextran T10-based D-MTX preparations at a dose of 40 mg/kg. Surprisingly, in spite of the distinct *in vitro* antiproliferative activity of these conjugates, the median survival times in the experimental groups were comparable. Statistical analysis did not reveal any significant differences in survival between the free MTX- and D-MTX-treated groups. There was no toxicity-caused lethality among the D-MTX-treated groups, since no mice died before the animals in the control group. Changes in average body weights after injection of the test preparation allowed us to suggest that the toxicity of all four conjugates was roughly the same (Figure 1). The results of the experiment are summarized in Table II.

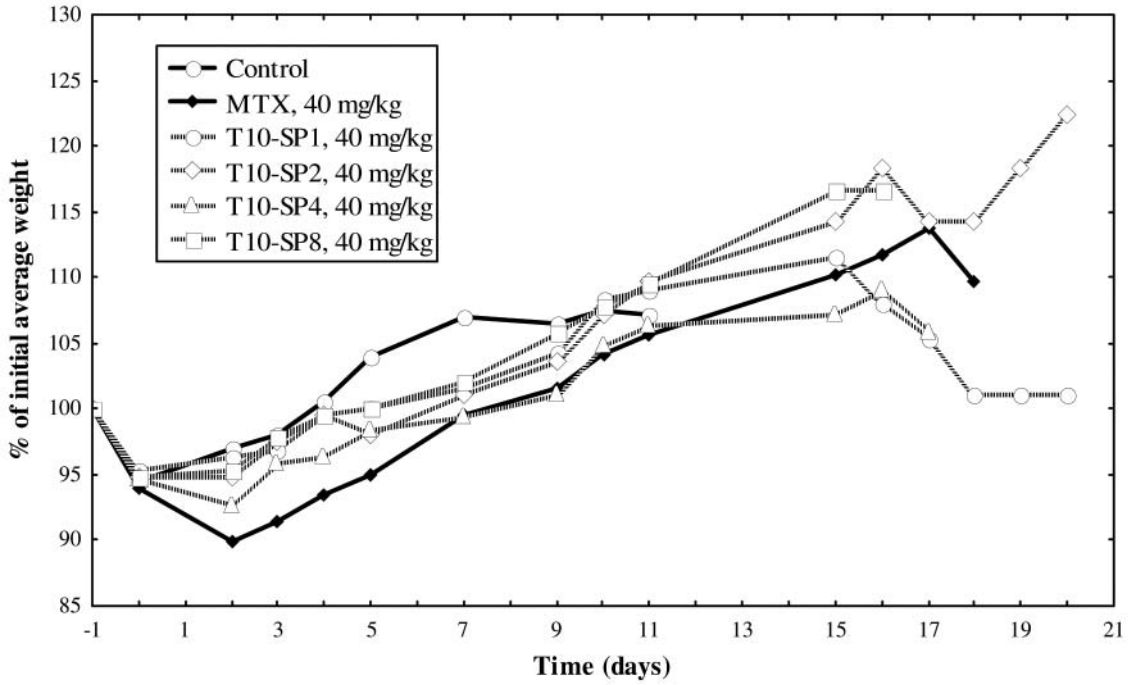

Figure 1. *Dynamics of body weight changes in leukemia-bearing mice treated with either MTX or one of the dextran T10-based D-MTX conjugates.*

Next, the same *in vivo* model was utilized as described above and the second, dextran T40-based set of conjugates was investigated. The results of the experiment are summarized in Table III. Again, there were no statistically significant differences in survival between groups treated with the conjugates. Noticeably, the median survival times and ILS in these groups tended to be even higher in comparison with the free MTX-treated group, but the effect of size was not sufficient to be statistically significant in this model. The toxicity of dextran T40-based D-MTX conjugates was approximately the same for all four preparations tested. However, it lasted longer and tended to be more profound in comparison with the free MTX-treated group (Figure 2).

Antitumor properties of T10-MTX and T40-MTX conjugates in a model with multiple-dose schedule. The previous experiment using the one dose schedule showed a marginally positive effect of conjugates on the survival of the leukemia-bearing mice, but not significant. To further assess the properties of these conjugates, a model with a multiple-dose schedule was applied. It was hypothesized that the differences between free MTX and conjugates would be more evident when they were applied several times. Leukemia P388-bearing mice were injected with free MTX or either of two selected conjugates at a dose of 20 mg/kg three times, on days 0, 3 and 6 of the experiment. The results of the experiment are summarized in Table IV.

Table III. *Survival data of leukemia-bearing mice treated with free MTX or dextran T40-based D-MTX conjugates, expressed as an ILS – increase in lifespan.*

Group	N[a]	ILS (%)	MST (days)[b]	L[c]
Control	8	0	11.5	
MTX, 40 mg/kg	7	39	16.0	0
T40-SP2 conjugate, 40 mg/kg	7	57	18.0	0
T40-SP3 conjugate, 40 mg/kg	7	65	19.0	0
T40-SP4 conjugate, 40 mg/kg	7	57	18.0	0
T40-SP6 conjugate, 40 mg/kg	7	65	19.0	0

[a]N, number of mice in group.
[b]Median survival time.
[c]Number of mice that died before control group.

Table IV. *Survival data of leukemia-bearing mice treated with free MTX or D-MTX conjugates in the multiple-dose schedule model.*

Group	N[a]	ILS (%)	MST (days)[b]	L[c]
Control	15	0	11.0	
MTX, 20 mg/kg x 3	22	136	26.0	0
T10-MTX conjugate, 20 mg/kg x 3	13	82	20.0	0
T40-MTX conjugate, 20 mg/kg x 3	23	145	27.0	0

[a]N, number of mice in group.
[b]Median survival time.
[c]Number of mice that died before control group.

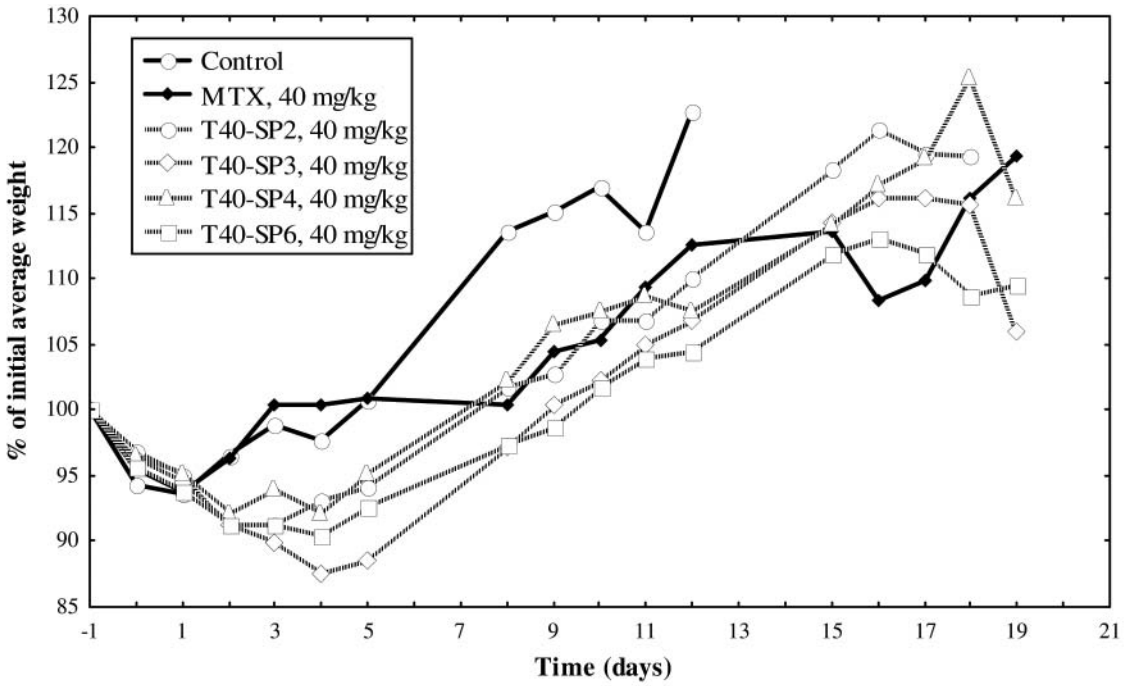

Figure 2. *Dynamics of body weight in leukemia-bearing mice treated with either MTX or one of the dextran T40-based D-MTX conjugates.*

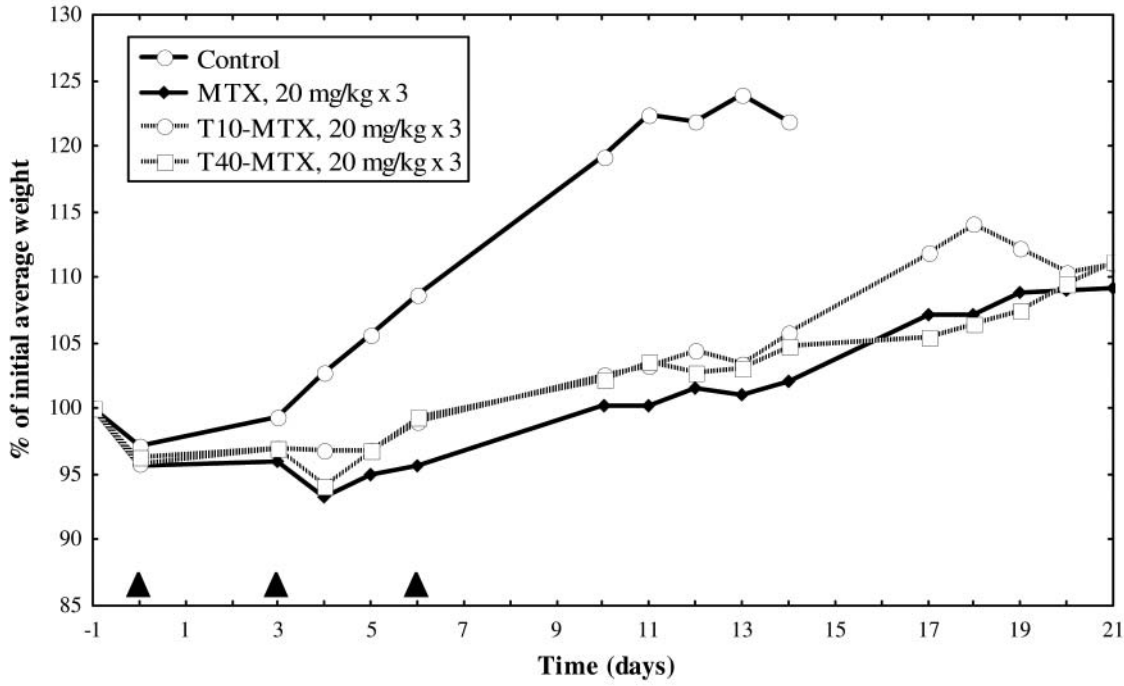

Figure 3. *Dynamics of body weight changes in leukemia-bearing mice treated with either MTX or one of the D-MTX conjugates in model with multiple-dose schedule. Triangles indicate drug injections (20 mg/kg/day of MTX, T10-MTX or T40-MTX).*

There were no early deaths due to toxicity in the groups treated with either of the conjugates. Data on weight changes showed that both tested preparations had approximately the same toxicity as free MTX when applied in low- and multiple-dose schedules (Figure 3). Notably, the antitumor effects of the conjugates were not the same. Mice

treated with the T10-MTX conjugate had a lower median survival time in comparison with both free MTX- and T40-MTX-treated groups and these differences were statistically significant ($p<0.01$ and $p<0.0001$, respectively). However, T40-MTX-treated mice had a median survival time similar to the free MTX-treated mice and there was no statistically significant difference in survival between these two groups.

Discussion

We recently published our study on the dependence of the antitumor properties and toxicity of the D-MTX conjugates *in vivo* and *in vitro* on the Mw of the carrier (14). On the basis of these results, we chose dextrans T10 and T40 with Mw of 10 and 40 kDa, respectively, for further investigations. Our choice was justified by the lower toxicity of the conjugates based on these dextrans as compared to preparations with higher Mw. The current report is a continuation of the investigations on the properties of the D-MTX conjugates.

We were interested in investigating whether there is any influence of the substitution level on the properties of the D-MTX conjugates. The substitution level is defined as the number of MTX molecules coupled to one glucose monomer or dextran polymer. When considered relative to glucose, the substitution level is a fraction lower than 1, because the dextran polymer consists of a number of the glucose molecules. The exact number of glucose monomers varies in the dextrans, depending on their average Mw. Therefore, two conjugates based on the different dextrans can have the same substitution levels relative to glucose, but differ in substitution levels relative to dextran and *vice versa*. This fact must be kept in the mind while analyzing and interpreting the results of this study.

The clear dependence of the *in vitro* antiproliferative effect on the substitution level was established only in the dextran T10-based set of conjugates. The IC_{50} values increased with the increasing substitution level of the respective preparations, implying that the antiproliferative effect of dextran T10-based conjugates diminished from the lowest to highest level of substitution. For the dextran T40-based set, no relationship between the substitution level and antiproliferative effect of the conjugates was observed. All IC_{50} values of dextran T40-based preparations in the tested range of substitution levels were approximately the same and neither ANOVA nor Cuzick's test revealed any differences between them (Table I). This discrepancy between the *in vitro* results of dextran T10- and T40-based sets can be explained by differences in the degradability of the respective preparations. It was postulated that, before acting, the conjugates have to be internalized to the tumor cell by fluid-phase endocytosis (25). Conjugates of MTX could also be internalized by binding of the D-MTX complex to the folate-binding protein, resulting in folate-binding protein-mediated endocytosis (8). Subsequently, the free drug is released *via* lysosomal degradation of the conjugate inside the cell (25, 26). Dextrans are hydrolyzed by α-1-glucosidases (dextranases) present in various organs in the body (3, 27). It has been shown that progressive chemical modification of dextrans results in the elimination of lysosomal degradation by these enzymes (3, 28). Therefore, we speculate that the degradation of our D-MTX conjugates depends on the number of MTX molecules coupled to a single oligosaccharide branch of the dextran (27). When there is only one MTX molecule, the branch is degraded relatively easily and the release of free drug progresses practically unhindered. However, if two or more molecules of MTX are coupled to one branch, the lysosomal degradation of this part of the macromolecule could be inhibited due to the mechanism of dextranase action. Our assumption is that the number of oligosaccharide branches was lower in our dextran T10 in comparison with T40, due to the smaller size of the former. Therefore, the probability of coupling two or more molecules of MTX to the same oligosaccharide branch is higher in dextran T10 than in dextran T40. With increased substitution and relative proportion of MTX coupled to one oligosaccharide branch by two or more molecules, the proportion of partially degraded D-MTX complexes could also increase. These complexes are significantly less active (or even not at all), so this results in diminished antiproliferative activity of the respective conjugates. If this hypothesis is true, the phenomenon would only be observed for conjugates with a low Mw carrier and high substitution levels. We assume that the macromolecule of dextran T40 is large enough and there is little possibility of coupling more than one MTX molecule to one oligosaccharide branch. Hence, the substitution level of the preparations does not significantly affect the extent of the degradation inside the cell for dextran T40-based conjugates. However, additional studies are needed to justify this rationale, since the mechanism of the *in vitro* antiproliferative effect of the conjugates is not entirely clear.

Next, both sets of conjugates in the P388 mouse leukemia were tested in the *in vivo* model. Quite contrary to the *in vitro* results, conjugates from the dextran T10-based set showed similar toxicity and antitumor effects. There were not any statistically significant differences in survival between groups of mice treated with them (Table II). This discrepancy between *in vitro* and *in vivo* results can be explained by differences in the action of the conjugates in these models. We suppose that the antiproliferative effect *in vitro* is primarily dependent on the rate of conjugate degradation inside the tumor cell, *i.e.,* the release of free MTX. However, it seems that the effectiveness of the D-MTX conjugates *in vivo* is dependent on their rate of

clearance from the body, making the speed and extent of conjugate degradation secondary (3). Clearance for dextran-based conjugates, in its turn, is believed to be mostly dependent on the Mw of the carrier and seems not to be significantly affected by the substitution level of the preparations (4). If this were true, the level of their substitution would have only a minor influence on the *in vivo* antitumor and toxicity properties of the conjugates.

All four conjugates from the dextran T40-based set revealed quite similar antitumor profiles *in vivo*. We did not observe any relationship between the biological properties of the preparations and their substitution levels. The conjugates tended to have even higher activity expressed as ILS and median survival time compared to free MTX (Table III). However, the differences in survival between these conjugates and free MTX were not statistically significant. The toxicity of the conjugates was also approximately the same for all four preparations tested, but lasted longer and tended to be more profound in comparison with the free MTX-treated group (Figure 2). Summarizing, for the tested range of dextran T40-based preparations, no clear dependency of the *in vitro* antiproliferative properties or *in vivo* antitumor effect and toxicity on the substitution level of the conjugates was observed.

We also attempted to test conjugates in the multiple-dose schedule. It is known that antimetabolites generally are schedule-dependent (29). Therefore, we hypothesized that differences between free MTX and the conjugates would be more evident in the multiple-dose schedule and would allow us to select more efficient carrier. Indeed, the T10-MTX conjugate had a significantly lower ILS and median survival time compared to either T40-MTX or free MTX. On the contrary, the antitumor activity of T40-MTX appeared to be quite similar to the effect of free MTX (Table IV). We explain this difference between the T10-MTX and T40-MTX conjugates by the diversity in the Mw of their carriers. It was shown that dextran T10 is excreted unrestrictedly, while dextran T40 is not able to pass unchanged through the pores of the glomerular capillary walls due to its larger molecular size (4). We suggest that the T10-MTX conjugate is excreted faster than T40-MTX. Therefore, a significantly lower amount of T10-MTX has time to degrade inside tumor cells in comparison with T40-MTX. The difference between the two conjugates becomes more pronounced in the multiple-dose schedule, when the advantage of T40-MTX over T10-MTX is cumulative during the administration course.

The results presented above allow us to conclude that the substitution level could have some importance only for conjugates based on low Mw dextrans. The impact was observed only during the *in vitro* studies. Data obtained in the P388 mouse leukemia *in vivo* model suggested that the substitution level did not significantly influence the antitumor properties or toxicity of the D-MTX conjugates *in vivo*. The latter observation was true for both dextran T10- and T40-based conjugates in the tested range of substitution levels. This fact, together with data from the multiple-dose schedule experiments, confirm our previously published results (14) that the Mw of the carrier is the critical parameter and should be taken into account while designing new conjugates. We are currently conducting studies in solid tumor models, to further investigate the possibility of application of the conjugates as prolonged forms of the parental drug. The advantage of dextran T40 over the dextran T10 carrier should also be confirmed in pharmacokinetics studies.

Acknowledgements

This work was supported by grants no. 1/2005 and 5/2005 from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences. We would like to thank Przemyslaw Boratynski for his suggestions concerning the manuscript.

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Received March 8, 2006
Accepted April 12, 2006