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Mechanism of Protein A-Induced Amelioration of Toxicity of Anti-AIDS Drug, Zidovudine

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Long-term treatment with 3-azido-3-deoxy thymidine (AZT) is often associated with myelosuppression. In AZT-treated Swiss mice, similar toxicological manifestations in terms of reduction of red blood and white blood cell counts and hemoglobin content had been observed as in AZT-treated AIDS patients. Pretreatment of animals with Protein A (PA) of *Staphylococcus aureus* Cowan I (1 μg/ml), twice a week for two weeks, alleviated such hematopoietic toxicity due to AZT. AZT-induced reduction in colony-forming unit–erythroid (CFU–E) and colony-forming unit–granulocyte monocyte (CFU–GM) were also reversed by the combined treatment of AZT and PA. PA treatment showed an increased level of erythropoietin in the blood plasma, and cellularity of spleen, thymus, and bonemarrow was also increased in the group receiving combined treatment (PA+AZT), higher than that in the AZT group. AZT or its metabolites inhibited the activities of liver microsomal monooxygenases, which, however, could be regenerated in an accelerated manner by pretreatment of mice with PA. Moreover, the PA-treated group showed an accelerated clearance of AZT and/or its metabolites. These results suggest that such an immunopharmacologic approach might substantially reduce the toxic effects of drugs, such as AZT.

Zidovudine, 3-Azido-3-Deoxy thymidine (AZT) is the FDA approved drug used widely in chemotherapy of AIDS patients (1). However, long-term treatment of AZT is often associated with hematological complications resulting in severe toxicity including anemia, leucopenia, neutropenia and thrombocytopenia (2). As a result of such myelosuppressive effects, long-term chemotherapy of AIDS has been problematic. Treatment with sub-therapeutic doses of AZT, to minimize the drug related toxicity, has resulted in less efficiency of the treatment (3).

The mechanism of anti-HIV activity of AZT, a thymidine analogue, involves metabolic activation to its corresponding triphosphate that is incorporated by the viral reverse transcriptase into the viral DNA, causing premature chain termination (4).There are some evidences of AZT anabolite incorporation into the cellular DNA also. Sommadossi and others (5) have demonstrated that the amount of AZT incorporated into the host DNA can be correlated with its initial extracellular concentration, which may be due to accumulation of its unmetabolized fraction in the system. Some other report suggests that 3-amino-3′-deoxythymidine, a reduced metabolite of AZT, may be potentially more toxic than AZT (6).

Significant dose related toxicity, associated with the administration of AZT, still remains a limiting factor in clinical management of AIDS (7,8). Such toxicity is often the single most complication ultimately requiring cessation of therapy. Drug related hemopoietic toxicity further complicates the already existing impaired hemopoiesis in AIDS patients (9). Moreover, it has been shown that there is a significant loss of hemopoietic progenitors in peripheral blood: this loss occurs earlier than that in bonemarrow of these patients (10). Arock et al., (11) have shown that the AZT sensitivity of marrow progenitors was different with IC50 of 10^{-8}M and 1^{-6}M for BFU-E and CFU-GM respectively. Assay of hematopoietic progenitors from bone marrow and spleen were performed to determine the hematopoietic toxicity associated with AZT in several cases (12). Harrison et al., (13) have reported that exposure of mice to AZT for 5 weeks produced marked anemia, thrombocytopenia, neutropenia and weight loss. Long term bonemarrow cultures (LTBMCS) established from mice exposed to AZT for 8 weeks showed a marked reduction in cellularity. Thus, AZT treatment suppresses the im-

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Abbreviations: AZT, 3-azido-3-deoxy thymidine; AIDS, acquired immunodeficiency syndrome; FDA, Food and Drug Administration; PA, protein A; CFU-GM, colony-forming unit–granulocyte monocyte; CFU-E, colony-forming unit erythroid; Epo, erythropoietin; LTBMCS, long-term bonemarrow cultures; RBC, red blood cells; WBC, white blood cells; rEpo, recombinant Epo.

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mune system further in AIDS patients, who are already immunodeficient, making them more susceptible to infection and premature death.

It is known that drug metabolism and pharmacokinetics are impaired during immunosuppressed condition (14), as may also be caused by AZT. Such reduction in drug metabolising capacity of the liver occurs along with a decrease in the cytochrome $_P$450 associated monooxygenase activities. Though both phase I and phase II biotransformation and detoxifying enzymes govern the metabolic disposition of any drug or chemical in the body, there is hardly any report regarding the fate of these enzymes during AZT treatment.

In this report we wanted to explore the possibility as to whether or not an accelerated regeneration and/or reconstitution of the abrogated metabolic, immunologic and biosynthetic status of the treated host may lead to overcome the toxic and/or immunosuppressive effects. Earlier studies from our laboratory demonstrated that Protein A (PA), of *Staphylococcus aureus* Cowan I, has the unique property of ameliorating toxicity of various drugs and chemicals (15-19). Though, use of such immunomodulators may have immense therapeutic benefit when used in combination with antiviral drug such as AZT, it has not been exploited in detail to date.

MATERIALS AND METHODS

AZT used in this study was a gift from Prof. K.C. Agrawal, Tulane University, U.S.A. Later it was obtained from Indian Institute of Chemical Technology, India. PA was purchased subsequently from Pharmacia Fine Chemicals, Stockholm, Sweden. Purified recombinant Epo (rEpo) was purchased from Genzyme (Cambridge, MA).

Treatment of animals. Male Swiss albino mice, 6-8 weeks old, weighing 20-25 g were used in these studies. The animals were maintained on a commercial pellet diet (Lipton India Ltd., Calcutta, India) and were provided ad-libitum The animals were divided into four groups of six animals each and treated separately. i) control (injected with sterile normal saline), ii) AZT group (injected with AZT, i.p. @ 100 mg/kg body weight for 5 consecutive days, iii) PA group (injected with PA, iv., @ 50 μg/kg body weight biweekly for 2 weeks. iv) PA+AZT group (administered with PA, i.v., @ 50 μg/kg body weight biweekly for 2 weeks followed by AZT, i.v., @ 100 mg/kg body weight for 5 consecutive days.

AZT injections were given 24 hrs. after the last PA injection. Effective dosage and time schedule of AZT inoculation in mice were determined by monitoring the hemoglobin content, since anemia is the typical toxic symptom of the drug. The dosage of PA that produced significant biological effects was determined on the basis of our previously published data (20).

All the animals were sacrificed by cervical dislocation 24 hrs. after the last inoculation. Before sacrificing, blood was collected from the retro-orbital plexus. Total RBC, WBC and hemoglobin concentrations were evaluated by standard methods. The smear was prepared from fresh blood for differential leukocyte counts in the light microscope using oil immersion lens. Trypan blue dye exclusion test was performed to determine viability of cells.

Total cell count in thymus, spleen, and bone marrow. Thymus and spleens were minced immediately after they were taken out of the animals in RPMI-1640 and passed through a fine mesh to make a single cell suspension. The cells were counted under light microscope using Haemocytometer (Neuberg, Germany). Bone marrow cells were collected from the right femur, single cell suspension was made and then the cells were counted.

Preparation of post-mitochondrial and microsomal fractions of liver cells. The livers were removed and washed with ice-cold saline to make it free from contaminating blood. The tissues were homogenized in a Potter Elvejem homogenizer in 50 mM Tris-HCl, pH 7.4, containing (1.15% w/v) KCl, 1 mM EDTA and (20% w/v) glycerol. Post-mitochondrial fraction and microsomal pellet were obtained for each group by differential centrifugation (9000g for 20 min) and by ultra centrifugation (100,000g for 1 hr) respectively. These fractions served as the source of biotransformation and detoxification enzymes.

Enzyme assay. Total cytochrome p450 content was determined by the method of Omura & Sato (21), wherein the concentrations were calculated from the difference in absorbance at 450 nm and 490 nm using a molar extinction coefficient of 91 $nM^{-1}cm^{-1}$. The activity of aniline hydroxylase was assayed by measuring the rate of formation of *p*-amino phenol (22). Aminopyrine-N-demethylase activity was determined by measuring the amount of formaldehyde formed (23). Glutathione-S-transferase activity was assayed according to the method of Habig and others (24) using 1-chloro-2,4-dinitro benzene (CDNB) as substrate.

Studies on bone marrow progenitor cells. CFU-E assay was performed by culturing bone marrow cells ($1x10^5$ cells/ml of 1.3% methyl cellulose solution in RPMI-1640) in duplicate of 35 mm petridishes, in the presence of rEpo (200 mU/ml) for 48 hrs at 37°C in an incubator maintained at 5% CO_2 in air. Cells were then stained with 3,3′-diaminobenzidine. Colonies containing eight or more benzidine positive cells were counted using an inverted microscope. For CFU-E assay, cells were cultured in 1% methyl cellulose solution in RPMI-1640 for 14 days and stained as mentioned above. Colonies containing 30 or more benzidine positive cells were counted. In case of CFU-GM assay, cells were cultured in 3% methyl cellulose solution in RPMI-1640 for 7 days and colonies containing more than 50 benzidine positive cells were only counted.

Quantification of erythropoietin by ELISA. Sandwich ELISA method was followed for Epo estimation using anti-Epo antibody (antibody titre 1:50,000) raised in our laboratory against standard Epo. A standard curve for Epo was generated using standard Epo and values of experimental samples were compared and contents were calculated.

HPLC analysis of AZT clearance from serum samples. AZT from serum samples of different groups of animals was extracted with three volumes of methanol and filtered separately. The concentrations of AZT were determined by HPLC using NOVAPAC C-18 column. AZT peak was confirmed by comparing the retention time with that of standard AZT. To understand the rate of metabolism, AZT was injected to the PA-pretreated as well as control animals and the rate of its clearance from the serum was analyzed by HPLC at different time intervals, that is, 1, 2, 6 and 24 hrs. intervals.

RESULTS

The results depicted in Fig. 1 show that AZT has high myelosuppressive effect. It drastically reduced RBC (Fig.1A) and hemoglobin (Fig. 1B) contents and the cellularity of bone marrow (Fig. 1C) and spleen (Fig. 1D). Effect of AZT on thymus (data not shown) was not very significant. PA increased the RBC and hemoglobin contents as well as the cellularity of both spleen and bone marrow, when applied prior to AZT inoculation. In this combination group (PA+AZT), the cellularity was reversed almost at the control level.

Results depicted in Fig. 2A show the effect of AZT

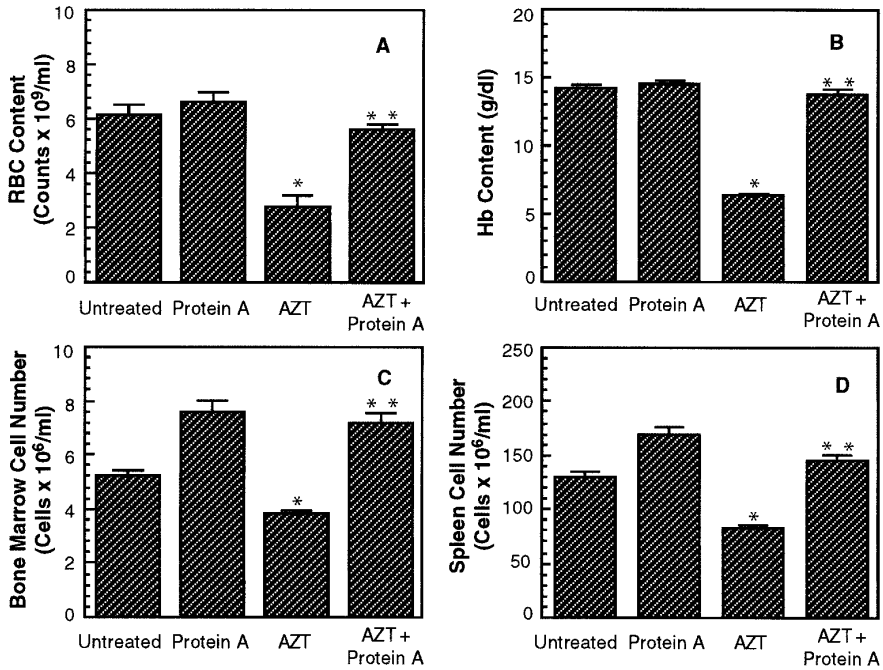

FIG. 1. Effect of AZT on RBC and hemoglobin contents as well as cellularity of spleen, thymus, and bonemarrow of mice in presence or absence of Protein A. Swiss albino mice were treated with AZT (100 mg/kg body weight) for five consecutive days in the presence or absence of PA (50 mg/kg body weight bi-weekly for two weeks). (A) RBC. (B) Hemoglobin content. (C) Total bonemarrow cells. (D) Total splenic cells. The values are mean ± SEM of six individuals. *$p<0.005$ compared with control. **$p< 0.005$ compared with AZT.

on the bonemarrow progenitor cells. AZT was found to reduce the number of CFU-E derived colony drastically. PA alone showed approximately 30% increase in CFU-E derived colonies compared to untreated controls. In PA + AZT group, number of colonies was 100% greater than that of AZT treated group. The results of BFU-E showed similar trend with higher recovery in case of combination group. Interestingly, the toxic effect of AZT was less towards CFU-GM compared to erythroid progenitors. However, PA could replenish the depleted cells in this case also. Moreover, our studies involving treatments with AZT and quantification of Epo have shown that serum Epo level was increased in the combination group (AZT + PA) (Fig. 2B). Interestingly, AZT treatment alone also could show some increase in the level of Epo, but not significantly. PA itself did not show any significant change when compared to AZT group, but did show major increase compared to control group. Thus Epo may have a role in the accelerated regeneration of the depleted cell pool in the combination group.

Since cytochrome p450 and its isoenzymes such as hydroxylases, demethylases, glutathione-S-transferases are important components of phase I biotransformation and phase II detoxification, we were interested to determine a) whether AZT alone has any effect on these enzymes, and b) whether PA can revert AZT-abrogated enzyme activity. Our results show that PA alone induced an increase in phase I and phase II biotransformation and detoxification enzyme activities. The content of cytochrome p450 (133% of control) (Fig. 3A), the enzyme activities of aniline hydroxylase (144% of control) (Fig. 3B) and aminopyrine-N-demethylase (109% of control) (Fig. 3C) were also increased in response to PA treatment. However, AZT was found to have inhibitory effects on cytochrome p450 content (54.8% of control), and also on the enzymatic activities of aminopyrine-N-demethylase (64% of control) and aniline hydroxylase (63.5% of control). A marked regeneration of activities of these enzymes was found in groups which were pretreated with PA followed by AZT administration. The level of cytochrome p450 reached as high as 82.3% while the enzyme activities of aniline hydroxylase and aminopyrine-N-demethylase were increased up to 84.0 and 90.2% of the respective control values of the AZT treated group.

The activity of Glutathione-S-transferase, an important phase II enzyme, was found to be decreased to the level of 30.3% of control in AZT treated group, whereas PA pretreatment resulted in a 100% recovery of this enzyme activity (Fig. 3D) in the PA+AZT group.

These observations led us to examine the serum clearance of AZT, in PA pretreated and untreated groups. HPLC analysis of serum samples indicated that there is an accelerated clearance of AZT from the blood upon pretreatment with PA. In AZT group (where no PA was given), the level of AZT was found to be 34 μg/ml of serum after 24 hrs. of the last injection. In the groups, where PA pretreatment was given, the corre-

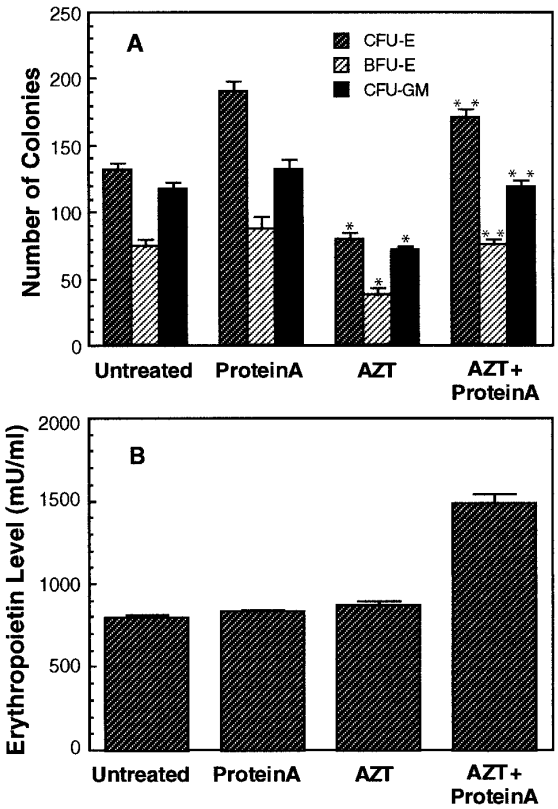

FIG. 2. Effects of AZT on bonemarrow progenitor cells and serum erythropoietin in presence or absence of Protein A. Swiss albino mice were treated with AZT (100 mg/kg body weight) for five consecutive days in the presence or absence of PA (50 mg/kg body weight biweekly for two weeks). After the last treatment the animals were sacrificed, blood was collected, and the bonemarrow cells were cultured for CFU-E assay (*$p<0.02$ compared with control; **$p<0.01$ compared with AZT group), BFU-E assay (*$p<0.05$ compared with control; **$p<0.02$ compared with AZT group), and CFU-GM assay (*$p<0.02$ compared with control; **$p<0.01$ compared with AZT group). The values are mean ± SEM of six sets of experiments. Serum was separated from blood and analyzed by ELISA for Epo (Fig. 2B).

sponding level of AZT was reduced to 1.4 μg/ml of serum after 24 hrs (Fig. 4A). These results indicated that the retention time of AZT in serum was appreciably reduced (about 24 times) upon PA treatment. When the level of residual AZT, and the activities of biotransformation enzymes under different treatment conditions were compared, a negative correlation was seen (data not shown).

Time course studies in HPLC (Fig. 4B) using serum samples drawn at different time intervals suggested that the rate of clearance of AZT from the circulation was very rapid for the animals pretreated with PA. The rate of clearance of AZT between 2 hrs. and 6 hrs. was 10 times higher for PA + AZT treated group, than the AZT group. In 24 hrs. there was no detectable amount of AZT in the serum of animals pretreated with PA, whereas 10.74 μg/ml was still present in the AZT group. These results suggest that the rates of clearance of AZT and/or its metabolites were faster in the PA plus AZT groups, than the groups where only AZT was administered. Thus, it appeared that the residency time for AZT and/or its metabolites was far less in PA + AZT group than the AZT group.

DISCUSSIONS

Chemical and drug toxicity are, to a great extent, determined by the metabolic disposition of the drug and/or its metabolites to the host, in terms of its biotransformation, detoxification and bioelimination mechanisms. Any disruption of such intrinsic ability of the host would result in a prolonged residency time of toxic metabolites, resulting in overt toxicity. As a result, the immune function of the host is also very often subjected to the toxic insults, resulting in immunodepression, increased susceptibility to infection and death.

Our results showed that RBC and hemoglobin contents were significantly reduced by AZT treatment leading to anemia in mice, which is the common toxic episode observed in AIDS patients treated with AZT. Cellularity of spleen, bone marrow and thymus were also drastically reduced in AZT treated mice. These findings are in conformity with that of Gogu et al. (25). An accelerated recovery from AZT induced anemia and leukopenia in PA+AZT treated mice suggests that PA was useful in protecting the host from AZT induced toxicity.

Our findings with CFU-E, BFU-E and CFU-GM assays confirm the hypothesis that the effect of AZT on progenitor stem cells may contribute to its associated hematopoietic toxicity. AZT treatment caused almost 50% reduction in the progenitor population as shown by the above mentioned assays. These findings are also in conformity with that of Scheding et al. (26) who reported that erythropoietic progenitors were the most sensitive cell type to AZT toxicity, and hematotoxicity increased with increasing proliferative activity. The toxic effect of AZT on progenitor cells had been reported by several other workers (27-30). Our studies showed that PA could regenerate the depleted pool of progenitor cells, to a large extent in the AZT treated mice.

Our results provide tacit support to the requirement of the measurement of the serum Epo levels of patients undergoing AZT treatment, so as to be able to treat them with Epo for combating anemia. It is known that Epo production increases only during stress conditions. Therefore, the slight increase in Epo production, as was observed in even AZT group, may be related to that significant increase in serum Epo levels, however, in the combination group (PA+AZT), indicates that PA treatment could maintain a steady level of Epo required for an accelerated regeneration of depleted hematopoietic cells.

It is well established that any disruption of the biotransformation, detoxification and bioelimination sys-

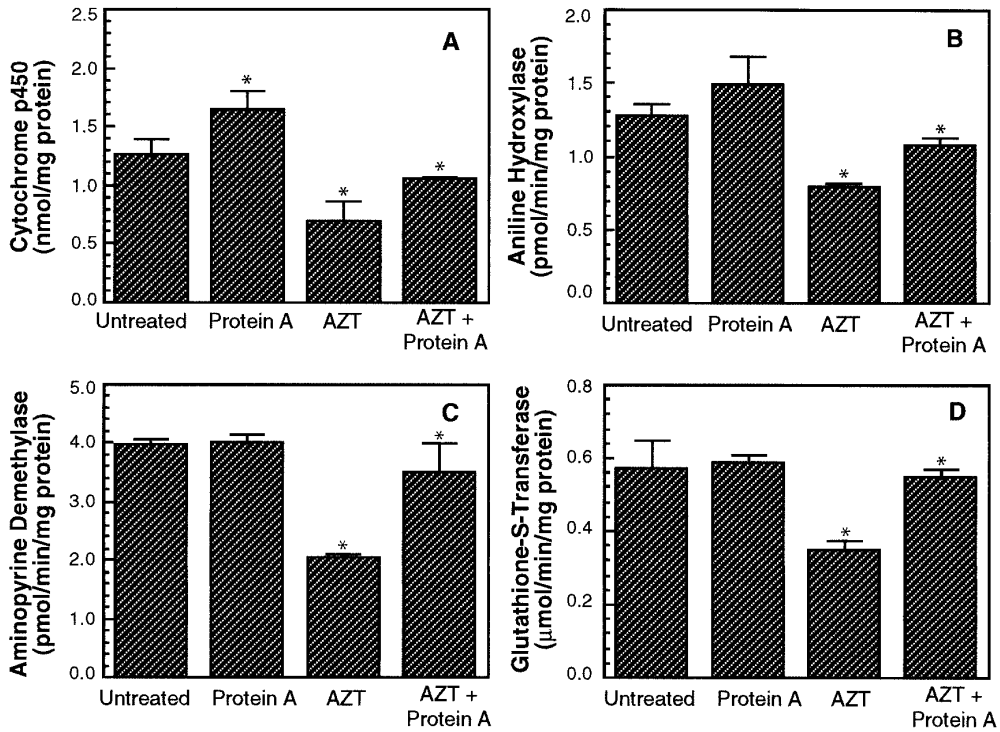

FIG. 3. Effect of AZT on different biotransformation enzymes when treated in presence or absence of Protein A in mice. Swiss albino mice were treated with AZT (100 mg/kg body weight) for five consecutive days in the presence or absence of PA (50 mg/kg body weight bi-weekly for two weeks). After the last treatment animals were sacrificed, microsomes were isolated from liver, and (A) Cytochrome $_P$450 content, (B) aniline hydroxylase activity, (C) aminopyrine demethylase activity, and (D) glutathione-S-transferase activity were determined. The values are mean ± SEM of six sets of experiments. *$p<0.001$ compared with control or AZT groups.

tems would result in a prolonged residency time of toxic metabolites causing overt toxicity. It was thus necessary to know about the metabolism of AZT to understand its cause of toxicity. After entering the cell by simple diffusion (31), AZT is anabolically converted to its corresponding TTP form by thymidylate kinase (1) to execute its antiviral properties within an hour. The excess amount of AZT, that is not anabolically converted, remains in the extracellular circulation to be catabolized by biotransformation enzymes. Increased AZT availability correlates with increased formation of 3-amino-3-deoxythymidine (AMT) (32), which is more toxic to bone marrow progenitor cells than AZT (6). Hence excess availability of AZT will lead to more hematological toxicity. We thought that it could be avoided by reconstituting the depressed biotransformation enzyme activity for rapid catabolism and clearance. Our results showed that PA could reconstitute the cytochrome $_P$450 dependent drug metabolizing enzymes as well as the phase II detoxification enzyme, glutathione-S-transferases, even after the abrogation of their activity by AZT and/or its metabolites. Depression of drug metabolising enzymes, in particular the phase I enzymes, may result in the inhibition of catabolism of AZT and its longer persistence. Moreover, the reduction of phase II enzyme activity will lead to reduced detoxification of toxic metabolite(s). This also indicates that the patients undergoing AZT treatment, may be more susceptible to toxicity of other drugs and chemicals that rely on glutathione conjugation for their detoxification and subsequent elimination. Such events would also increase the total toxin load in the body, leading to severe deleterious effects.

It has been reported that co-admission of L-2-oxothiazolidine-4-carboxylate, and intracellular stimulator of glutathione, can reduce or prevent the toxicity associated with AZT (33). It has also been reported that phenobarbitone pretreatment of rats resulted in 5.5 fold increase in AZT clearance (34). In our studies, pretreatment of mice with PA has resulted in rapid clearance of AZT leading to a reduction of the associated hematological toxicity. This happens as early as 2 hours, which is sufficient for anabolic conversion of AZT to the active form. PA has been reported to induce specific isoenzymes of glutathione-S-transferases, which indicates decreased retention of cytotoxic metabolites in the system (35). Considering the enzymatic and serum clearance data, it can be concluded that PA decreases the toxicity of AZT by reconstituting both the phase I and II biotransformation and detoxification enzyme activities in one hand, and accelerating the bioelimination process on the other. Further, an accelerated reconsti-

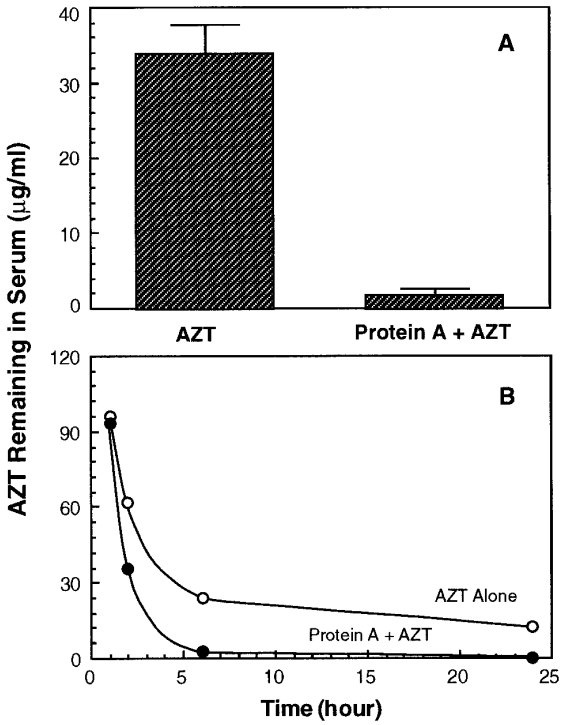

FIG. 4. Effect of Protein A on AZT residency in serum and its clearance from serum of treated mice. Swiss albino mice were treated with AZT (100 mg/kg body weight) for five consecutive days in the presence or absence of PA (50 mg/kg body weight bi-weekly for two weeks). (A) After the last treatment blood was collected from retro-orbital plexus and serum was separated. The samples were analyzed for AZT in HPLC using reverse phase NOVAPAC C-18 column with mobile phase consisting of acetonitrile (15%) and ammonium phosphate buffer (85%, pH 2.2). (B) After the last treatment, blood was drawn at different time intervals. Serum was separated and quantitatively analyzed for AZT in HPLC as mentioned above. Values are average of six different mice.

tution of depleted cell pool of the hematopoietic system in PA treated mice may have helped them to reduce considerably the toxic effects of AZT.

Quantitative analysis of AZT and its metabolites using HPLC increases the convenience and accuracy of measurement and thus can be widely exploited. It has been reported that mean apparent clearance of AZT is 1.40 L/hr/kg measured in plasma by HPLC (36) indicating the rate of its metabolism.

It has been proposed that AZT-induced hematological toxicity and antiviral activity are mediated by different biochemical mechanisms (37). As is already known, anabolites of AZT, i.e., AZTTP is having antiviral activity, whereas reduced catabolite, i.e., AMT, is associated with toxicity. Our results indicate that PA stimulates the catabolic pathway of AZT, through the activation of biotransformation enzymes. Hence the present results appear to be significant in that without compromising the AZT antiviral capacity, one can reduce its associated toxicity by using PA. Since PA is an interferon inducer (38), it is also logical to expect that it will synergise the antiviral capacity with that of AZT, and thus increase the overall therapeutic index of the drug significantly.

Since PA is a bacterial protein, it is therefore required to be assessed whether or not it can be used as such in the human patients at a low concentration without any deleterious effects. Our results in rodents did not show any adverse effects at the dosage level tested over a period of time. Very careful attention is required in this direction. Studies on various peptide segments of PA are going on to avoid any possible complications.

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REFERENCES

1. Furman, P. A., Fyfe, J. A., St. Clair, M. H., Weinhold, K., Rideout, J. L., Freeman, G. A., Nusinoff Lehrman, S., Biolognesi, D. P., Borders, S., Mitsya, H., and Barry, D. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8333–8337.
2. Eichhoff, T. C. (1988) *Ann. Intern. Med.* **108,** 575–580.
3. Jackson, G. G., Paul, D. A., Falk, L. A., Rubenis, M., Despotes, J. C., mack, D., Knigge, M., and Enison, E. E. (1988) *Ann. Intern. Med.* **108,** 175–180.
4. De Clercq, E. (1991) *J. Acquired Immune. Syndr.* **4,** 207–218.
5. Sommadossi, J. P., Cartisles, R., and Zhow, Z. (1989) *Mol. Pharmacol.* **36,** 9–14.
6. Stagg, M. P., Cretton, E. M., Kidd, B. S., Diasio, R. B., and Sommadossi, J. P. (1992) *Clin. Pharmacol. Ther.* **51,** 668–676.
7. Fischl, M. A., Richman, D. D., Grieco, M. H., Gottlieb, M. S., Volberding, P. A., Laskin, O. L., Leedom, J. L., Groopman, J. E., Mildvan, D., Schooley, R. T. et al. (1987) *N. Engl. J. Med.* **317,** 185–191.
8. Richman, D. D., Fischl, M. A., Grieco, M. H., Gottlieb, M. S., Volberding, P. A., Laskin, O. L., Loedon, J. M., Groopman, J. E., Mildvan, D., Hirsch, M. S., Jackson, G. G., Furack, D. T., and Nusineff, L. (1987) *N. Engl. J. Med.* **317,** 192–197.
9. Schneider, D. R., and Picker, L. J. (1985) *Am. J. Clin. Pathol.* **84,** 144–152.
10. Bagnara, G. P., Giorgio, Z., Giovannini, M., Carbe Re, J., Furlini, G., and Placa, M. L. (1990) *Exp. Hematol.* **18,** 426–430.
11. Arock, M. Dedenon, A., Damais, C., Averlant, G., and Guillosson, J. J. (1992) *Comples Rendus des Scanes de la Societe de Biologie et de Ses Filiales.* **186,** 206–214.
12. Gallicchio, V. S., Hughes, N. K., and Tse, K. F. (1993) **5,** 62–71.
13. Harrison, J., Kappas, A., Levere, R. D., Lutton, J. D., Chertkov, J. L., Jiang, S.,and Abraham, N. G. (1994) *Blood* **83,** 3829–3831.
14. Langouet, S., Corcos, L., Abdel-Razzak, Z., Loyer, P, Ketterer, B., and Guillouzo, A. (1995) *Biochem. Biophys. Res. Commun.* **216,** 793–800.
15. Zaidi, S. L., Singh, K. P., Raisuddin, S., Saxena, A. K., and Ray, P. K. (1990) *Immunopharmacol. Immunotoxicol.* **12,** 479–512.
16. Singh, K. P., Zaidi, S. A., Raisuddin, S., Saxena, A. K., Dwivedi,

P. D., Seth, P. K., and Ray, P. K. (1990) *Toxicol. Lett.* **51,** 339–351.

17. Shankar, U., Kumar, A., Rao, G. S., Dwivedi, P. P., Pandya, K. P., and Ray, P. K. (1993) *Biochem. Pharmacol.* **46,** 517–524.
18. Raisuddin, S., Singh, K. P., Zaidi, S. A., and Ray, P. K. (1994) *Int. J. Immunopharmacol.* **16,** 977–984.
19. Dwivedi, P. D., Verma, A. S., Misra, A., Prasad, A. K., Saxena, A. K., Dutta, K. K., and Ray, P. K. (1989) *Toxicol. Lett.* **49,** 1–13.
20. Kumar, S., Shukla, Y., Prasad, A. K., Verma, A. S., Dwivedi, P. D., Mehrotra, N. K., and Ray, P. K. (1992) *Cancer Lett.* **61,** 105–110.
21. Omura, T., and Sato, R. (1964) *J. Biol. Chem.* **239,** 2370–2378.
22. Kato, R., and Gillette, J. R. (1965) *J. Pharmacol. Exp. Ther.* **10,** 279–284.
23. Nash, T. (1953) *Biochem. J.* **55,** 416–421.
24. Habig, H., Pabst, M. J., and Jecoby, W. B. (1974) *J. Biol. Chem.* **249,** 7130–7139.
25. Gogu, S. R., Lertora, J. J. L., George, W. J., Hyslar, N. E., and Agrawal, K. C. (1991) *Exptl. Hematol.* **19,** 649–652.
26. Scheding, S., Media, J. E., and Nakeff, A. (1994) *Exptl. Hematol.* **22,** 60–65.
27. Du, D. L., Volpe, D. A., Grieshaber, C. K., and Murphy, M. J., Jr. (1990) *Exptl. Hematol.* **18,** 832–836.
28. Du, D. L., Volpe, D. A., Grieshaber, C. K., and Murphy, M. J., Jr. (1992) *Intern. J. Cell. Clon.* **10,** 87–93.
29. Gogu, S. R., Beckman, B. S., and Agrawal, K. C. (1992) *Antimicrob. Agents Chemother.* **36,** 2370–2374.
30. Chan, T. C., Boon, G. D., Shaffer, L., and Redmond, R. (1992) *Dur. J. Hematol.* **49,** 71–76.
31. Kong, X. B., Zhu, Q. Y., Vidal, P. M., Watanabe, K. A., Polsky, B., Armstrong, D., Ostrander, M., Lang, S. A., Jr., Muchmore, E., and Chou, T. C. (1992) *Antimicrob. Agents Chemother.* **36,** 808–818.
32. Cretton, E. M., and Sommadossi, J. P. (1991) *Biochem. Pharmacol.* **42,** 1475–1480.
33. Goldberg-Dennis-I, White-Randy, D., and Wilson-Daniel, M. (1993) *Can. Pat. Appl.* **61,** 209–211.
34. Besek, S. (1994) *J. Pharma-Pharmacol.* **46,** 575–580.
35. Dwivedi, P. D., Kumar, A., Prasad, A. K., Pandya, K. P., and Ray, P. K. (1990) *Biochem. Biophys. Res. Commun.* **169,** 470–480.
36. Qian, M. S., Swagler, A. R., Mehta, M., Viswanathan, C. T., and Gallo, J. M. (1992) *Pharm. Res.* **9,** 224–227.
37. Sommadossi, J. P., Carlisle, R., Schinanzi, R. E., and Zhou, Z. (1988) *Antimicrob. Agents Chemother.* **32,** 977–1001.
38. Catalona, W. J., Ratliff, T. L., and McCool, R. E. (1981) *Nature* **291,** 77–79.