Anti-Cancer Drugs 1994, **5**, pp. 57–63

Anti-tumor efficacy of glutaminase–copper–ATP combination in mice bearing Ehrlich ascites carcinoma

Smarajit Pal and Putul Maity

Department of Cell Biology, Chittaranjan National Cancer Institute, 37 SP Mukherjee Road, Calcutta 700026, India.
Tel: (+91) 33 475 2750; Fax: (+91) 33 475 5001.

Glutaminase is a hematotoxic anti-tumor agent, and copper–ATP complex (Cu–ATP) is both anti-neoplastic and hematostimulatory. Combination chemotherapy with these two agents has been performed in mice bearing Ehrlich ascites carcinoma, to elucidate whether this could result in augmented tumor inhibition with reduced hematotoxicity. Glutaminase–Cu–ATP combination (glutaminase 250 IU/kg per day intraperitoneally for 10 days and Cu–ATP 2.5 mg/kg per day intraperitoneally for 10 days) was observed to be more effective in inhibiting tumor growth and in increasing the life span of the tumor hosts, compared with the individual efficacies of these two agents. Moreover, addition of Cu–ATP successfully prevented the hematotoxic effects of glutaminase in normal and in tumor-bearing animals. Thus glutaminase in combination with Cu–ATP holds promise for an effective cancer chemotherapeutic regimen.

Key words: CFU-S, combination therapy, Cu–ATP complex, DNA, glutaminase, mice, proteins, RNA, tumor inhibition.

Introduction

The anti-tumor activity of glutaminase, a glutaminolytic enzyme, is well established,[1–3] However, glutaminase is also known for its suppressive effects on normal lymphocyte blastogenesis,[4] peripheral blood cell counts and hematocrit.[5] These adverse effects on normal cell systems often limit the optimal use of this enzyme as an anti-tumor agent.

We have recently demonstrated that a synthetic copper–ATP compound $[Cu_3(ATP)_26H_2O]^{2-}$ (Cu–ATP) inhibits tumor growth[6,7] and concomitantly stimulates murine hematopoiesis at the pluripotent stem cell level.[8] We were interested to know, therefore, whether a combination of glutaminase with Cu–ATP could result in augmented anti-tumor efficacy with minimal hematotoxicity. We investigated the tumor growth and hematological response of mice bearing transplantable Ehrlich ascites carcinoma (EAC) following combination chemotherapy with glutaminase and Cu–ATP. The hematotoxic effects of this combination were also evaluated in tumor-free normal mice.

Correspondence to P Maity

Materials and methods

Animals

Closed-colony-bred male Swiss mice, aged 6–7 weeks and weighing 20–22 g, were used throughout this study. The animals were obtained from our Institute's vivarium, and were maintained in metal cages (five mice per cage) with alternate 12 h dark/light cycles. Food (standard mouse pellet; Hind Lever, Bombay, India) and water were given *ad libitium*.

Tumor

EAC was maintained by serial intraperitoneal (i.p.) transplantation of 1×10^6 viable tumor cells suspended in 0.2 ml of sterile phosphate-buffered saline (PBS, pH 7.4). Control animals received 0.2 ml of PBS only.

Drug and treatment schedule

Purified glutaminase (grade II from *Escherichia coli*) was purchased from Sigma Chemical Co. (St Louis, MO) Cu–ATP was a gift from Professor R. G. Bhattacharya and Dr K. K. Nayak, Department of Chemistry, Jadavpur University, Calcutta, India.

Glutaminase, dissolved in 0.1 M sodium acetate buffer (pH 6), was injected (0.2 ml volume) intraperitoneally at a dose of 250 IU/kg body weight per day for 10 consecutive days. Control mice received 0.2 ml of buffer only. For combination therapy, Cu–ATP was dissolved in sterile isotonic saline and

injected (0.2 ml volume) intraperitoneally 3 h after glutaminase administration at a dose of 2.5 mg/kg per day for 10 consective days. Control animals were injected intraperitoneally with 0.2 ml volumes of buffer followed by normal saline. In the case of tumor hosts, drug administration started 24 h after tumor transplantation.

Tumor growth response

Tumor growth response to therapy was assessed by determining the viable tumor cell count and percentage increase in host life span (% ILS) following the standard procedures.[9]

Measurements of DNA, RNA and protein synthesis

The rates of DNA, RNA and protein synthesis of EAC tumor cells were measured by [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine incorporation studies, respectively. Mice bearing EAC received a single i.p. injection of glutaminase (250 IU/kg) followed by Cu–ATP (2.5 mg/kg). Tumor cells were harvested at different time intervals, up to 96 h after drug administration, washed and resuspended in RPMI 1640 media (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Gibco). Tumor cells were incubated either with 5 μCi/ml of [^{3}H]thymidine (specific activity 17000 mCi/mmol), or [^{3}H]uridine (specific activity 16000 mCi/mmol) or [^{3}H]leucine (specific activity 7000 mCi/mmol) (all from BARC, Bombay, India) for 20 min at 37°C, after which the reaction mixture was precipitated by the addition of equal volumes of 10% ice-cold trichloroacetic acid (TCA). The precipitates were collected on glass fibre filters (Whatman, CF/C) on a suction filtration apparatus (Millipore), and washed with TCA, ether and ethanol, respectively. After drying, the filters were counted (radioactivity) in LKB 1202, Rackbeta liquid scintillation apparatus. Incorporation of radioactive isotopes in the tumor cells of treated mice was expressed as percentage of controls.

Glutaminase enzyme assay

Nine mice from each group were killed by cervical dislocation on days 0, 5, 10 or 15 following tumor inoculation. Liver samples of these animals were collected on ice and placed quickly into chilled homogenization medium (5 mM HEPES, 0.33 mM sucrose, 1 mM EDTA; pH 7.4) which contained 5 mM protease inhibitor phenyl methyl sulfonyl fluride. The liver was minced and homogenized in 9 vol of ice-cold medium. Glutaminase amidohydrolase (GNase) (EC 3.5.1.2) enzyme assay was carried out from liver homogenate.

The incubation mixture for the GNase contained 154 mM NaCl, 10 mM Tris–HCl buffer, 5 mM glutamine, 0.1 ml of liver homogenate and 5 mM NaH_2PO_4, pH 7.4, in a total volume of 3.0 ml. All incubations were carried out at 37°C for 30 min and the amount of ammonia produced was estimated following the procedure of Braganca *et al.*[10] All values were corrected for the necessary substrate and enzyme blanks. Protein estimations were carried out according to the method of Lowry *et al.*[11]

Hematological studies

Routine hematological studies were done from free-flowing tail vein blood by standard procedures.[12]

Bone marrow and splenic cellularity

The animals were killed by cervical dislocation and the femur and spleen were removed. Marrow cells were flushed from the femoral shaft into ice-cold RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). A single-cell suspension was prepared by sequentially passing the cell suspension through 20-, 22- and 26-gauge needles. Single-cell suspension of the spleen was prepared in ice-cold RPMI 1640 plus 10% FCS by using a sterile steel wire mesh. Total nucleated cell count was done using a Neubaur counting chamber under light microscope.

CFU-S assay

Pluripotent hematopoietic stem cells (CFU-S) in control and treated mice were assayed following the spleen colony technique of Till and McCulloch.[13] The assay mice were lethally irradiated with 8.1 Gy from a ^{137}Cs source at a dose rate of 0.75 Gy/min. Within 2 h of irradiation, the animals were injected intravenously with 1.0×10^5 bone marrow cells or 5.0×10^5 spleen cells collected from the donor mice. The spleen of the recipients were dissected out 10 days later and fixed in Bowins fluid. Macroscopic colonies at the surface of the spleens

were counted as CFU-S. Thereafter, the spleens were processed for histology. Microscopic colonies in hematoxilin and eosin stained sections (5 μm thickness) were identified and classified according to the criteria of Curry and Trentin.[14]

Statistical analysis

The results were analyzed by students *t*-test and statistical significance was assigned when $p < 0.05$.

Results

Tumor growth pattern and hosts life span

The combination of glutaminase and Cu–ATP appeared to be highly effective in inhibiting EAC tumor growth and in increasing the life span of the tumor hosts. It is evident from Table 1 that the combination chemotherapy elicited 80, 94 and 93% reduction in tumor cell count at day 5, 10 and 15 post-transplantation, respectively, which was followed by about 122% increment in host survival when compared with that of untreated controls.

Protein and nucleic acid synthesis

Tumor inhibition by glutaminase–Cu–ATP combination was observed to be associated with suppression of DNA, RNA and protein synthesis. Figure 1 shows marked reduction in DNA, RNA and protein synthesis in EAC tumor cells which is evident from radioactive thymidine, uridine and leucine incorporation studies respectively following *in vivo* administration of a single dose of the drug combination. A detectable reduction in the extent of incorporation of radioactive precursors in EAC tumor cells was observed at 6 h after treatment and the change was more pronounced at 12 h post-treatment. However, an increment in uridine and thymidine incorporation was found at 30–36 h without any appreciable radioactive leucine incorporation.

Glutaminase activity in liver

It is evident from Table 2 that growth of EAC tumor was associated with a progressive increase in liver GNase activity. The degree of elevation in liver GNase activity in the treated group, however, was significantly lower ($p < 0.001$) than the matched controls.

Hematological parameters

Glutaminase treatment has a suppressive effect on Hb, RBC and platelet values of normal mice, but the addition of Cu–ATP with glutaminase restored the normal levels of these hematological parameters (Table 3). Similarly in EAC-bearing mice, combined action of glutaminase and Cu–ATP resulted in improvements in Hb concentration and erythrocyte count when compared with that of untreated EAC-bearing mice. The fall in platelet count that accompanies glutaminase treatment both in normal and tumor-bearing mice was also prevented substantially by the addition of Cu–ATP. Glutaminase in combination with Cu–ATP induced an appreciable increase in total leukocyte count in normal mice. Differential counts of WBC in drug-treated normal mice show that while glutaminase alone caused a mild decrease in relative distribution (%) of neutrophils with an increment in lymphocyte population, the reverse was documented following combination therapy. Neutrophilic leukocytosis that parallels EAC tumor growth in mice was checked to

Table 1. Combination chemotherapy with glutaminase and Cu–ATP compound: effects on tumor growth and survival of EAC-bearing mice

Group	Tumor cell count ($\times 10^6$), mean ±SE			Tumor inhibition (%)			Hosts' survival	
	day 5	day 10	day 15	day 5	day 10	day 15	median (days)	% of ILS
EAC, control	55.0 ± 4.1	223.0 ± 14.0	322.0 ± 22.0				27	—
				80	94	93		
EAC, treated[a]	10.25 ± 1.3[b]	14.2 ± 0.9[b]	19.3 ± 1.3[b]				60	122

[a] Glutaminase and Cu-ATP were injected i.p. at a dose of 250 IU and 2.5 mg/kg body weight/day, respectively, for 10 consecutive days. A total number of 10 animals were studied in each group.
[b] $p < 0.001$ compared with matched control.
ILS = Increased life span.

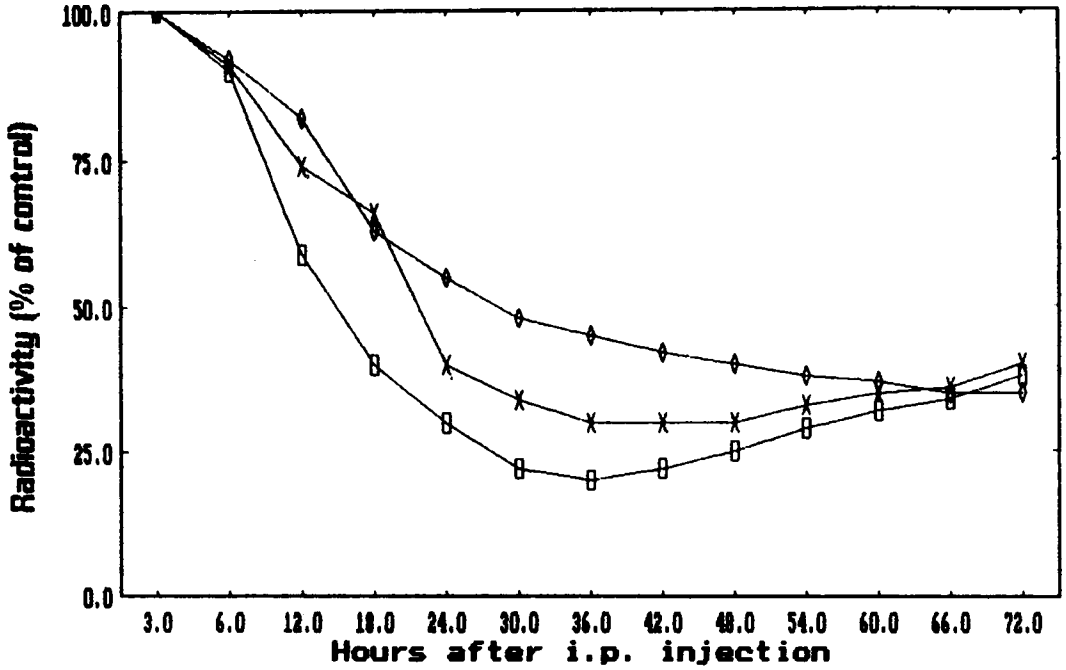

Figure 1. Effects of glutaminase–Cu–ATP combination on DNA, RNA and protein synthesis of EAC tumor cells expressed in terms of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine incorporation, respectively. ⊟, thymidine; ✳, uridine; ◇, leucine.

some extent in glutaminase treated group and a combination of glutaminase and Cu–ATP was found to be more effective in this regard than the action of glutaminase alone.

Bone marrow and splenic CFU-S

Treatment of normal mice with glutaminase alone was followed by significant reduction ($p < 0.05$) in spleen weight and total number of nucleated cells in the femoral marrow and spleen. In contrast, combination therapy did not significantly alter the nucleated cellularity of bone marrow and spleen, although moderate increase in spleen weight was observed like neutrophilic leukocytosis, splenomegaly with increased number of nucleated cells and CFU-S in the spleen accompanied EAC tumor growth. Glutaminase treatment in combination with Cu–ATP brought down these values substantially (Table 4). Glutaminase alone caused about 59 and 25% depletion of CFU-S in the femoral marrow of normal and EAC-bearing mice respectively, whereas combination chemotherapy could bring the number of CFU-S in bone marrow of normal and tumor bearing mice towards the normal range.

Histological evaluations of CFU-S colonies (Table 5) revealed that glutaminase treatment of normal and EAC-bearing mice resulted in inhibition of differentiation of bone marrow and splenic CFU-S towards erythroid and megakaryocyte lineage. Interestingly such inhibition was less apparent following combination therapy.

Table 2. Glutaminase activity in the liver of EAC-bearing mice following glutaminase–Cu–ATP (mean ± SE)

Group	Glutaminase activity (μmol ammonia produced/min/mg of protein			
	days after tumor transplantation			
	0	5	10	15
EAC, control	0.04 ± 0.001	0.046 ± 0.002	0.082 ± 0.003	0.091 ± 0.002
EAC + glutaminase + Cu–ATP	0.043 ± 0.001	0.039 ± 0.001[a]	0.048 ± 0.001[a]	0.051 ± 0.002[a]

[a] $p < 0.001$, when compared with respective control.
A total number of nine animals were studied for each experiment.

Table 3. Hematological changes (mean ± SE) in normal and EAC-bearing mice following treatment with glutaminase alone and in combination with Cu–ATP

Group	Hemoglobin (g/dl)	RBC ($\times 10^6$/μl)	Platelets ($\times 10^5$/μl)	WBC (total) ($\times 10^3$/μl)	Neutrophil (%)	Lymphocyte (%)
Normal, control	15.9 ± 0.1	5.6 ± 0.3	7.4 ± 1.7	6.7 ± 1.3	41.0 ± 2.0	56.0 ± 1.6
Normal + glutaminase	13.8 ± 0.2[a]	4.6 ± 0.2[a]	4.1 ± 1.2[a]	7.1 ± 2.1	34.0 ± 1.2[a]	65.0 ± 1.2[a]
Normal + glutaminase + Cu–ATP	16.1 ± 0.3	5.4 ± 0.1	6.2 ± 1.3	10.2 ± 1.6	57.0 ± 1.2[a]	41.0 ± 1.3[a]
EAC, control	14.2 ± 0.2	4.7 ± 0.1	10.2 ± 0.4	21.3 ± 1.6	68.0 ± 3.0	29.0 ± 1.3
EAC + glutaminase	14.0 ± 0.3	4.5 ± 0.2	6.1 ± 0.2[a]	18.6 ± 1.1	52.0 ± 1.9[a]	44.0 ± 2.1[a]
EAC + glutaminase + Cu–ATP	15.8 ± 0.1	5.5 ± 0.1	9.6 ± 0.2	16.6 ± 0.3	50.0 ± 0.3[a]	48.0 ± 0.3[a]

[a] $p < 0.05$, when compared with respective controls.
A total number of 12 animals were studied for each experiment.

Discussion

The use of glutaminase as an antitumor agent is limited by its immunosuppressive and hematotoxic effects.[3,4] When glutaminase is used in combination with either azaserine or azaguanine significant tumor-inhibition resulted in mice bearing transplantable tumors.[15] Unfortunately, these combinations have failed to prolong the host life span significantly.[15] In this perspective the tumor inhibitory and hematostimulatory effects of Cu–ATP provided the rationale for combining it with glutaminase in an attempt to reduce the tumor burden successfully and to diminish the adverse hematologic effect of glutaminase. As expected, the present study has demonstrated that a combination of glutaminase with Cu–ATP is highly effective in inhibiting EAC tumor growth and increasing the host life span. Glutaminase and Cu–ATP alone are known to increase the hosts life span by 46% and 73% respectively.[2,7] Thus the observed 122% increase in hosts life span following combination therapy suggests that glutaminase and Cu–ATP have additive effects on tumor inhibition and hosts' survival. Glutaminase is thought to act primarily by inhibiting protein synthesis with consequent effects on DNA and RNA synthesis.[16] But the mechanism of Cu–ATP-induced tumor inhibition is yet to be elucidated, although interaction of this compound with DNA has been reported.[6] We made an attempt to understand the mechanism of tumor inhibition by a combination of these two drugs by evaluating the DNA, RNA and protein synthesis of the tumor cells following treatment. Our data clearly demonstrated inhibition of synthesis of all these three macromolecules by EAC cells of the treated animals. It is apparent therefore that tumor inhibition by glutaminase-Cu–ATP combination is mediated by inhibition of both nucleic acid and protein synthesis.

Tumor-associated alterations of glutaminase enzyme in the host tissues are well established.[17,18] We have recently reported that apart from the enzymological significance, this enzyme assay could also provide a method for monitoring host response to

Table 4. Nucleated cellularity and total CFU-S of femoral marrow and spleen of normal and EAC-bearing mice following treatment with glutaminase alone or in combination with Cu-ATP (mean ± SE)

Group	Femoral Marrow		Spleen		
	nucleated cell count ($\times 10^6$)	total CFU-S ($\times 10^3$)	nucleated cell count ($\times 10^7$)	total CFU-S ($\times 10^4$)	spleen weight (mg)
Normal, control	25.6 ± 0.8	7.8 ± 0.9	17.5 ± 0.9	4.65 ± 0.8	109 ± 25
Normal + glutaminase	20.2 ± 0.6[a]	3.2 ± 0.5[a]	9.5 ± 0.6[a]	3.21 ± 0.6[a]	60 ± 11[a]
Normal + glutaminase + Cu–ATP	24.9 ± 2.1	6.7 ± 2.1	19.3 ± 2.3	5.2 ± 2.2	138 ± 14[a]
EAC, control	20.2 ± 0.7	3.9 ± 0.6	25.9 ± 0.5	12.3 ± 1.6	270 ± 31
EAC + glutaminase	14.4 ± 0.6[a]	2.8 ± 0.9	20.6 ± 0.3[a]	6.7 ± 2.1[a]	172 ± 12[a]
EAC + glutaminase + Cu–ATP	18.3 ± 0.4	3.7 ± 2.1	17.2 ± 1.7[a]	8.3 ± 2.3[a]	150 ± 16[a]

[a] $P < 0.05$, when compared with matched control.
A total number of 12 animals were studied in each group.

Table 5. Histological evaluation of spleen colonies formed by marrow and splenic CFU-S of drug-treated mice (mean ± SE)

Source of CFU-S	Colony type (%)			
	erythroid	granulocyte	megakaryocyte	mixed/ undifferentiated
Femoral Marrow				
Normal, control	59.4 ± 2.3	21.7 ± 1.7	5.7 ± 0.9	13.2 ± 0.8
Normal + glutaminase	46.1 ± 1.6[a]	30.9 ± 2.1[a]	2.3 ± 0.6[a]	21.7 ± 0.6[a]
Normal + glutaminase + Cu–ATP	51.3 ± 2.2	30.0 ± 1.9[a]	6.3 ± 0.3	12.7 ± 0.3
EAC, control	20.2 ± 0.8	55.6 ± 0.9	12.2 ± 1.2	12.0 ± 0.1
EAC + glutaminase	21.6 ± 0.6	56.3 ± 0.7	4.8 ± 2.1[a]	17.3 ± 1.3[a]
EAC + glutaminase + Cu–ATP	17.0 ± 2.1	59.6 ± 2.1[a]	5.3 ± 0.9[a]	28.1 ± 0.6[a]
Spleen				
Normal, control	26.5 ± 2.2	50.2 ± 1.8	10.3 ± 1.2	13.0 ± 0.7
Normal + glutaminase	23.4 ± 0.3[a]	45.3 ± 0.6[a]	5.3 ± 0.6[a]	26.0 ± 0.3[a]
Normal + glutaminase + Cu–ATP	25.0 ± 1.6	49.1 ± 2.3	8.2 ± 1.3	16.7 ± 1.3
EAC, control	25.5 ± 3.3	44.4 ± 2.6	15.4 ± 2.6	14.7 ± 1.1
EAC + glutaminase	22.3 ± 1.2[a]	51.1 ± 1.7[a]	9.5 ± 1.6[a]	18.1 ± 2.1[a]
EAC + glutaminase + Cu–ATP	24.2 ± 1.6	54.3 ± 1.2[a]	12.1 ± 1.2[a]	9.4 ± 0.4[a]

[a] $p < 0.05$, when compared with respective control.
A total number of six animals were studied in each experiment.

therapy.[2,19] Results of this study also clearly indicate that glutaminase-Cu–ATP combination successfully kept the glutaminase activity in the hosts' liver within the normal range.

In addition to antitumor activity, the effect of this drug combination on hematopoietic system was evaluated as a great majority of antineoplastic agents suppress erythropoiesis leading to the development of severe anemia.[20] It is particularly encouraging in this context that glutaminase with Cu–ATP inhibits tumor growth and at the same time attenuates the degree of anemia in the host animals. Myelosuppression resulting in leukopenia and thrombocytopenia is another frequent and major complication of cancer chemotherapy[21] but this drug combination did not show any adverse effect on circulating leukocytes. On the contrary, the suppressive effect of glutaminase on circulating platelates was prevented to a great extent by combining it with Cu–ATP. Blood cells of all the lineages originate ultimately from pluripotent stem cells (CFU-S). Drug induced changes in peripheral blood cell counts may therefore be attributed to the action of any of these antineoplastic agents on CFU-S. The hematoxic activity of glutaminase is further evident from its suppressive effects on nucleated cellularity, absolute number of CFU-S and differentiation towards erythroid and megakaryocyte lineages. As expected, addition of Cu–ATP with glutaminase substantially prevented these adverse hematological effects at the level of pluripotent stem cells and their progenitors.

Conclusions

The central objective of this study was to ascertain whether glutaminase–Cu–ATP combination could result in augmented tumor inhibition with reduced hematotoxicity when compared to their individual efficacies. The results are quite encouraging in this experimental tumor model. However, since the majority of human cancers are slow growing and solid in nature, this study needs to be extended to primary tumors which are better representatives of human malignancies. In addition, systemic effect of this drug combination on hematopoietic tissues also needs careful evaluations for future trials. Nevertheless, results of the present study suggest that glutaminase–Cu–ATP combination shows encouraging results as a possible cancer chemotherapeutic regimen.

Acknowledgements

The authors are thankful to Dr (Mrs) J Roychowdhury, Director, Chittaranjan National Cancer Institute, Calcutta, for providing facilities. Authors are grateful to Professor RG Bhattacharya and Dr KK Nayak, Chemistry Department, Jadavpur University, Calcutta for providing us with Cu–ATP complex and to Dr MR Ray, Chittaranjan National Cancer Institute for helpfull suggestions.

References

1. Schimid FA, Roberts J. Antineoplastic and toxic effects of *Acinetobactor* and *Pseudomonas* glutaminase asparaginase. *Cancer Chemother Rep* 1974; **88**: 829–40.
2. Pal S, Maity P. Investigation on glutamine amidohydrolase (EC 3.5.12) and glutamine aminotransferase (EC 2.5.1.15) activity in liver and plasma of EAC-bearing mice following glutaminase therapy. *Cancer Lett* 1992; **66**: 225–31.
3. Spiers AS, Wade HE. *Achromobector* L-glutaminase–L-asparaginase: human pharmacology, toxicology and activity in acute leukemias. *Cancer Treat Rep* 1979; **63**: 1019–24.
4. Hersh EM. L-Glutaminase: suppression of lymphocyte blastogenic response *in vitro*. *Science* 1971; **172**: 736–38.
5. Holcenberg JS, Roberts J, Dolowy WE. Glutaminase as antineoplastic agents. In: Prusiner S, Stadman ER, eds. *The enzyme of glutaminase metabolism*. New York: Academic Press 1973: 227–92.
6. Nyak KK, Maity P, Bhattacharrya RG, *et al.* Antitumor activities of copper–ATP complex on transplantable lymphoma. *Pharmacology* 1990; **41**: 350–56.
7. Pal S, Roychowdhury TN, Maity P. Toxicity and inhibition of tumor growth in relation to Cu–ATP therapy. *Med Sc Res* 1993; **21**: 491–92.
8. Pal S, Ray MR, Maity P. Tumor inhibition and hematopoietic stimulation in mice by a synthetic copper–ATP complex. *Anti-Cancer Drugs* 1993; **4**: 505–10.
9. Wick MM. Dopamine: novel antitumor agent against B-16 melanoma *in vivo*. *J Invest Dermatol* 1978; **71**: 163–64.
10. Braganca BM, Questal JH, Schucher R. Note on microestimation of ammonia using the Warburg manometric apparatus. *Arch Biochem Biophys* 1954; **52**: 18–21.
11. Lowry OH, Rosenbrough NJ, Farr AL, *et al.* Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–75.
12. Kolmer JA, Spaulding EH, Robinson HW. *Approved laboratory technic*, 5th edn. New York: Appleton-Century-Crofts 1969; 39–126.
13. Till JE, McCulloch EA. A direct measurement of radiation sensitivity of normal mouse bone marrow cells. *Radial Res* 1961; **14**: 213–21.
14. Curry JL, Trentin JJ. Hemopoietic spleen colony studies 1. Growth and differentiation. *Dev Biol* 1967; **15**: 395–413.
15. El-Asmer FA, Greenberg DM. Studies on the mechanism of inhibition of tumor growth by the enzyme glutaminase. *Cancer Res* 1966; **26**: 116–22.
16. Cooney DA, Handschumacher RE. L-asparaginase and L-asparagine metabollism. *Ann Rev Pharmacol* 1970; **10**: 421–40.
17. Chakraborty P, Shrivastav GC. Regulation of glutamine-aminohydrolase in Ehrlich cells and fibrosarcoma mice and effect on plasma of host mice. *Ind J Med Res* 1972; **60**: 1082–88.
18. Bhattacharya M, Maity P, Chaudhury L, *et al.* Glutaminase and glutamine synthase in 20-methylcolanthrene treated mice. *Ind J Exp Biol* 1976; **15**: 52–3.
19. Pal S, Nayak KK, Maity P. Investigation on phosphate dependent glutaminase (EC.3.5.1.2) activity in host tissues of EAC-bearing mice and response of liver EC 3.5.1.2 on Cu–ATP therapy. *Cancer Lett* 1991; **58**: 151–3.
20. Doll DC, Weiss RB. Chemotherapeutic agents and erythron. *Cancer Treat Rep* 1983; **10**: 185–200.
21. Hoagland HC. Hematological complications of cancer chemotherapy. *Semin Oncol* 1982; **9**: 95–102.

(Received 9 September 1993; accepted 24 September 1993)