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Effect of Succinylated *Acinetobacter* Glutaminase–Asparaginase Treatment on an Acute Myeloid Leukemia in the Rat (BNML)*

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Abstract—*The effect of succinylated* Acinetobacter *glutaminase–asparaginase (G–A) treatment was investigated in the Brown Norway myeloid rat leukemia (BNML). It was observed that a daily treatment schedule of* 400 i.u./kg *for* 7 days *was effective when liver and spleen weight were used as criteria. However, no effect was seen in the bone marrow, which indicates that either pharmacokinetic distribution parameters or differences in sensitivity may influence the outcome of this treatment. Since similar results were observed in children with acute lymphocytic leukemia, the BNML offers a good experimental model to study the effects of G–A treatment on leukemic cells.*

INTRODUCTION

L-ASPARAGINASE has been shown to possess antitumor activity in leukemias and lymphomas [1]. However, its activity against solid tumors is limited. Attempts have been made to improve the biological activity of enzymes against nonhaematological neoplasms. In 1974, Roberts *et al.* [2] purified a glutaminase–asparaginase enzyme with activity in tumors resistant to asparaginase. It was called G–A: 1.2, indicating that the enzyme has a ratio of 1.2:1.0 of glutaminase and asparaginase activity. Schrek *et al.* demonstrated that the glutaminase activity of the enzyme was more important than asparaginase in reducing both the survival of chronic leukemic lymphocytes and the transformation of normal lymphocytes [3]. The physical properties were described by Holcenberg *et al.* [4]. Since the biochemical and physical effects of L-glutamine and L-asparagine levels on tumor cells *in vitro* were studied extensively, some valuable animal models were selected to investigate the *in vivo* efficacy. It was shown that succinylation of the enzyme greatly prolongs its half-life in animals and man [5, 6].

This study describes the results of treatment with *Acinetobacter* glutaminase–asparaginase in the acute myeloid leukemia in the Brown Norway rat (BNML). This model is quite similar to the human acute myeloid leukemia in respect to growth pattern and reaction to chemotherapy.

MATERIALS AND METHODS

Glutaminase–asparaginase was isolated from *Acinetobacter glutaminasificans* and purified and succinylated by Dr. Holcenberg [5]. The enzyme used in these studies was from a single batch and had a specific glutaminase activity of 150 i.u./mg of protein. The ratio between specific glutaminase activity and asparaginase activity in this batch was ascertained to be 1.2. Enzyme activity in the plasma was assayed by ammonia formation from asparagine, as measured spectrophotometrically using Nessler solution (Harleco No. 64092). Enzyme activity was calculated from the ratio of absorbance as compared with the absorbance produced by ammonium chloride.

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Abbreviations used—G–A: succinylated *Acinetobacter* Glutaminase Asparaginase. BNML: Brown Norway Myeloid Leukemia. CFU-s: Colony Forming Units in the Spleen.

Animals

Male rats of the Brown Norway strain at the age of 12 weeks (body weight 230 g) were inoculated i.v. with 10^7 leukemic spleen cells in Hanks' Balanced Salt Solution at a final volume of 1 ml. The experiments started at day 15 after transplantation when liver and spleen were greatly increased in size, the bone marrow contained more than 90% leukemic cells and leukemic blasts started to appear in the peripheral blood. This stage of the disease is comparable with that at the time of clinical diagnosis in man.

Treatment

Leukemic animals were injected with G–A daily at a dose of either 200 i.u./kg or 400 i.u./kg. Enzyme solutions were prepared daily. The treatment was given i.v. during a 7-day period. Immediately after this period, animals were sacrificed and the dry liver and spleen weights were determined. In addition, the bone marrow cells from one femur were collected and counted after suspending in Hanks' Balanced Salt Solution.

The plasma G–A activity was assayed in a separate group of animals treated with a single dose of 400 i.u./kg i.v. Blood was obtained by aortic puncture, centrifugated and frozen (−20°C). The G–A content was later determined, using the plasma of 4 animals for each point on the curve. The procedure for determination of G–A employed in this study has been extensively described by Roberts *et al.* [2].

RESULTS

The effect of succinylated *Acinetobacter* glutaminase–asparaginase treatment is shown in Table 1. Former experiments with other antitumor agents have demonstrated that liver and spleen weights are sensitive parameters for the tumor mass in the BNML [7, 8]. Effective drug administration reduces the spleen and liver weights and results in a decrease in the total number of bone marrow cells. Therefore, these 3 criteria were used in the determination of the antileukemic effect of G–A.

As shown in Table 1, a 7-day treatment period with 400 i.u./kg G–A is very effective in reducing the leukemic infiltration of the liver and spleen ($P<0.01$). If the lower dose of 200 i.u./kg is administered, the results are moderate and not significant. The remarkable lack of effect in bone marrow is difficult to explain and will be discussed later. In these experiments no deaths due to toxicity have been observed, neither at the high dose, nor at the lower one. In view of the observed effect on the leukemic cells population, application of higher dosages seems to be desirable. However, those experiments should be accompanied by studies regarding the effect of high doses on normal haemopoiesis as measured by the content of CFU-s.

Figure 1 shows the plasma G–A activity following the administration of 400 i.u./kg i.v. The observation period was 24 hr. Within this period, an initial half-life of ± 5 hr followed by a long second half-life is observed. This might indicate that the drug penetrates well into the tissues. The rather long second phase may be the result of a slow excretion and a minor breakdown and is probably of major relevance for the efficacy of the drug.

DISCUSSION

As judged by the remarkable decrease in liver and spleen weights following the

Table 1. Effect of glutaminase–asparaginase on BNML

Treatment	Liver (g) M ± S.D.	Spleen (g) M ± S.D.	Femoral bone marrow (cells × 10^7) M ± S.D.
Glutaminase–asparaginase 400 i.u./kg/day i.v. for 7 days; ($n=8$)	8.92 ± 0.50	1.55 ± 0.14	5.82 ± 0.38
Glutaminase–asparaginase 200 i.u./kg i.v. for 7 days; ($n=8$)	12.78 ± 2.38	2.92 ± 0.41	6.48 ± 1.04
NaCl 0.9% for 7 days ($n=8$)	26.2 ± 5.05	3.66 ± 1.06	5.95 ± 1.50
Untreated non-leukemic rats; ($n=5$)	8.21 ± 0.50	0.65 ± 0.12	9.70 ± 2.15

Autopsy at day 22 after inoculation of 10 spleen cells.

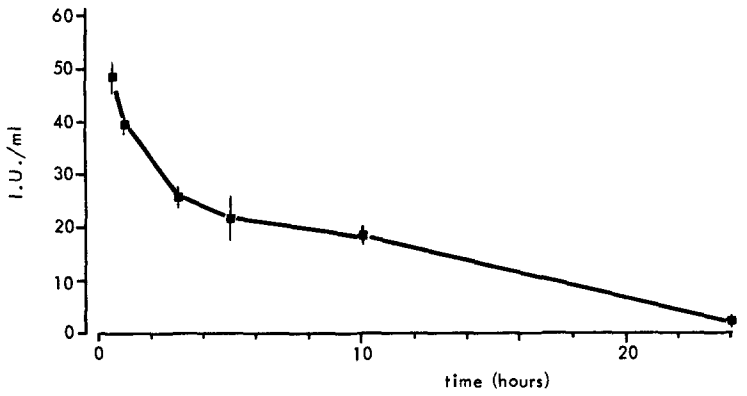

Fig. 1. Plasma succinylated Acinetobacter *glutaminase–asparaginase in the rat (M ± S.D.). Dose:* 400 i.u./kg *i.v.*

400 i.u./kg dose, G–A is an effective drug in the BNML. However, no effect can be observed in the bone marrow. In comparison with the effects of other drugs such as methotrexate and Ara-C [8], this observation is very strange. It may possibly be explained by either: (1) pharmacokinetic variables such as an incomplete penetration of G–A in the bone marrow; or (2) a reduced glutamine and asparagine requirement of the leukemic bone marrow blasts when compared with blast cells which have migrated to the liver and spleen. Similar observations have been made in children with acute lymphocytic leukemia. In these children, treatment with G–A resulted in a decrease in spleen and liver size and often clearing of the peripheral blasts but rarely of marrow blasts [6]. Since the maximally tolerated dose has not been determined in man or in this rat model, experiments are in progress to investigate the problem of partial lack of response to G–A. Some of these investigations will concern the pharmacokinetic properties of the drug. When observing the plasma curve after an i.v. administration of 400 i.u./kg, a two-compartment system seems feasible to explain the distribution of the drug. However, it is impossible to conclude from this curve that an incomplete penetration into the bone marrow would occur. Therefore, future experiments have been planned to study tissue distribution, *in vitro* sensitivity of isolated leukemic blast cells, and the effect of higher doses of G–A.

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