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Antileukemic Effect of High-dose Thymidine in a Rat Model for Acute Myeloid Leukemia (BNML)*

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Abstract—*The efficacy of high-dose thymidine (HD-TdR) was investigated as a function of various routes and schedules of administration in a rat model for acute myelocytic leukemia (BNML). The drug was administered either intravenously (i.v.) or intraperitoneally (i.p.) or by continuous infusion (c.i.) at different intervals. Five-day treatment with HD-TdR at a daily dosage of* 20–40 g/m^2 *did not lead to an increase in survival when compared with saline-treated controls. Among the investigated regimens c.i. led either to a significant reduction of survival or death during treatment. As can be derived from assays for the estimation of clonogenic bone marrow cells (CFU-S), HD-TdR treatment during a 5-day period is strongly toxic to the haemopoietic organ. It can be concluded that the lack of efficacy of HD-TdR in acute myeloid leukemia is due to haematotoxicity.*

INTRODUCTION

HIGH concentrations of thymidine (TdR) have been repeatedly reported to affect a variety of tumors *in vitro* [1, 2], and several studies suggest a selective antitumor action by high dose thymidine (HD-TdR) *in vivo*, when given at frequent intervals or by continuous infusion [3–6]. Early evaluation of phase I-II trials on the clinical pharmacology of HD-TdR in patients with haematologic malignancies and solid tumors indicates that the toxicity of HD-TdR (75 g/m^2/day as a continuous infusion for 5 days) is considerable and that the anti-tumor effect is not impressive [7]. The need for exact determination of the optimal dose of HD-TdR has led to pharmacokinetic studies on its distribution and elimination during and after continuous intravenous infusions [8]. At present, little information is available on the antileukemic effect of HD-TdR *in vivo*. One reason is that the drug has not been adequately tested in previously untreated leukemia patients. Secondly, preclinical investigations have not been performed in animal models for acute leukemia, which exhibit a slow net growth rate, as is the case with human acute myelocytic leukemia (AML). Therefore, this study was started, the aim of which was to evaluate the antileukemic effect of HD-TdR by various routes of administration, in a rat model for acute myelocytic leukemic (BNML), which has been reported to exhibit a response to chemotherapy identical to that in human AML [9, 10].

MATERIALS AND METHODS

Female rats of the Brown Norway inbred rat strain aged 12 weeks were used and kept on food and water *ad libitum* during the experiments. Animals were intravenously inoculated with 10^7 leukemia (BNML) cells, obtained from spleens of leukemic rats in a terminal stage of the disease. Treatment always started at day 15 after inoculation, a stage which is comparable with that of first clinical admittance of AML patients. The characteristics of the leukemia at day 15 are listed in Table 1. TdR was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) and dissolved in a mixture of glucose 10%, NaCl 0.15%, KCl 0.1% at a final concentration ranging from 2.75 to 66 mg per ml. Drug solutions were administered either intravenously (i.v.) or intraperitoneally (i.p.) in ether anaesthesized rats or by continuous infusion (c.i.) in unanaesthesized rats using a method described previously [11]. With this method rats are placed in a fixation cage

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*Table 1. Kinetic parameters of BNML leukemia cells at day 15 after transplantation**

Organ	Labeling index (%)	Growth fraction (%)
Bone marrow	25±2	35
Spleen	35±2	49
Liver	34±3	48
Peripheral blood	21±2	

*Mean±S.D. of 6 animals/point.

which makes long-lasting anaesthesia unnecessary. Using a Bard I Cath infusion system (International, U.K.) which was inserted into a lateral tail vein, the infusion fluid was administered by an infusion pump (Braun, Melsungen, F.R.G.) at a flow rate of 20 ml/24 hr, which represents the normal daily fluid requirement of the rat. Assays for the quantification of clonogenic haemopoietic cells (CFU-S) were performed from rat bone marrow cells transplanted into irradiated BC3 mice [9]. For this assay femoral cell suspensions from treated rats were intravenously injected into lethally irradiated BC3 mice. At 9 days after transplantation mice were sacrificed and the spleens were fixated in Telleznicky's. The number of colonies counted on the surface of the spleen represented the number of CFU-S injected. As endpoints for the effect of HD-TdR treatment we used the survival, number of CFU-S and the reduction of liver and spleen weight, which have been previously shown to be sensitive parameters for the leukemic cell load and the reduction of it by chemotherapy in the BNML [9].

RESULTS

The outcome of HD-TdR treatment in the BNML is summarized in Table 2. Various dosages, routes and schedules of administration were investigated and compared with a scheduled treatment of 1-β-D-arabinosidecytosine (Ara-C) which has a significant antileukemic effect in the BNML [9, 10]. For good interpretation of the data it is necessary to realize that all treatment started at day 15 of the disease, which represents a stage in which the percentage of S-phase BNML cells, as determined with autoradiography, amounts to 42%. Apparently a high percentage of BNML cells is a proliferating population which should theoretically be sensitive to S-phase specific drugs like HD-TdR and Ara-C. However, the results of HD-TdR treatment show a very moderate inhibitory effect on the development of the leukemia. With respect to the lower dosage regimens no significant increase in survival time is obtained with either schedule of administration. If the daily dosage is administered by two scheduled injections, HD-TdR turns out to be toxic. This is also evident if the total dosage is administered by continuous infusion. In those treatment groups in which the survival is reduced, the liver and spleen weight at death

*Table 2. Effect of HD-TdR treatment on BNML leukemia**

Schedule of administration (daily)	Total dose	Liver (g)	Spleen (g)	Survival (days after inoculation)
i.v., 20 g/m² × 5 (q = 24 hr)	20 g/m²/day × 5	9.78±2.10	1.24±0.40	29.3±7.7
i.v., 10 g/m² × 10 (q = 12 hr)		12.30±2.70	1.46±0.19	25.8±5.6
i.v., 10 g/m² × 10 (q = 6 hr, 18 hr)		10.21±3.42	1.21±0.54	23.8±5.1
i.p., 20 g/m² × 5 (q = 24 hr)		10.67±2.23	1.49±0.29	25.1±6.7
c.i., 20 g/m² × 5		14.94±2.90	1.71±0.53	21.0±0.5
controls i.v., NaCl 0.9% (q = 24 hr)		12.23±2.33	1.37±0.33	28.0±5.6
i.v., 40 g/m² × 5 (q = 24 hr)	40 g/m²/day × 5	10.69±3.31	1.92±0.76	26.9±2.4
i.v., 20 g/m² × 10 (q = 12 hr)		10.70±2.70	2.04±0.37	27.5±1.6
i.v., 20 g/m² × 10 (q = 6 hr, 18 hr)		9.80±1.25	1.94±0.39	26.4±1.5
i.p., 40 g/m² × 5 (q = 24 hr)		9.39±1.15	1.57±0.31	26.0±6.4
c.i., 40 g/m² × 5		—	—	dead during treatment
controls i.v., NaCl 0.9% (q = 24 hr)		12.23±2.33	1.37±0.33	28.0±5.6
i.v. Ara-C, 200 mg/kg × 5 (q = 12 hr) 1000 mg/kg		5.37±0.16	0.30±0.01	41.0±2.0

*Results are indicated as mean±S.D. of 10 treated animals per group. Start of treatment at day 15 following inoculation with 10^7 splenic leukemia cells.

is equal or higher than in the control group. When using the higher dosage of HD-TdR, some effect on liver weight can be observed. However, spleen weight at autopsy is significantly higher in all treatment groups, when compared with controls, while survival is less. Animals treated with continuous infusion have not even survived the treatment period. Although the antileukemic effect of the higher dosage of HD-TdR appears to be superior to that by the lower dosage, toxicity is equal or even more evident, as can be concluded from the survival time. When the results of HD-TdR treatment are compared with those obtained by five scheduled administrations of Ara-C, it becomes clear that this drug has a significant effect on BNML leukemia. However, this drug has been chosen from a number of clinically employed antileukemic agents which are active in the BNML rat leukemia, including 6-thioguanine, vincristine, daunorubicin, adriamycin and cyclophosphamide. Therefore, it may be concluded that HD-TdR is not active as an antileukemic drug in this leukemia whatever the administration.

In order to investigate whether the toxicity of HD-TdR is due to suppression of normal haemopoiesis, the effect of various schedules of administration on bone marrow CFU-S was investigated (Table 3). For this study all

Table 3. Effect of HD-TdR on bone marrow CFU-S in BNML rats

Treatment	Number of CFU-S (mean ± S.D.) per 10^5 bone marrow cells
HD-TdR i.v. $3.0\,g/m^2 \times 2$; q = 12 hr	9.7 ± 4.1
HD-TdR i.p. $3.0\,g/m^2 \times 2$; q = 12 hr	2.2 ± 1.6
HD-TdR c.i. $6.0\,g/m^2$	7.9 ± 2.8
NaCl 0.9% c.i.	1.9 ± 1.0
HD-TdR c.i. $6.0\,g/m^2$/day for 5 days	not detectable

groups were treated for 24 hr with either i.v., i.p. or c.i. of HD-TdR. Twenty-four hours following termination of the treatment, the rats were killed and femoral cell suspensions were intravenously injected into lethally irradiated BC3 mice. When compared with saline-treated rats, the number of CFU-S in HD-TdR-treated animals had increased significantly. Although the mechanism behind this effect is presently not known, it is possible that HD-TdR recruits non-proliferating CFU-S into the cycle.

DISCUSSION

At present, little information is available with respect to the optimal dosage and scheduling of HD-TdR treatment. In human tumors xenografted into nude mice, HD-TdR showed significant activity only in a tumor with a high proliferation rate [6]. In contrast to these results, other investigators found HD-TdR to be very active against heterotransplanted human tumors in nude mice [4]. Although clinical experience with HD-TdR is limited, early phase I–II reports mention considerable toxicity and a low efficacy of the treatment [7]. Especially, the clinically observed haematological toxicity of HD-TdR suggests that the drug does not represent a new class of agents in chemotherapy. Its usefulness in the treatment of acute leukemia seems limited, as can be concluded from the present study. Since the BNML leukemia has a proliferating fraction of ±40%, phase specific cytostatic treatment theoretically should be effective. While Ara-C produces an impressive reduction of the leukemic tumour load, which leads to a significant increase in lifespan, HD-TdR is ineffective. It is suprising that modifications of either the dosage or the route or scheduling of administration does not improve the antileukemic action. This might be due to either toxicity or to high endogenous thymidine levels in BNML rats, as has been described in nude mice. Although CFU-S data of 24-hr treatment with HD-TdR do not support the idea of strong haematological toxicity, it is possible that the increase of CFU-S following 24-hr treatment with HD-TdR is to be attributed to recruitment of non-proliferating bone marrow precursor cells. Subsequent treatment with HD-TdR (for example for 5 days) may possibly kill these proliferating CFU-S. In fact, no bone marrow CFU-S can be detected following 5 days of continuous infusion with HD-TdR. These findings indicate that the poor survival of HD-TdR-treated animals is due to haematological toxicity. This toxicity can be avoided by employing short treatment periods. As far as the route of administration is concerned, no significant differences in treatment efficacy can be detected. Since c.i. and i.p. administration lead to significantly reduced survival, the long existence of thymidine in the blood produced by these schedules of administration is probably of major relevance for the in-

duction of toxic side effects. It should be noted, however, that no HD-TdR treatment schedule is effective in this leukemia. HD-TdR does not appear to represent a valuable treatment modality in acute myelocytic leukemia.

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