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Effect of tubercidin on nucleoside incorporation in human tumors*

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TUBERCIDIN (7-deazaadenosine; 4-amino pyrrolo (2,3-*d*)pyrimidine-β-D-ribofuranoside) is an analogue of adenosine produced by *Streptomyces tubercidicus*.[1] Growth inhibition has been demonstrated in some experimental tumors.[2] In tissue culture, Smith *et al.* found that the ID_{50} concentration in KB cells was 0·02 μg/ml.[3] Acs *et al.*[4] demonstrated marked inhibition of mouse fibroblast multiplication in tissue culture with 0·01 μg/ml and complete inhibition with 0·05 μg/ml. They found that mouse fibroblasts converted 80–90% of the tubercidin to the ribotide, primarily the triphosphate, although some was incorporated into RNA and DNA.

Clincial studies have shown that the maximum tolerated human dose is 200 μg/kg/day when given by rapid i.v. injection over a ten-day period. Such a schedule results in proteinuria followed by azotemia. In this paper we will describe the effect of tubercidin on nucleoside uptake in 17 human tumors of various types studied *in vitro*.

The experimental method has been described in detail in previous publications.[5] In short, it consisted of an incubation of fresh, human tumor slices in 1·5 ml of Eagle's medium containing 10% bovine serum and antibiotics. Tubercidin was added to find concentrations of 0·01, 0·3, 3·0, and 60 μg/ml, and 1 hr later was added 1 μc of tritiated nucleosides (thymidine 3·0 c/mmole, uridine 2·9 c/mmole, or adenosine 5·3 c/mmole). The total time of incubation was 24 hr. Tissues were fixed in formalin. The acid-soluble compounds were removed with 2% perchloric acid at 4° for 4 hr and RNA was removed with ribonuclease (0·25 mg/ml for 2 hr at 37°). Autoradiographs were made with NTB 2 liquid emulsion and exposed for seven days.

Uridine and adenosine uptakes without ribonuclease were evaluated and reported as grains per tumor cell. Uridine and adenosine, after ribonuclease, and thymidine uptakes were recorded as per cent labeled tumor cells. Because grain counts were frequently impossible, owing to the dense uptake with tritiated thymidine, the uptake per labeled tumor cell was evaluated on a 0 to 4 scale: 0 = no uptake, +1 = uptake such that all grains were discrete, +2 = some grains being confluent, +3 = approximately half the cell covered with confluent grains, and +4 = confluent label over the entire cell.

The nucleoside uptake in the control specimens emphasizes the biochemical diversity of the human tumors being studied (Table 1). No consistent pattern of uptake was present even in tumors arising from the same organ and of similar histology. Under the present experimental conditions, the normal tissue elements which were removed together with the tumor specimens incorporated relatively small amounts of nucleosides. It was therefore not possible to assess the effect of tubercidin on the accompanying normal tissue.

Four tubercidin concentrations were studied (Table 1). The 0·01 μg/ml concentration was chosen because it had been shown to be inhibitory to mouse fibroblast proliferation;[4] 3·0 μg/ml and 0·3 μg/ml were felt to represent concentrations that might be attained in tumors during clinical chemotherapy. The 60 μg/ml concentration was selected in hopes of attaining a maximal inhibitory effect. These selections were empirical—no drug distribution studies were done. At 60 μg/ml, the nucleoside uptakes were profoundly inhibited, although no morphologic cytotoxicity was apparent. At the smaller doses, 3·0, 0·3, and 0·01 μg/ml, the inhibitory effect was less uniform and less consistent. In one tumor, however, there was marked inhibition of nucleoside uptake at 0·01 μg/ml.

The studies by Acs *et al.*[4] demonstrated that tubercidin rapidly inhibited DNA, RNA, and protein synthesis in mouse fibroblast cultures. Our present study indicates that some human tumors are as sensitive *in vitro* to tubercidin as are mouse fibroblasts. However, consistent inhibition was attained only at the higher drug concentrations. It would appear that the clinical usefulness of tubercidin

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TABLE 1. EFFECT OF TUBERCIDIN ON NUCLEOSIDE INCORPORATION*

Histology	Nucleoside uptake	Control (%)	Control (uptake)	Tubercidin 60 μg/ml (%)	Tubercidin 60 μg/ml (uptake)	Tubercidin 3·0 μg/ml (%)	Tubercidin 3·0 μg/ml (uptake)	Tubercidin 0·3 μg/ml (%)	Tubercidin 0·3 μg/ml (uptake)	Tubercidin 0·01 μg/ml (%)	Tubercidin 0·01 μg/ml (uptake)
1. Melanosarcoma	Tdr→DNA	12·0	+2					9·0	+3	12·5	+2
	Ar →DNA	12·0	7·5					17·0	4·4	8·8	3·2
	Ur →DNA	20·0	4·1					10·0	4·5	8·5	3·8
	Ar →NA		2·6						1·0		5·9
	Ur →NA		1·4						2·6		1·9
2. Synovial sarcoma	Tdr→DNA	24·6	+4			12·9	+3				
	Ar →DNA	23·2	3·7			3·7	2·7				
	Ur →DNA	16·6	4·7			0	0				
	Ar →NA		3·6				1·6				
	Ur →NA		2·6				2·3				
3. Fibrosarcoma	Tdr→DNA	4·6	+3			5·0	+3				
	Ar →DNA	13·0	5·0			7·7	3·0				
	Ur →DNA	0	0			0	0				
	Ar →NA		1·3				0				
	Ur →NA		0				0				
4. Synovial sarcoma	Tdr→DNA	21·0	+4					10·0	+4	16·6	+4
	Ar →DNA	0	0					0	0		
	Ur →DNA	0	0					0	0	0	0
	Ar →NA		1·4						1·2		
	Ur →NA		1·8						1·7		2·1
5. Lung, sq. cell	Tdr→DNA	30·0	+4					29·0	+4	43·1	+3
	Ar→DNA	13·0	5·0					11·1	4·5		
	Ur→DNA	0	0					0	0	0	0
	Ar→NA		3·1						2·8		3·6
	Ur→NA		2·7						2·4		2·7
6. Lung, sq. cell	Tdr→DNA	35·0	+2			26·6	+2				
	Ar→DNA	11·0	4·3			0	0				
	Ur→DNA	6·6	4·0			0	0				
	Ar→NA		2·8				0				
	Ur→NA		2·5				0				

7. Lung, sq. cell	Tdr→DNA	22·8	+1	0	0							
	Ar→DNA	50·0	4·7	0	0							
	Ur→DNA	40·0	4·3	0	0							
	Ar→NA		4·3		0							
	Ur→NA		4·5		0							
8. Pancreas, adenoca.	Tdr→DNA	28·0	+4			24·0	+2			17·4	+4	
	Ar→DNA	34·0	1·8			7·0	6·3			59·0	7·3	
	Ur→DNA	21·0	8·9			12·5	4·4			64·0	3·1	
	Ar→NA		2·6				1·9				2·2	
	Ur→NA		4·9				4·8				3·6	
9. Pancreas, adenoca.	Tdr→DNA	0·7	+1	0	0							
	Ar→DNA	0	0	0	0							
	Ur→DNA	0	0	0	0							
	Ar→NA		3·0		0							
	Ur→NA		2·4		0							
10. Stomach, adenoca.	Tdr→DNA	67·5	+4	7·0	+2							
	Ar→DNA	23·0	6·0	0	0							
	Ur→DNA	18·5	3·3	0	0							
	Ar→NA		3·6		0							
	Ur→NA		3·3		0							
11. Stomach, adenoca.	Tdr→DNA	2·5	+1					5·0	+2	12·0	+2	
	Ar→DNA	14·6	4·6					14·0	2·0	10·0	5·2	
	Ur→DNA	18·0	5·6					10·0	40·0	22·4	45·0	
	Ar→NA		2·5						0·5		2·1	
	Ur→NA		3·6						1·6		2·2	
12. Stomach, adenoca.	Tdr→DNA	28·0	+3							27·0	+2	
	Ar→DNA	0	0					0	0	0	0	
	Ur→DNA	0	0					0	0	0	0	
	Ar→NA		2·4						2·7		1·2	
	Ur→NA		3·6						3·2		3·6	
13. Breast, adenoca.	Tdr→DNA	19·0	+3					6·0	+1	5·0	+1	
	Ar→DNA	40·0	5·0					0	0	0	0	
	Ur→DNA	20·0	3·8					0	0	5·0	5·0	
	Ar→NA		2·0						1·0		0·2	
	Ur→NA		2·3						3·6		0·5	

TABLE 1—*continued*

Histology	Nucleoside Uptake	Control		Tubercidin 60 μg/ml		Tubercidin 3·0 μg/ml		Tubercidin 0·3 μg/ml		Tubercidin 0·01 μg/ml	
		*%	Uptake	%	Uptake	%	Uptake	%	Uptake	%	Uptake
14. Breast, adenoca.	Tdr→DNA	16·6	+4					7·1	+2		
	Ar→DNA	0	0					0	0	0	0
	Ur→DNA	0	0					0	0	0	0
	Ar→NA		3·7						3·6		1·4
	Ur→NA		1·4						2·0		1·3
15. Rectum, adenoca.	Tdr→DNA	11·0	+3							10·0	+2
	Ar→DNA	10·7	6·3							0	0
	Ur→DNA	19·1	14·4							0	0
	Ar→NA		4·1								1·2
	Ur→NA		3·9								1·2
16. Colon, adenoca.	Tdr→DNA	15·0	+2					28·0	+3	16·0	+2
	Ar→DNA	20·4	4·0					11·8	5·0	23·0	3·3
	Ur→DNA	28·0	6·0					7·5	4·4	29·0	7·0
	Ar→NA		1·8						1·6		3·0
	Ur→NA		2·8						2·6		3·1
17. Cervix, sg. cell	Tdr→DNA	23·4	+2	0	0						
	Ar→DNA	41·3	4·3	0	0						
	Ur→DNA	39·0	3·4	0	0						
	Ar→NA		78·3		0						
	Ur→NA		3·6		0						

*Tdr=thymidine, Ar=adenosine, Ur=uridine, DNA=uptake after ribonuclease extraction, NA=uptake before ribonuclease extraction.
%=Per cent tumor cells incorporating Tdr, Ar, or Ur into DNA.
Uptake (for Tdr label): 0=no label; +1=all grains discrete; +2=some grains confluent; +3=approximately half; and +4=over half the tumor cell covered with confluent grains.
Uptake (for Ar and Ur): values represent grains per tumor cell for Ar and Ur without ribonuclease and grains per labeled tumor cell after ribonuclease treatment.

will depend upon the ability to bring adequate concentrations of the drug into contact with the tumor. As was the case with other drugs studied in human tumors,[5] individual tumor sensitivity to tubercidin varied greatly irrespective of tissue of origin or of histologic type.

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The chick embryo as a biologic indicator: Structure–function relationship of sympathomimetic agents*

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MANY different compounds have been introduced as vasopressor agents over the years. This group includes both aromatic and aliphatic compounds of saturated and unsaturated linkages. A substituted amine group in the terminal position of the aliphatic chain is, however, a feature common to all agents in this group.

Epinephrine, norepinephrine, and phenylephrine have been previously shown to produce distinctive lesions in the chick embryo.[1] Application of 50 μg of either of the agents to the chorio-allantoic membrane induced cephalic hematoma and/or skin and extremity hemorrhage. The cephalic hematoma occurred within the subcutaneous mesenchyme of the head, cephalad to the optic lobes in the area of the epiphysis. Grossly, the lesion was elevated, blue-black, and occupied the greater portion of the dorsum of the head. Microscopically, the area of hemorrhage extended beneath the developing membranous bone of the skull and into the subdural space. Extremity hemorrhage in its most advanced stage resembled hemorrhagic infarction; its mechanism differed from that of the cephalic lesion.[1] Pretreatment of the embryos with cortisone resulted in complete inhibition of the cephalic hematoma. Cortisone did not inhibit skin and extremity hemorrhage induced by norepinephrine or phenylephrine.[1] The lesions were distinctive, reproducible, and evolved rapidly. This model has been suggested as a measure of the pharmacologic activity of the sympathomimetic agents on blood vessels.[1]

The purpose of this paper is to report an evaluation of additional vasopressor agents by means of this biologic system.

METHOD

The drugs were dropped directly upon the chorio-allantoic membrane of the 10-, 11- or 12-day chick embryos as previously described.[1]

RESULTS AND DISCUSSION

Table 1 lists thirteen adrenergic drugs and indicates the response of the chick embryo to them both before and after pretreatment with cortisone. Four of these drugs have been included in a previous report.[1] By observing the structural formulas it can be noted that those compounds with β-hydroxyl substitution of the side chain, a relatively free amine group on the α-carbon of the side chain, and one or two hydroxyl or methoxy groups attached to the aromatic ring produced lesions. Aliphatic compounds were inactive.