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ANTITUMOUR ACTIVITY OF LIPOSOMAL ENCAPSULATED TIAZOFURIN AND ITS NAD-ANALOGUE

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SUMMARY

Liposomal encapsulation of the antitumour drug Tiazofurin and its active metabolite Thiazole-4-carboxamide adenine dinucleotide (TAD) which is a NAD-analogue, potentiated the anticancer activity of the drug. Tiazofurin at concentrations upto 100 mgs/kg body weight and the TAD at concentration 5 mg/kg body weight were found ineffective while the encapsulated Tiazofurin at a concentration of 10 mgs/kg body weight produced a 31% increased life span and the encapsulated TAD prolonged the life span by 85.7 percent at a concentration of 1 mg/kg body weight in Dalton's lymphoma bearing mice.

INTRODUCTION

Tiazofurin [(2-B-D ribofuranosyl thiazole-4-carboxamide) NSC 286193] is a potent oncolytic C-nucleoside. It was effective in increasing the life span of rats bearing Lewis lung carcinoma and L 1210 and p388 leukemia in mice systems (Jayaram et al 1982, Robins et al 1982). Tiazofurin has been shown to be phosphorylated to Tiazofurin-5-monophosphate and then further to Thiazole-4-Carboxamide adenine dinucleotide (TAD). This metabolite (TAD) has been isolated from Tiazofurin treated tumours, synthesised chemically and enzymatically and is a potent inhibitor of Inosine monophosphate Dehydrogenase (IMP-DH), a key enzyme in guanine nucleotide synthesis (Cooney et al 1982, Kuttan et al 1982).

It has been shown that only a small fraction of the administered drug, Tiazofurin is converted to the active metabolite (Susan et al 1984). In order to circumvent the problems of low entry and conversion rate of Tiazofurin to TAD we have prepared lipsomal encapsulated Tiazofurin and its active metabolite TAD. Our initial results indicate that liposomally encapsulated TAD effectively reduced the tumour growth in experimental animals at much lower concentrations than Tiazofurin.

MATERIALS AND METHODS

Neutral, small unilamellar vesicles were prepared by the procedure of Bangham et al (1974). Di-palmitoyl phosphatidyl choline (DPPC) and cholesterol were purchased from Sigma. Tiazofurin and TAD were received as gift from Dr. R.K. Robins, Bringham Young Univ., Utah. DPPC and cholesterol in molar ratio 1:4 in chloroform was evaported under vaccum on to walls of the flask. The drug in 0.01 M PBS pH 7.4

was added to the dried lipid film; mixture vortexed and sonicated for thirty minutes. Liposomes were separated from free drug by column chromatography on Sephadex G-50. The encapsulation efficiency was calculated by disrupting the liposomes with chloroform and spectrophotometrically estimating the drug released at 238 nm for Tiazofurin and 252 nm for the TAD. Liposomes containing 0.01 M PHS and no drug were prepared in the same manner (plain liposomes). Dalton's lymphoma was grown in ascites form in the peritoneal cavity of Swiss albino mice. Groups of Six weighing 20-25 grams were injected intraperitoneally with 1 x 10^6 cells/mouse in normal saline. After 24 hours, animals were treated with different doses of free drug, plain liposomes and encapsulated drug intraperitoneally daily for nine days. Weight on day seven, mortality and increased life span as compared to nontreated group calculated (Ahluwalia et al 1984). The experiment was done thrice and the mean value taken. The percent survival was calculated as

$$\frac{\text{Median survival time of treated tumours}}{\text{Median survival time of control tumours}} * 100$$

The percent increase life span (ILS) is = percent survival - 100.

RESULTS

The encapsulation efficiency of Tiazofurin and its active metabolite TAD in neutral, small unilamellar vesicles was calculated by the UV absorption at 238 and 252 nm respectively. It is found that the encapsulation efficiency of Tiazofurin is rather low (5%) compared to that of TAD (60%). The reason for this difference may be due to the polar groups that are present in the TAD molecule as well as its size.

The effect of various concentrations of Tiazofurin (Free and encapsulated) on the tumour reduction and increased life span of animals with Dalton's lymphoma ascites tumour was studied (Table I). We have used four different concentrations of free Tiazofurin, 10 mg, 25mg and 100mg/kg body weight of the animal. However, at these concentrations studied all the animals developed ascites tumour, were dead within thirty days and there was no increase in life span as compared to that of untreated animals. This could be expected so, as effective concentration of free Tiazofurin needed in the case of p388 animals was 200 mgs/kg. Higher concentrations could not be used to determine the effective dose of Tiazofurin in our experiment due to the limited availability of the drug.

Encapsulated Tiazofurin on the other hand was found to effectively reduce the ascites tumour induced by Dalton's lymphoma cells. Thus even at 10 mgs/kg body weight it produced an increased life span of 31 percent. Moreover 5/6 animals were alive on 30th day as against no survivors in control group, animals given plain liposomes and animals treated with 100 mgs/kg body weight of free Tiazofurin. In all these experiments there was no change in body weight of the animals indicating no toxicity due to the drug.

The result was pronounced in the case of encapsulated TAD. TAD encapsulated in neutral, small unilamellar vesicles injected intraperitoneally into mice transplanted with Dalton's lymphoma cells effectively reduced the tumour formation. There was an increased life span of 75 percent in the case of animals treated with 2.5 mg/kg body weight. As in the case of Tiazofurin there was no weight loss showing no drug toxicity in the treated animals. Unencapsulated TAD at a concentration of 5 mgs/kg body weight did not show any effect in increasing the life span of tumour bearing animals.

DISCUSSION

Liposomes have been extensively exploited as drug delivery system. Uptake of

TABLE-I

Effect of free and liposomal encapsulated drug on span of Dalton's lymphoma ascites tumour bearing mice.

Drug	Dose in mgs / kg body weight	Survival of animals 30 days	60 days	Increased life span
Control	No drug	0 / 6	-	-
Tiazofurin	100 mgs	0 / 6	-	-
	50 mgs	1 / 6	-	-
	25 mgs	0 / 6	-	-
	10 mgs	0 / 6	-	-
Liposome encapsulated Tiazofurin	10 mgs	5 / 6	0 / 6	31%
TAD	5 mgs	0 / 6	-	-
Liposome encapsulated TAD	2.5 mgs	6 / 6	2 / 6	75%
	1 mgs	6 / 6	3 / 6	85.7%

Animals were given one million lymphoma cells intraperitoneally. After 24 hrs., animals were administered different forms of the drug I.P. daily for nine days.

liposomes by cells in generally by endocytosis and tumours are known to have high endocytic capacities. The cell membrane is impermeable to the active metabolite of Tiazofurin i.e. Tiazofurin-5-monophosphate and TAD. The conversion of Tiazofurin to TAD is stringently dependent on the extracellular concentration Tiazofurin (Cooney and Kutsmitz, 1984) and the relative activity of the enzymes. Studies on the pharmacokinetics and metabolism of Tiazofurin in rodents have revealed that only less than fifteen percent of the administered Tiazofurin is converted to its active metabolite (Susan et al 1984). Moreover studies on human tumour cell lines and human lung cancer lines in culture have proved the anabolism of Tiazofurin to TAD (Earle and Glazer, 1983). Hence liposomal encapsulation of TAD is ideally suited as a drug delivery system as it can circumvent the above problems.

In the present study we have liposomally encapsulated Tiazofurin as well as TAD in neutral, small unilamellar vesicles and compared the efficacy of encapsulated drug over the non-

encapsulated drug. Encapsulation of both Tiazofurin and TAD had definite advantage in tumour reduction, but was more pronounced in the case of encapsulated TAD as the efficiency of encapsulation was higher as well as the effective concentration needed was very low, The Tiazofurin concentration needed to effectively increase the life span in p388 mouse tumours was found to be 200 mgs/kg body weight (Gebeyehu et al 1983). In our system also we have found that at 100 mg/kg body weight of Tiazofurin there was no increase in life span of Dalton's lymphoma ascitis tumour mice. However the life span was increased by 85.7 percent when 1 mg/kg body weight of encapsulated TAD was given. The dose is almost 1/500th of the dosage needed with Tiazofurin, as such. Similarly, with encapsulated Tiazofurin 10 mg/kg. the life span was increased by 31 percent . However the encapsulation efficiency of Tiazofurin was only five percent which may be due to its glycosidic nature.

Although Tiazofurin showed promising anticancer activity in experimental animals Phase one clinical trials of the drug have not been very successful, mainly due to the large dosage needed (Trump et al 1985). Moreover at this dosage it produced toxic symptoms like neurotoxicity, myelosuppression, desquamation of palms and soles, stomatitis, drug fever and increased creatine phosphokinase levels. Resistance to Tiazofurin treatment in which TAD is not synthesised in the resistant cells has been reported. Encapsulation is proved to prolong the plasma half life of drugs and increase their tissue retention. This will be an added advantage for Tiazofurin encapsulation whose half life is only 150-180 minutes.

Liposomal encapsulation of TAD as shown in this manuscript can effectively circumvent most of the problems related to Tiazofurin administration and could be an effective tool in tumour therapy with this drug. More studies on the pharmacokinetics of liposome encapsulated TAD are needed for full evaluation of its therapeutic potential.

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