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TIAZOFURIN-INDUCED SELECTIVE DEPRESSION OF NAD CONTENT IN HEPATOMA 3924A

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The NAD content in hepatoma 3924A was approximately 40% of that in the liver of ACI/N rats bearing this hepatoma. Treatment of tumor-bearing rats with tiazofurin decreased NAD pools in the hepatoma, but no change was apparent in the liver. In a dose-response study, injection of varying amounts of the drug decreased NAD pools in the hepatoma in a dose-dependent fashion. In time-sequence studies, a single drug dose (200 mg/kg) depressed NAD pools in the hepatoma from 2 to 24 h after injection to approximately 50% of control at the lowest point before returning to control range at 48 h. The tiazofurin-induced depletion of NAD pools in the hepatoma to approximately 20% of that of normal liver might play a role in the anti-cancer action and toxicity of this drug.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC-286193) exhibits potent oncolytic activity against several murine leukemias and Lewis lung carcinoma (1) and hepatoma 3924A (2). Evidence has been provided that the drug is metabolized first to tiazofurin-5'-monophosphate and then to thiazole-4-carboxamide adenine dinucleotide (TAD), an analogue of NAD (3). TAD was shown to inhibit IMP dehydrogenase with a subsequent depletion of guanylate pools which was proposed to be the cause of the oncolytic action of the drug (4). Since TAD is presumably synthesized in vivo from tiazofurin-5'-monophosphate by NAD pyrophosphorylase (5), the enzyme that synthesizes NAD from nicotinamide mononucleotide, and since NAD is a coenzyme of IMP dehydrogenase and may protect it from tiazofurin action, an investigation of the behavior of NAD pools should be helpful in elucidating the mechanism of action and selectivity of tiazofurin. The results of our studies on NAD pools in hepatoma 3924A and rat liver, and on the influence of tiazofurin treatment on the pools are presented here.

MATERIALS AND METHODS

Materials: Tiazofurin was provided by Dr. V. L. Narayanan, N.C.I., N.I.H., Bethesda, MD. Pyrazofurin was obtained through courtesy of Eli

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Lilly Co., Indianapolis, IN, and acivicin was from the Upjohn Co., Kalamazoo, MI. Hydroxyurea and dipyridamole were purchased from Sigma Co., St. Louis, Mo. Lycurim was obtained through courtesy of Dr. S. Eckhardt and dibromodulcitol through courtesy of Dr. E. Olah, Hungarian Oncological Institute, Budapest, Hungary. All reagents were of the highest purity available.

In Vivo Studies: Rapidly growing solid hepatoma 3924A was maintained as bilateral s.c. transplants in male ACI/N rats (180-200 g). The biological and biochemical properties of this hepatoma have been reported (6). Animals carrying 14-day-old tumors or normal rats were injected with a single dose of the drug. Controls received a saline injection. At the indicated time after injection, the animals were lightly anesthetized with ether, and the hepatoma and liver were freeze-clamped as previously described (7).

Ribonucleotide Analysis: Freeze-clamped tissues were extracted with perchloric acid, the extracts neutralized with $KHCO_3$ or trioctylamine in freon, and ribonucleotides were determined by high pressure liquid chromatography as previously cited (2) except that 5 mM ammonium phosphate, pH 3.5 and 0.5 M ammonium phosphate, pH 4.5 buffers, and a Radial-PAK SAX cartridge in a Waters Associates Z-Module were used. NAD eluted before AMP in this system. Data were statistically evaluated by the t-test for small samples. Differences between means giving a probability of less than 5% were considered as significant.

RESULTS AND DISCUSSION

The concentration of NAD in freeze-clamped samples of hepatoma 3924A and rat liver was determined by high pressure liquid chromatography. Similar NAD content was found in normal and host rat liver, 892 ± 10 and 917 ± 10 nmol NAD/g tissue respectively (Tables 1 and 2). The NAD content

Table 1: Tiazofurin dose-response effect on NAD concentrations in hepatoma 3924A and normal liver

Tiazofurin dose (mg/kg)	Hepatoma 3924A		Normal liver	
	nmol NAD per g	% of control	nmol NAD per g	% of control
0, control	410 ± 12	100	892 ± 10	100
50	351 ± 27	86	875 ± 11	98
100	263 ± 28	64*	879 ± 8	99
150	244 ± 39	60*	925 ± 24	104
200	206 ± 22	50*	844 ± 50	95

Means ± SE of 2 or 3 samples are given. Tiazofurin was administered i.p. to rats bearing the hepatoma or normal rats; controls received saline. Six hours after injection, rats were anesthetized with ether, and the hepatoma and liver were freeze-clamped. NAD was determined by HPLC analysis as outlined in Methods.

*Significantly different from control ($p < 0.05$).

Table 2: In vivo effect of tiazofurin on NAD concentrations in hepatoma 3924A and host liver

Time after injection	Hepatoma 3924A nmol NAD per g	Hepatoma 3924A % of control	Host liver nmol NAD per g	Host liver % of control
0, control	345 ± 2	100	917 ± 10	100
10 min	351 ± 3	102	947 ± 22	103
30 min	371 ± 9	108	992 ± 12	108*
2 hr	273 ± 16	79*	948 ± 30	103
6 hr	150 ± 5	43*	993 ± 49	108
12 hr	225 ± 6	65*	957 ± 6	104*
24 hr	165 ± 2	48*	1104 ± 16	120*
48 hr	326 ± 8	94	921 ± 19	100
72 hr	354 ± 4	103	1021 ± 38	111

Mean ± SE of 3 samples are given. Tiazofurin (200 mg/kg) was administered i.p. to rats bearing the hepatoma. The rats were anesthetized with ether, and the hepatoma and host liver were freeze-clamped at the indicated times. NAD was determined by HPLC analysis as outlined in Methods. Controls received a saline injection.
*Significantly different from control ($p < 0.05$).

in hepatoma 3924A ranged from 345 to 411 nmol NAD/g tissue (Tables 1-3), a decrease to approximately 40% of the liver. The decreased NAD content in hepatoma 3924A is in line with the decreased NAD concentration reported for several other Morris hepatomas (8) and also for other tumors (9).

Treatment of tumor-bearing and normal rats with tiazofurin decreased NAD pools in the hepatoma, but no effect was observed in normal or host livers. In an in vivo dose-response study, tiazofurin (50-200 mg/kg) decreased NAD pools in the hepatoma 6 h after injection in a dose-dependent fashion (Table 1) to as low as 50% of control; normal liver was unaffected. The decrease in NAD pools with tiazofurin dose is in contrast to the constant extent of depletion of GTP pools and increase in IMP pools in the hepatoma over the same dosage range observed previously (2).

In a time-course study, a single i.p. injection of tiazofurin (200 mg/kg) decreased NAD pools in the hepatoma with time (Table 2) reaching a low point of approximately 50% of control during 6 to 24 h before returning

to the control range by 48 to 72 h. This parallels the decrease in guanylate pools and increase in IMP pools in the hepatoma resulting from tiazofurin treatment (2). NAD pools in the host liver were essentially unchanged or slightly increased at all time periods (Table 2).

Various drugs that inhibit purine and pyrimidine metabolism (pyrazofurin, acivicin, and hydroxyurea), alkylate DNA (lycurim and dibromodulcitol), or block nucleoside transport (dipyridamole) were examined for their ability to alter NAD pools. The drug doses were selected as those most effective for hepatoma 3924A in chemotherapeutic studies for pyrazofurin and lycurim (10) and for inhibiting activities of key enzymes (11) and nucleoside transport (12). The results in Table 3 show that apart from tiazofurin none of the drugs influenced NAD content in hepatoma 3924A.

The results of these experiments indicate that tiazofurin selectively decreased in a dose- and time-dependent manner the NAD pools in hepatoma 3924A without affecting the NAD pools in the liver. As NAD pools in hepatoma 3924A were approximately 40% of that in the liver, tiazofurin

Table 3; *In vivo* effect of drugs on NAD concentrations in hepatoma 3924A

Drugs	Dose (mg/kg)	nmol NAD/g		
		Control	Drug treated	% of control
Tiazofurin	200	410 ± 12	206 ± 22	50*
Pyrazofurin	10	360 ± 7	365 ± 17	101
Acivicin	1	411 ± 42	383 ± 14	93
Hydroxyurea	1000	411 ± 42	390 + 10	95
Lycurim	15	411 ± 42	422 ± 58	103
Dibromodulcitol	600	396 ± 3	413 ± 13	104
Dipyridamole	400	411 ± 42	397 ± 11	97

Mean ± SE of 3 samples are given The drugs or saline were administered i.p. to rats bearing the hepatoma. Control for dibromodulcitol was dimethylsulfoxide. Six hours after injection, the rats were anesthetized with ether, and the hepatoma was freeze-clamped. NAD was determined by HPLC analysis as outlined in Methods.

*Significantly different from control ($p < 0.05$).

treatment further decreased them to approximately 20% of that observed in the liver. This profound alteration of NAD metabolism may reveal a novel aspect in the action of tiazofurin and might play a role in the therapeutic action and selectivity of this anti-cancer agent.

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