INHIBITION OF MACROMOLECULAR SYNTHESIS IN TUMORS BY L-1-TOSYLAMIDO-2-PHENYLETHYL CHLOROMETHYL KETONE

by

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Summary

L-1-tosylamido-2-phenylethyl chloromethyl ketone was observed to inhibit the incorporation of [^{3}H] amino acids into protein and [^{3}H] thymidine incorporation into DNA in Novikoff hepatoma ascites cells in vitro. Similar effects were seen with several Morris hepatomas and a transplanted colon tumor in rats, and were accompanied by decreased uptake of isotope into acid soluble tissue fractions. Under the same conditions, there was no significant inhibition in regenerating liver and there was an increased uptake of [^{3}H] amino acids in the livers of normal and tumor bearing rats.

Introduction

TPCK[1] was initially studied as an inhibitor of chymotryptic activity and was later found to inhibit the initiation of protein synthesis (1,2). A report of the selective inhibition of growth of transformed cells in culture by TPCK (3) was followed by observations of growth inhibition in both normal and virally transformed cells (4). There have been few studies of the effects of TPCK in animals although it has been shown to inhibit tumor promotion in mouse skin (5) and to prevent blastocyst implantation in mice (6). We wish to present evidence that TPCK can exert tissue - selective effects on macromolecular synthesis in the rat.

Materials and Methods

Novikoff hepatoma ascites cells were maintained in female Sprague-Dawley rats and animals of this type were used for experiments on control liver and regenerating liver. Partial hepatectomies consisted of removal of two thirds of the liver (7). Morris hepatomas (8) and the LK1 colon tumor were transplanted in a subcutaneous position in male Buffalo rats. The LK1 colon tumor was induced by sixteen weekly subcutaneous injections of 1,2-dimethylhydrazine dihydrochloride according to the procedure of McCall and Cole (9). Deter-

[1]Abbreviation: TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone

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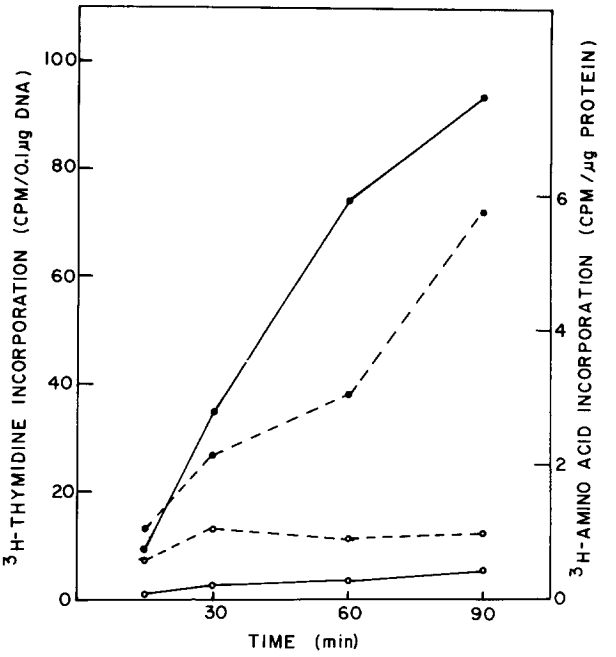

Fig. 1. Effects of TPCK on the incorporation of [^{3}H] thymidine and [^{3}H] amino acids into Novikoff hepatoma ascites cells. The incubations at 37° included 4 x 10^6 cells and 1 µCi [^{3}H] thymidine (continuous lines) or 2 µCi of the mixture of 15 [^{3}H]L-amino acids (broken lines) in 0.4 ml Eagles minimal essential medium containing 50 mM HEPES buffer pH 7.4. The incubations contained either 10 µl dimethylsulfoxide (closed circles) or 100 µg TPCK dissolved in 10 µl dimethylsulfoxide (open circles). The reaction was stopped by the additon of 4 ml 0.5 N $HClO_4$. DNA and protein were extracted as previously described (12). Each point represents the mean of two experiments performed with triplicate incubations.

minations of isotope incorporation into acid soluble and insoluble fractions were performed by adjusting to 0.5N with respect to $HClO_4$ at 0°. The acid insoluble material was washed three times with 0.5N $HClO_4$ (twenty times the original tissue volume) and dissolved in 1N NaOH at 70° for 10 minutes. Protein was assayed by the procedure of Lowry et al (10). Procedures for the measurement of [methyl-^{3}H] thymidine into DNA were as described previously except volumes were reduced to one fourth (11). Radioactivity was determined with a Beckman LSC-250 liquid scintillation counter with an efficiency of approximately 35% for ^{3}H. The mixture of 15 [^{3}H] amino acids (catalog number TRK.440) and [methyl-^{3}H] thymidine (42 Ci/mmole) were obtained from Amersham/Searle,Arlington Heights, Ill. TPCK was purchased from Sigma Chemical Co., St. Louis, Mo. Statistical significance of the results was determined by the t test with a probability of less than 5% being considered significant.

Results and Discussion

TPCK at a concentration of 250 µg per ml inhibited the incorporation of [^{3}H] amino acids into protein and of [^{3}H] thymidine into DNA in Novikoff hepatoma ascites cells (Fig. 1) with the latter incorporation showing the greater

Table 1: Effect of TPCK on [^{3}H] amino acid uptake in rat liver and hepatomas

Tissue	Number of Rats	mg TPCK per kg body weight	Hours	Acid soluble counts/min per mg tissue	Acid insoluble counts/min per 100 µg protein
Control liver	3	0	24	249 ± 8	132 ± 8
	3	100		412 ± 34	177 ± 20
	1	0	2	278	125
	3	200		363 ± 56	142 ± 30
Regenerating liver (24 hrs)	3	0		328 ± 18	362 ± 49
	4	100		398 ± 17	440 ± 17
Hepatoma 9618A$_2$	5	0		226 ± 10	60 ± 8
	5	100		120 ± 19	11 ± 4
9618A$_2$ host liver	5	0		280 ± 20	201 ± 18
	5	100		405 ± 39	280 ± 19
Hepatoma 5123C	2	0		308 ± 11	148 ± 18
	3	100		158 ± 12	12 ± 3
5123C host liver	2	0		286 ± 6	219 ± 4
	3	100		409 ± 3	278 ± 41

Rats received i.p. injections of TPCK solution in dimethylsulfoxide (100 mg/ml) or dimethylsulfoxide alone at the stated time before i.p. injection of [^{3}H]amino acids (50 µci/100 g body weight) and were killed one hour after the isotope injection. Results are presented as means ± standard errors for the stated number of rats.

inhibition. Thus, after 90 minutes incubation there was a 77% inhibition of [^{3}H] amino acid incorporation and a 98 % inhibition of [^{3}H] thymidine incorporation.

Further studies *in vivo* showed that administration of TPCK at a level of 100 mg per kg body weight resulted in a large inhibition of [^{3}H] amino acid incorporation into protein in Morris hepatomas 9618A$_2$ and 5123C (Table 1). In the livers of host animals, normal rats and in regenerating liver there was a tendency for [^{3}H] amino acid incorporation into protein to be increased under these conditions with a 40% increase being seen in the livers of rats

Table 2. Effect of TPCK on [^{3}H] thymidine uptake in normal and neoplastic tissues

Tissue	Number of Rats	mg TPCK per kg body weight	Hour	Acid soluble counts/min per mg tissue	DNA counts/min per 10 μg nucleic acid
Regenerating liver (24 hrs)	6	0	2	561 ± 74	743 ± 123
	6	100		507 ± 77	545 ± 92
Hepatoma 7288CTC	4	0		124 ± 6	163 ± 48
	4	20		83 ± 5	31 ± 10
	4	100		31 ± 6	4 ± 2
	2	0	15	117 ± 5	127 ± 13
	2	100		15 ± 7	1 ± 1
7288CTC host liver	4	0	2	229 ± 9	26 ± 5
	4	20		305 ± 25	29 ± 3
	4	100		339 ± 27	24 ± 3
	2	0	15	188 ± 13	21 ± 2
	2	100		381 ± 38	12 ± 2
Hepatoma 5123C	3	0	2	181 ± 24	104 ± 31
	3	200		16 ± 6	0
5123C host liver	3	0		297 ± 7	18 ± 4
	3	200		388 ± 28	14
LK1 colon tumor	3	0		137 ± 5	144 ± 11
	4	100		93 ± 19	37 ± 22
Colon mucosa	3	0		87 ± 6	304 ± 38
	4	100		110 ± 6	164 ± 27

Rats received i.p. injections of TPCK solution in dimethylsulfoxide (100 mg/ml) or dimethylsulfoxide alone at the stated time before i.p. injection of [^{3}H] thymidine (20 μCi/100 g body weight) and were killed one hour after the isotope injection. Results are presented as means ± standard errors for the stated number of rats.

bearing hepatoma 9618A$_2$. The uptake of [^{3}H] amino acids into acid soluble fractions showed changes in the same direction as those seen for incorporation into acid insoluble fractions after treatment with TPCK. These constituted a decrease in the hepatomas and an increase in normal, host and regenerating liver.

Inhibitory effects on [^{3}H] thymidine uptake into the acid soluble frac-

tions of hepatomas 7288CTC and 5123C and the LK1 colon tumor were seen after treatment of rats with TPCK (Table 2). The reverse effect was found in host livers and colon mucosa where increased uptake into the acid soluble fraction was observed after drug treatment. In regenerating liver no significant changes were seen in [^{3}H] thymidine uptake into the acid soluble fraction and incorporation into DNA after administration of TPCK. [^{3}H] thymidine incorporation into DNA of hepatoma 7288CTC was greatly inhibited after treatment of rats with 20 or 100 mg TPCK per kg body weight and determinations 15 hours after drug administration suggested that this was a prolonged effect. There were relatively small effects on [^{3}H] thymidine incorporation into DNA of host livers of rats bearing hepatomas 7288CTC and 5123C. Decreased incorporation was seen in both the LK1 colon tumor (74% inhibition) and the colon mucosa (46% inhibition) after TPCK treatment.

Inhibition by TPCK of protein synthesis in rat hepatoma cells in culture was reported by McIlhinney and Hogan (13). Our studies in vivo suggest some tissue specificity for the effects of TPCK and reveal effects resembling those of cyanate in hepatomas and normal liver (14,15). These drugs have the potential to inhibit amino acid uptake by hepatomas under conditions in which there is no effect or an increased uptake in host livers. They are reactive molecules which may have multiple sites of action. It is known that DNA synthesis in eukaryotic cells including the liver requires concomitant protein synthesis (16) and it is possible that inhibition of DNA synthesis is secondary to effects on protein synthesis. However the action of TPCK on [^{3}H] thymidine uptake into the acid soluble fraction of hepatomas suggests that metabolite uptake by cells may be a critical site of action for TPCK.

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