Effect of some Synthetic α-*N*-Alkyl Glutamine Derivatives on Transplantable Mouse Tumours

AYENGAR et al. suggested that metabolic mechanisms in tumours might be disturbed by interference with glutamine metabolism[1,2]. ROBERTS et al.[3] have shown that tumour cells are generally distinguishable from normal tissues by their low levels of free glutamine. The role of glutamine in the amino acid metabolism of malignant cells has been emphasized by PASIEKA and MORGAN[4].

From these findings, the speculation arose whether derivatives of glutamine, which may act as glutamine antimetabolites, can adversely influence tumour cell growth. This preliminary report describes the action of 4 synthetic α-*N*-alkyl derivatives of L-glutamine on a spectrum of transplantable rodent tumour systems. The mouse tumours used were sarcoma 180 (S-180), sarcoma Black (SBL_1), mammary adenocarcinoma E0771 and MMC_1A, Ehrlich ascites carcinoma (LETTRÉ) and radiation leukemia 105 (RL105)[5]. The compounds used for treatment of the tumours described above were α-*N*-ethyl-L-glutamine, α-*N*-propyl-L-glutamine, α-*N*-butyl-L-glutamine and α-*N*-Amyl-L-glutamine. The 4 compounds injected i.p. into mice (single administration) at dose levels up to 2 g/kg body weight produced no toxic signs.

In our experiments each compound was injected i.p. into groups of 10 mice, 24 h after tumour implantation. Injections were repeated every 24 h during a period varying from 6–12 days. The dose used was 500 mg/kg. For evaluation of the carcinostatic effect, the animals were sacrificed one day after the last injection. Tumour volume (TV) and total packed cell volume (TPCV) for the Ehrlich ascites carcinoma and tumour weights for the solid tumours, were determined. The ratios of tumour weight or volume of treated and control animals, T/C, were established according to CCNSC specifications[6]. In a

[1] P. AYENGAR and E. ROBERTS, Proc. Soc. exp. Biol. Med. *79*, 476 (1952).
[2] P. AYENGAR and E. ROBERTS, Growth *17* (1953).
[3] E. ROBERTS and P. R. F. BORGES, Cancer Res. *15*, 697 (1955).
[4] A. E. PASIEKA, J. H. MORTON and J. F. MORGAN, J. natn. Cancer Inst. *16*, 995 (1956).
[5] L. SACHS, J. natn. Cancer Inst. *29* (1962).
[6] Cancer chemotherapy service center (CCNSC), Cancer Chemother. Rep. *1*, 56 (1953).

Table I. Effect of glutamine derivatives on growth of transplanted mouse tumours

Treatment group 10 mice each	Tumour	No. of treatments[a]	% inhibition of tumour growth 100 (1 − T/C)	Significance[b]
α-*N*-ethyl-L-glutamine	S-180	12	52	S.
α-*N*-ethyl-L-glutamine	SBL_1	12	18	N.S.
α-*N*-ethyl-L-glutamine	EO771	10	5	N.S.
α-*N*-ethyl-L-glutamine	Ehrlich ascites carcinoma	6	15	N.S.
α-*N*-propyl-L-glutamine	S-180	12	51	S.
α-*N*-propyl-L-glutamine	SBL_1	12	21	N.S.
α-*N*-propyl-L-glutamine	MMC_1A	10	34	N.S.
α-*N*-propyl-L-glutamine	EO771	10	60	N.S.
α-*N*-butyl-L-glutamine	S-180	12	62	S.
α-*N*-butyl-L-glutamine	SBL_1	12	50	S.
α-*N*-butyl-L-glutamine	MMC_1A	12	40	S.
α-*N*-butyl-L-glutamine	EO771	12	35	N.S.
α-*N*-butyl-L-glutamine	Ehrlich ascites carcinoma	6	30	N.S.
α-*N*-amyl-L-glutamine	S-180	12	24	N.S.
α-*N*-amyl-L-glutamine	SBL_1	12	17	N.S.
α-*N*-amyl-L-glutamine	MMC_1A	10	17	N.S.
α-*N*-amyl-L-glutamine	EO771	10	16	N.S.
α-*N*-amyl-L-glutamine	Ehrlich ascites carcinoma	6	16	N.S.

[a] Daily dose 500 mg/kg body weight. [b] Significance of tumour growth inhibition calculated by *t* test; $p \leq 0.05$; S, significant; N.S., non significant.

Table II. Effect of glutamine derivatives on the survival time of radiation leukemia 105 of mice

Treatment group 10 mice each	No. of treatments[a]	Mean survival time in days		% increase in survival time over control	Significance[b]
		Treated	Control		
α-*N*-ethyl-L-glutamine	10	18 ± 1.5	15 ± 1.2	20	S.
α-*N*-propyl-L-glutamine	10	17.5 ± 1.5	14 ± 1.2	25	S.
α-*N*-butyl-L-glutamine	10	18 ± 1.5	14.5 ± 1.5	24	S.

[a] Daily dose 500 mg/kg body weight. [b] Significance of tumour growth inhibition calculated by *t* test. $p \leq 0.05$; S, significant; N.S., non significant.

Table III. Effect of combined treatment with α-*N*-butyl-L-glutamine and 6-mercaptopurine on growth of transplanted mouse tumours

Tumour	Group (10 mice each)	Treatment	Compound dose mg/kg daily	Compound dose mg/kg total	Mean tumour weight or volume mg ± S.D. or ml ± S.D. TV	Mean tumour weight or volume mg ± S.D. or ml ± S.D. TPCV	% inhibition of tumour growth 100 (1 − T/C) TV	% inhibition of tumour growth 100 (1 − T/C) TPCV	Significance[a] TV	Significance[a] TPCV
Ehrlich ascites carcinoma	I	Control 0.5% CMC in saline			4.4 ± 1.3	1.9 ± 0.3	0		–	–
Ehrlich ascites carcinoma	II	α-*N*-butyl-L-glutamine	200	1200	4.2 ± 1	2.1 ± 0.2	5	− 6	N.S.	N.S.
Ehrlich ascites carcinoma	III	6-mercaptopurine	5	30	3.1 ± 1	1.5 ± 18	30	25	N.S.	N.S.
Ehrlich ascites carcinoma	IV	α-*N*-butyl-L-glutamine + 6-mercaptopurine	200 plus 5	1200 30	2.3 ± 1.4	1.2 ± 1	50	39	S.	N.S.
S-180	I	Control 0.5% CMC in saline			595 ± 291		0		–	–
S-180	II	α-*N*-butyl-L-glutamine	200	2000	440 ± 283		27		N.S.	
S-180	III	6-mercaptopurine	5	50	293 ± 224		51		S.	
S-180	IV	α-*N*-butyl-L-glutamine + 6-mercaptopurine	200 plus 5	2000 50	180 ± 117		70		S.	

[a] Significance of tumour growth inhibition calculated by *t* test. $P \leq 0.05$; S, significant; N.S., non significant.

separate experiment the survival of RL105 treated bearing mice was observed and compared to that of untreated controls. Furthermore, also the combined action of 6-mercaptopurine and α-*N*-butyl-L-glutamine on tumour growth was tested. Data were statistically analyzed using the Student's *t* test. Table I summarizes the data on inhibition of growth of S-180, SBL_1, MMC_1A, E0771 and Ehrlich ascites carcinoma. It may be seen that α-*N*-butyl-L-glutamine is the most active carcinostatic agent among the 4 compounds tested. Growth rate inhibition of 62% for S-180 and of 50% for SBL_1 were obtained.

These data were found to be significant, $p \leq 0.05$. α-*N*-amyl-L-glutamine did not produce growth inhibition. Out of the 5 mouse tumours tested, S-180 was the most sensitive: 3 of the compounds namely; α-*N*-ethyl-L-glutamine, α-*N*-propyl-L-glutamine and α-*N*-butyl-L-glutamine inhibited its growth above 50% (see Table I).

These results are in accordance with the findings of DAVIDOV et al.[7], who demonstrated that the growth of glutamine requiring strains of hemolytic streptococci was markedly inhibited by α-*N*-butyl-L-glutamine and to a lesser degree by α-*N*-propyl-L-glutamine. The inhibition was abolished by increasing the concentration of glutamine in the medium.

A significant prolongation of the survival time of mice with radiation induced leukemia treated by these compounds was observed (see Table II).

6-Mercaptopurine is known to effect the synthesis of nucleotides from preformed purines[8] while glutamine antimetabolites inhibit mainly the de novo purine nucleotide biosynthesis. A combination of α-*N*-butyl-L-glutamine with 6-mercaptopurine was thought to be effective. For this purpose 4 groups of 10 mice were used for each of the 2 tumours tested. The results are summarized in Table III. The combination of subeffective doses of α-*N*-butyl-L-glutamine with small doses of 6-mercaptopurine yielded a more effective retardation of tumour growth than each agent separately.

Considering the non-toxic character of these glutamine derivatives, the tumour growth inhibition obtained with α-*N*-butyl-L-glutamine is of interest. A further investigation on the anti-tumour activity of this derivative is now in progress[9,10].

Zusammenfassung. Es wurde die Wirkung von 4 synthetischen α-*N*-alkyl Derivaten des L-Glutamin auf die Entwicklung von implantierten Maustumoren geprüft und α-*N*-butyl-L-Glutamin als aktivste Substanz ermittelt. Ihre Kombination mit 6-Merkaptopurin verursacht eine stärkere Wachstumshemmung der Tumoren als die Behandlung der Versuchstiere mit Einzelsubstanzen.

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[7] E. DAVIDOV, E. ROSEN, N. SHALITIN and N. LICHTENSTEIN, Biochim. biophys. Acta. *117*, 73 (1966).

[8] A. D. WELCH, Cancer Res. *21*, 1475 (1961).

[9] Our thanks are due to Prof. N. LICHTENSTEIN of the Hebrew University, Jerusalem, for his kindness in preparing and supplying the α-alkyl-derivatives of glutamine, and to Mrs. ZELIKSON-SINGER, and Mr. D. STRASBERG (Labour Sick Fund Statistics Department) for their assistance in statistical evaluation.

[10] This work is supported by a research grant from the National Council for Research and Development of Israel.