Growth Inhibition of Kirkman-Robbins Hepatoma by 1-(1,3-Dihydroxy-2-propoxymethyl)-5,6-tetramethyleneuracil and Possible Mechanism of its Biological Activity

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1-(1,3-Dihydroxy-2-propoxymethyl)-5,6-tetramethylene-uracil (DHPTU) is a newly synthesized acyclonucleoside which shows cytostatic properties. It was tested in Syrian hamster 6 days after heterotransplantation of Kirkman-Robbins hepatoma. A reduction of tumour weight by 61% was found 48 h after its intraperitoneal (i.p.) administration in doses of 20 mg per kg of body weight. Inhibition of tumour growth is accompanied by a reduction of dThd, dGuo and dTMP kinase activities in tumour cytosol (by 91% and 74% and 55%, respectively) and decrease in contents of dTTP, dGTP and dATP (by 92%, 77% and 67%, respectively) in dNTP pool. DHPTU is not phosphorylated by any tumour dN kinases, but undergoes cleavage with TU release in reaction catalyzed by the tumour cell enzyme, competitively inhibited by FA. After [^{14}C]DHPTU or [^{14}C]TU had been given i.p. to the animals with the tumour, 90% of the subcellular fraction labelling fell into the nuclear fraction. However, if [^{14}C]DHPTU was administered with FA and DCF, 27% of radioisotope was found in the nuclear fractions and 68% in cytosol. Since DCF which prevented FA deamination to FB (which is not an inhibitor of the mentioned enzyme) reduces DHPTU-induced changes in activity of dN kinases and dTMP kinase in hepatoma cells, the cytostatic activity of DHPTU seems to be connected to an enzyme which releases TU from DHPTU.

Introduction

Discovery of antiviral and antitumorous properties of acyclonucleosides [1, 2] became the rationale for search of their derivatives. DHPTU, one of the pyrimidine acyclonucleosides recently synthesized by Dramiński *et al.* [3] appears to be of particular interest as it shows cytostatic properties in Kirkman-Robbins hepatoma.

Data concerning the molecular mechanism of acyclonucleosides *e.g.* acycloguanosine suggest, that after phosphorylation by the virus genome coded dThd kinase, acycloguanosine monophosphate undergoes further phosphorylation to triphosphate in reaction catalyzed by cellular kinases [4]. Therefore, the antiviral action of acycloguanosine seems to result from the virus DNA polymerase inhibition [5]. However, the above-mentioned mechanism has not been confirmed for DHPTU. Consequently, this work was undertaken in an attempt to shed more light on the mechanisms of cytostatic action of this compound in mitotically active cells.

Abbreviations: DHPTU, 1-(1,3-dihydroxy-2-propoxymethyl)-5,6-tetramethyleneuracil; TU, 5,6-tetramethyleneuracil; dAdo, 2′-deoxyadenosine; dGuo, 2′-deoxyguanosine; dCyd, 2′-deoxycytidine; dThd, thymidine; dUrd, 2′-deoxyuridine; dN, 2′-deoxynucleoside; dNMP, 2′-deoxyribonucleoside-5′-monophosphate; dNTP, 2′-deoxyribonucleoside-5′-triphosphate; PNP, purine nucleoside phosphorylase; FA, formycin A; FB, formycin B; DCF, deoxycoformycin.

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Materials and Methods

Chemicals

[^{14}C]dN's and [^{14}C]dTMP were obtained from Amersham Corp. (England). DHPTU, TU [^{14}C]DHPTU and [^{14}C]TU were prepared according to Dramiński *et al.* [3]. Unlabelled dN's, dNMP's, dNTP's, FA, FB and DCF were purchased from Sigma Chemical Company (U.S.A.), Calbiochem-Beh-

ring Corp. (U.S.A.) and from Boehringer (Mannheim, F.R.G.). Other reagents used were highest quality commercially available products of Fluka AG, Loba-Chemie (Wien), Koch-Light Lab. and POCh (Poland).

Animals and subcellular fractions preparation

Groups of four female Syrian hamsters weighing 80–90 g were used for each experiment. Kirkman-Robbins hepatoma (hepatoblastoma) was maintained as a subcutaneous transplant and transplanted at 10 days intervals. Six days after the heterotransplantation the animals were given intraperitoneally DHPTU (20 mg/kg of body weight). 48 h later they were sacrificed by sectioning the cervical spinal cord. The tumours were excised, separated from surrounding tissues and homogenized at 0 °C in a Potter-Elvehjem apparatus. For dN kinases and PNP activities assays, the tumours were cultured in the medium A (25 mM Tris-HCl buffer pH 7.4, containing 250 mM sucrose, 25 mM KCl and 5 mM $MgCl_2$). For the assay of dTMP kinase activity, the above-mentioned medium A contained additionally 0.002 mM dTMP. Nuclei and mitochondria were spun down at $10{,}000 \times g$ for 15 min. The resulting pellet was homogenized with medium A, the slurry was underlayered with 440 mM sucrose and centrifuged at $800 \times g$ for 15 min. Upper layer consisted of mitochondria, whereas the pellet contained the nuclei [6]. Purified mitochondria were recovered from the upper layer as described previously [6]. The cytosol and microsomal fractions were obtained from the postmitochondrial supernatant after centrifugation at $105{,}000 \times g$ for 60 min (Beckman ultracentrifuge, swing-out rotor SW 21, 6×38 ml). Supernatant represents cytosol and the pellet microsomes. The subcellular fractions from Syrian hamster liver were obtained using the same method.

Chemicals and enzyme assays

All enzyme activities were assayed under optimal conditions and with linear kinetics. dThd kinase activity was assayed according to Cheng and Prusoff [7], but phosphocreatine and creatine kinase was omitted and ATP–Mg^{+2} (1:1) concentration was increased to 10 mM in the incubation mixture for 5′-nucleotidase inhibition [6]. For dCyd as a substrate, the reaction mixture was the same as for dThd kinase. dAdo and dGuo kinase activities were determined by the method of Durham and Ives [8]. The substrate concentration according to K_m values were: [^{14}C]dThd, [^{14}C]dCyd and [^{14}C]dGuo 0.5 mM (0.2 μCi per sample), [^{14}C]dAdo 0.2 mM (0.1 μCi per sample). After incubation at 37 °C in water bath (30 min for dThd, dCyd, dGuo kinases and 20 min for dAdo kinase), the reactions were arrested by immersion of the tubes in boiling water for 2 min, denatured protein was removed by centrifugation and dNMP's were isolated from incubation mixture by descending paper chromatography (Whatman DE 81) at room temperature in 2 mM ammonium formate. The spots corresponding to standards were counted in LKB scintillation counter, using toluene scintillator (with R_f for dAdo, dGuo, dCyd and dThd of 0.65, 0.43, 0.88 and 0.72, respectively). The phosphorylation of DHPTU was performed in conditions matching all dN kinase activities, using [^{14}C]DHPTU (0.2–0.5 mM, 0.1–0.5 μCi per sample) and the phosphorylation products were isolated and counted as described above (with R_f for DHPTU of 0.82, while DHPTU phosphates similarly as dNMP's remains in the start position). dTMP kinase activity were carried out as described previously [6]. PNP activity was measured by the formation of adenine (A) from [^{14}C]dAdo or guanine (G) from [^{14}C]dGuo (with substrate concentration 0.5 mM, 0.2 μCi per sample) by the method described by Millman *et al.* [9]. Reaction mixtures were incubated at 37 °C for 15 min, terminated by immersing tubes in boiling water for 2 min and after removing denatured protein, [^{14}C]A or [^{14}C]G were separated from the [^{14}C]dAdo or [^{14}C]dGuo and counted as given above (with R_f for A of 0.34 and for G of 0.29). The conversion of [^{14}C]DHPTU to [^{14}C]TU and aliphatic orthophosphate residue was assayed by the same method. After agitation in water bath (37 °C for 30 min), the reaction was arrested by immersing the samples in a boiling water for 2 min and [^{14}C]DHPTU was separated from [^{14}C]TU and counted as shown above (with R_f for DHPTU and TU of 0.82 and 0.61, respectively). The aliphatic orthophosphate residue was extracted from the chromatogram according to Meggio *et al.* [10] and determined spectrophotometrically according to Muszbek *et al.* [11]. The enzyme activities were expressed in μmol of products formed per minute per milligram of protein (*i.e.* U per mg of protein).

dNTP's were assayed in acid-soluble fractions obtained according to Letnansky [12] from 20% (v/w)

homogenates of hepatomas in the medium A, 48 h after i.p. administration of [^{14}C]dN's (2 mCi per animal). The acid-soluble fraction was then passed with dNTP standard through a column of Dowex 1×8 (200–400 mesh, formate type, 0.8×10.0 cm) and eluted from the column by formic acid [13]. dNTP was assayed in 2 ml fractions spectrophotometrically and the fractions corresponding to standard were counted in 5 ml of toluene scintillator containing 30% (v/v) Triton X-100.

DNA was determined by the Burton method [14], protein by Lowry *et al.* [15].

Results

dN Kinases, dTMP kinase and PNP activities in hepatoma cells subject to DHPTU action

The Kirkman-Robbins hepatoma (hepatoblastoma) is one of fast growing tumours. The most intensive growth occurs 6 to 10 days after transplantation. From the 12th to 14th day regressive changes take place and within the following 2–3 days the tumour is eliminated [16]. Six days after tumour transplantations to Syrian hamsters, acyclonucleosides were administered i.p. in doses of 10–50 mg/kg body weight, and tumour weight changes were determined 48 h after drug administration. Out of the recently synthesized derivatives of acyclonucleoside [3], this was DHPTU which produced the strongest cytostatic effect. If applied in dose of 20 mg/kg of body weight it brought about the reduction in tumour weight by 61% ± 4% (SD for four experiments). Therefore this compound became the subject of our studies. Experiments were carried out both *in vivo* and *in vitro*. In the *in vivo* experiments DHPTU (in doses as above) was administered i.p. six days after tumour transplantation and 48 h later the enzyme activities were assayed in tumour cytosol. In the *in vitro* experiments enzyme activities were determinated in cytosol of six days hepatoma in incubation mixture contained 1 mM of DHPTU. In the *in vitro* experiments we found no influence of DHPTU on dN kinases, dTMP kinase and PNP activities. Also no substantial changes of PNP activities were found in hepatoma cells, subject to DHPTU action *in vivo*. The results of dN and dTMP kinase activities after i.p. DHPTU administration presented in Table I, shows the reduction of dThd, dGuo and dTMP phosphorylation by 91%, 74% and 55%, respectively.

Table I. dN kinase and dTMP kinase activities (U/mg of protein × 10^{-5}) in cytosol of growing hepatoma treated with DHPTU. DHPTU was administered i.p. (20 mg per kg of body weight) six days after tumour transplantation and 48 h later the enzyme activities were assayed.

Substrate	Treated with DHPTU	Control	% of Inhibition
dAdo	7.4 ± 0.8 (NS)	9.2 ± 1	18.6
dGuo	7.9 ± 0.6 (p = 0.001)	29.0 ± 3	73.8
dCyd	1.1 ± 0.1 (NS)	1.2 ± 0.3	8.8
dThd	8.6 ± 0.8 (p = 0.001)	94.0 ± 16	90.8
dTMP	37.3 ± 4.4[a] (p = 0.02)	82.0 ± 12	54.6

Each value: means ± SEM for four experiments. The values of p (in parentheses) were calculated using two tailed student's test. NS – means non-significant ($p > 0.05$). Control: dN and dTMP kinase activities in the cytosol of 6–8 days old hepatoma.
[a] Expressed as sum of dTDP and dTTP formed.

Effect of DHPTU on dNTP pool in acid-soluble fraction

Changes in activity of dN and dTMP kinases subject to DHPTU action, become the reason for undertaking further studies of dNTP's content in acid-soluble fraction, obtained from the hepatoma. Six days after tumour transplantation the animals were given i.p. [^{14}C]dN's with and without DHPTU (20 mg per kg of body weight). 48 h later dNTP's were assayed in acid-soluble fractions obtained from the tumour

Table II. dNTP's content in Syrian hamster six days growing hepatoma, treated with DHPTU (for detail see the text).

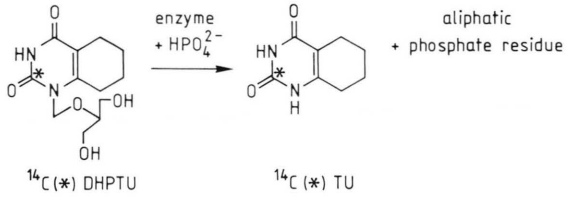
	dNTP – nmol/g of wet weight Treated with DHPTU	Control	% of Control
dATP	5.5 ± 0.8	16.8 ± 3.2	32.7 (p = 0.01)
dGTP	1.3 ± 0.2	5.2 ± 0.8	23.1 (p = 0.005)
dCTP	47.3 ± 4.3	48.0 ± 4.6	98.5 (NS)
dTTP	0.7 ± 0.09	8.9 ± 0.7	7.8 (p = 0.001)

Each value: means ± SEM for four experiments. The values of p (in parentheses) were calculated as in Table I. NS – means non-significant ($p > 0.05$).

homogenate (for detail see Materials and Methods). The result of that experiments (Table II) shows the reduction of dTTP, dGTP and dATP in dNTP pool by 92%, 77% and 67% (respectively).

Metabolism of DHPTU

The ability of HSV-induced dN kinases to phosphorylate acyclonucleoside [4, 5], prompted investigation of synthesis of DHPTU phosphate. Accordingly [^{14}C]DHPTU was incubated with all subcellular fractions obtained from the 6 days growing tumour. In reaction mixtures used for determination of the all dN kinase activities none of the dN kinase present in subcellular fractions of hepatoma catalyzed the DHPTU phosphate synthesis. On the contrary [^{14}C]-DHPTU was quickly degraded to [^{14}C]TU and aliphatic phosphate residue in reaction catalyzed by an enzyme present in this fractions, according to equation:



The activities of the DHPTU phosphorolyzing enzyme in hepatoma and in Syrian hamster liver subcellular fractions are shown in Table III. It is worth mentioning that DHPTU phosphorolyzing enzyme shows 40 times higher activity in hepatoma as compared to similar activity in Syrian hamster liver.

DHPTU phosphorolyzing enzyme shows Michaelis-Menten type of kinetics. The apparent K_m value for DHPTU calculated from Lineweaver-Burk plots was 210 μM (Fig. 1A). The release of TU from DHPTU is the first-order reaction with k $2.1 \times 10^{-3} \times \text{min}^{-1}$. The reaction rate was constant at 30 min in case of all subcellular fractions of hepatoma. Although the apparent K_m values for dAdo and dGuo in reaction catalyzed by PNP in 6 days hepatoma cytosol are 21 μM and 48 μM, respectively, the V_{max} value for DHPTU, dAdo and dGuo are practically the same (Fig. 1A). In 50 mM Tris-HCl buffer containing 2 mM dithiotreitol, the highest activity of DHPTU phosphorolyzing enzyme was obtained at

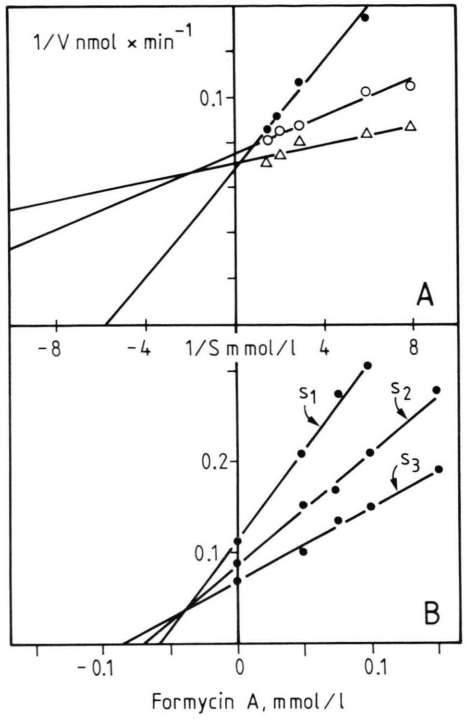


Fig. 1A. Lineweaver-Burk plots for the effect of DHPTU concentrations on DHPTU phosphorolyzing enzyme (●) and of Ado (○) and dGuo (△) concentrations of PNP activity in cytosol of six days growing hepatoma. B: Dixon plots for the effect of FA concentrations on DHPTU phosphorolyzing enzyme activity in cytosol of six day growing hepatoma. S_1 – 0.3 mM, S_2 – 0.4 mM, S_3 – 0.5 mM.

Table III. The DHPTU phosphorolyzing enzyme activity in 6 days growing hepatoma and in Syrian hamster liver subcellular fractions (U/mg of protein $\times 10^{-5}$).

Enzyme source	Hepatoma	Liver	Hepatoma: liver activity ratio
Cytosol	76 ± 4.2	2.0 ± 0.3	38.0
Mitochondrial fraction	83 ± 6.3	0.0	–
Nuclear fraction	96 ± 8.0	2.3 ± 0.4	41.7
Microsomes	61 ± 3.4	0.0	–

Each value: means ± SD for four experiments.

pH 7.5–8.0. In concentration of 1 μM of DCF which inhibits FA deamination and dAdo phosphorylation in hepatoma cells [17, 18], DHPTU phosphorolyzing enzyme present in six days cytosol of hepatoma is competitively inhibited by FA (Fig. 1B), with K_i 36 μM, with total enzyme activity preserved by FB.

The release of TU from DHPTU as a dominating metabolic pathway of this acyclonucleoside in hepatoma cells, prompted us to investigate the *in vivo* and *in vitro* influence of TU and DHPTU phosphorolyzing enzyme activity on dN's and dTMP phosphorylation. In the *in vitro* experiments when incubation mixture contained 1 mM TU or DHPTU, any changes of all dN and dTMP kinase activities in six days tumour cytosol were found. In the *in vivo* experiments 6 days after tumour transplantation the animals were administered i.p.:

DHPTU or [^{14}C]DHPTU, DHPTU or [^{14}C]-DHPTU with FA and DCF, TU or [^{14}C]TU. The dN and dTMP kinase activities in cytosol of hepatoma and distribution of radioisotope in subcellular fractions of hepatoma 48 h after application of non-labelled or ^{14}C-labelled compounds, are presented in Table IV and in Fig. 2. Those results demonstrate that inhibition of dThd, dGuo and dTMP phosphorylation calculated per micromole of the tested compound is practically the same for DHPTU and TU (Table IV, A, C). When DHPTU was adminis-

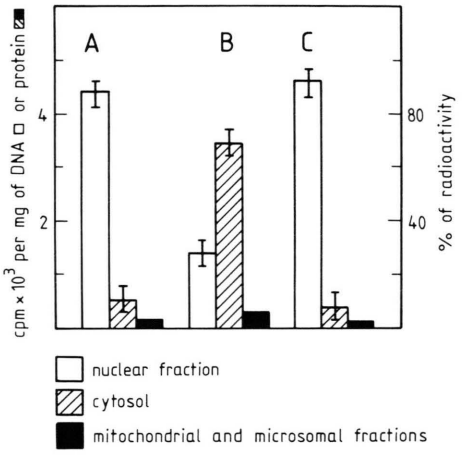


Fig. 2. Distribution of: A – [^{14}C]DHPTU, B – [^{14}C]DHPTU with FA and DFC, C – [^{14}C]TU in subcellular fractions of growing hepatoma, 48 h after ^{14}C-compounds i.p. administration (at doses as in Table IV and 3×10^6 cpm per animal) to animals six days after tumour transplantation. Each data: the mean ± SD for four experiments.

Table IV. dN and dTMP kinase activities (U/mg of protein $\times 10^{-5}$) in cytosol of growing hepatoma six days after tumour transplantation, when the animals were administered i.p.: A – DHPTU 20 mg per kg/body weight *i.e.* 1.8 mg per animal = 6.6 μM; B – DHPTU at dose as in A with 2 μM of FA and 0.5 μM of DCF per animal; C – TU 5.5 mg per kg/body weight *i.e.* 0.5 mg per animal = 3 μM (due to hydrophobic properties of TU making impossible its use in a dose corresponding to 20 mg per kg/body weight). 48 h after drug administrations the enzyme activities were assayed.

Substrate	A	B	C	Control a	Control b
dAdo	7.8 ± 0.7	3.9 ± 0.4	8.7 ± 0.8	4.6 ± 0.4	9.6 ± 0.7
	2.8[1] (18.8)	2.3[2] (15.3)	3.1 (9.4)		
dGuo	8.4 ± 0.8	25.0 ± 2.2	20.0 ± 1.7	32.0 ± 2.6	30.0 ± 2.3
	10.9 (72.0)	2.5 (16.7)	11.1 (33.4)		
dCyd	1.2 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
	1.4 (9.0)	–	–		
dThd	9.8 ± 0.9	77.0 ± 6.5	52.0 ± 4.5	93.0 ± 8.2	96.0 ± 8.3
	13.6 (89.8)	3.0 (19.8)	15.3 (45.8)		
dTMP	37.0 ± 3.1	76.0 ± 6.3	62.0 ± 5.1	85.0 ± 7.7	84.0 ± 7.3
	8.6 (56.6)	1.5 (9.6)	8.7 (26.2)		
Tumour weight (g)	0.51	1.1	0.88	1.1	1.3
	60.8[3]	15.4	32.4		

Each value: the mean ± SD for four experiments. Control: enzyme activities in cytosol of 6–8 days hepatoma, a – in presence of FA and DCF, b – without FA and DCF. dTMP kinase activity was expressed as in Table I.

[1] % of inhibition in comparison to control b, calculated per 1 μM of tested compound (in parentheses: % of inhibition of control b value).

[2] In comparison to dAMP synthesis with control a.

[3] % of growth inhibition in comparison with control b.

tered with the inhibitor of TU releasing enzyme (Table IV, B), the synthesis of dTMP, dGMP, dTDP and dTTP decreased from 89.8 to 19.8%, from 72 to 16.7% and from 56.6 to 9.6% respectively. [^{14}C]-DHPTU and [^{14}C]TU accumulate the label up to 90% in the nuclear fraction (Fig. 2A, C). However, when [^{14}C]DHPTU was administered with FA, which inhibits TU release from DHPTU, only 27% of the activity was found in the nuclear fraction and 70% in the cytosol.

Discussion

The results presented so far give rise to a number of observations. Our attention has been drawn to the fact that the inhibition of dTMP, dGMP, dTDP and dTTP synthesis in cytosol of the growing hepatoma and respective decrease in contents of dTTP, dGTP and dATP in dNTP's pool occurs only, when DHPTU is administered *in vivo* (Tables I, II). Incubation of DHPTU *in vitro* with cytosol of growing hepatoma, has no effect on the synthesis of mentioned above dN's. This may suggest that DHPTU undergoes metabolic activation *in vivo* and the results presented in Table IV show, that DHPTU may acquire biological activity when TU is released. On the other hand, the incubation of DHPTU *in vitro* with cytosol of growing hepatoma, in which the enzyme releasing TU from DHPTU is present, has no effect on dThd and dGuo nucleotide synthesis. Also no effects on dN's and dTMP kinase activities were found when TU was incubated with six days hepatoma cytosol *in vitro*. Thus it may be stated that TU is not a direct inhibitor of the synthesis of these dN's, though the release TU from DHPTU *in vivo* plays an important role of the mechanism of inhibition of dThd, dGuo and dTMP phosphorylation. Such conclusion may be also drawn from the fact that the inhibition of dTMP, dGMP, dTDP and dTTP synthesis occurs, when the animals were given DHPTU or TU. This inhibition decreased from about 90 to 10% when animals had been given DHPTU with the inhibitor of TU releasing enzyme (Table IVA, B). The *in vivo* administration of [^{14}C]TU or [^{14}C]DHPTU leads to selective accumulation of the radioisotope in nuclear fraction. However, when [^{14}C]DHPTU is injected simultaneously with FA, which inhibits TU releasing enzyme, a level of drug in the cytosol increases to 70% of the administration activity and correspondingly decreases in the nuclear fraction (Fig. 2A, B). This suggest an interaction between TU and the nuclear fraction, and may point to the fact that such interaction results from inhibition of *de novo* dThd, dGuo and dTMP kinase synthesis. There is no proof that this really takes place, however the *de novo* dN and dTMP kinase synthesis occurs only in mitotically active cells [19, 20] and the fact that DHPTU is biologically active only in the *in vivo* experiments (*i.e.* after TU release), supports this contention. Though the cytostatic effect by means of *de novo* kinase synthesis inhibition is still controversial, the changes in dTTP, dGTP and dCTP level in hepatoma cells after DHPTU administration (Table II) are connected with the tumour growth inhibition. Similar changes in dNTP's contents, occurring in Novikoff hepatoma cells and in L 1210 tumour cells treated with methotrexate or 5-fluoro-dUrd [21, 22] makes us believe that the decrease of these dNTP's concentration in Kirkman-Robbins hepatoma cells, plays an important role in growth inhibition of this tumour. The decrease of dATP content in dNTP pool is due to the elevated concentration of dGuo. As dGuo is a strong inhibitor of dAdo kinase [23], the correspondingly reduced dAMP synthesis is most likely responsible for the reduced level of dATP in the acid-soluble fraction of hepatoma.

The cytostatic activity of DHPTU is regulated by an enzyme, which compared to the normal (*i.e.* non-regenerating) liver, releases TU from DHPTU in hepatoma cells with 40 times higher activity. The enzymes catalyzing the release of TU from DHPTU possesses properties of PNP (distinct differences between K_m values for DHPTU and dAdo/dGuo, but the same V_{max} values, type of kinetics and kind of catalyzed reactions). Although it is not certain whether this enzyme is PNP indeed, the following facts supported this idea: 1. Phosphorylases present in the tumours exhibit preferences to modify ribo- and deoxyribonucleosides [24]. 2. Uridine derivatives (DHPTU among them) are inhibitors of pyrimidine nucleoside phosphorylase, but not of PNP [3, 25]. 3. FA inhibiting DHPTU phosphorolyzing enzyme belongs to specific inhibitors of adenosine phosphorylase [26].

The lack of DHPTU phosphorylation and presence of enzyme releasing TU from DHPTU in hepatoma cells is the distinct metabolic feature of this tumour. To take such a statement as plausible it is enough to say that 9-(1′,3′-dihydroxy-2′-propoxymethyl)-guanine (DHPG), differing from

DHPTU only by presence of guanine in the DHPG molecule, is phosphorylated by both kinases present in calf thymus and in human cytomegalovirus-infected lung cells [27].

The majority of acyclonucleosides revealing antiviral properties are phosphorylated to monophosphates not by cellular dN kinases, but by virus genome coded dN kinases [1, 2, 4, 5]. Since, as it has been shown in the present paper, also acyclonucleosides showing cytostatic activity are also not substrates for cellular dN kinases, such an acyclonucleoside should be found because it undergoes metabolic activation in a different way than phosphorylation. The discovery in hepatoma cells of an enzyme, which catalyzing the phosphorolysis of DHPTU turning this compound into cytostatically active metabolites, would not only confirm that DHPTU is searched structure, but also given grounds for the view that metabolism of acyclonucleosides with cytostatic properties may differ from that of acyclonucleosides with antiviral activity.

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