# METABOLISM OF 1-(*S*)-(3-HYDROXY-2-PHOSPHONOMETHOXYPROPYL)-CYTOSINE (HPMPC) IN HUMAN EMBRYONIC LUNG CELLS

Tomáš CIHLÁŘ, Ivan VOTRUBA, Květoslava HORSKÁ, Radek LIBOSKA, Ivan ROSENBERG and Antonín HOLÝ

*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

Received February 3, 1992
Accepted February 12, 1992

The acyclic nucleotide analogue HPMPC is in human embryonic lung cells, cultured in vitro, transformed to its mono- and diphosphates (HPMPCp and HPMPCpp) and HPMPCp-choline; the synthesis of HPMPCp is catalysed by pyrimidine nucleoside monophosphate kinase (EC 2.7.4.14); HPMPCp-choline (analogue of CDP-choline) is formed from HPMPCpp and choline phosphate in the presence of CTP:phosphorylcholine cytidylyltransferase (EC 2.7.7.15). These metabolites persist for a long time in the cellular pool even after HPMPC has been removed from the medium; on the other hand, they efflux to the medium. Neither HPMPC, nor any of its metabolites interfere with intracellular CDP-choline level.

The acyclic nucleotide analogue 1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)cytosine (HPMPC) possesses a potent antiherpetic activity in vitro while exhibiting a low cytotoxicity in the host cells[1 - 4]. The drug is much more efficient in vivo against systemic HSV-1 infection in mice than acyclovir[2,3] and against murine cytomegalovirus than ganciclovir[3,5].

HPMPC is very active above all against HCMV* in vitro not only following a single dose after virus adsorption but also when administered 48 h after infection[8], or even on mere pretreatment of host cells[7,8]. It was suggested[8] that it not only inhibits viral DNA synthesis[6] but also interferes with cellular processes associated with virus replication. Bronson et al.[9] reported that HPMPC in analogy to HPMPA (refs[10,11]) is transformed by cellular enzymes to the corresponding mono- and diphosphates and, furthermore, to

* Abbreviations used: HPMPCp and HPMPCpp, mono- and diphosphoryl derivatives of HPMPC; HSV-1, herpes simplex virus type 1; HCMV, human cytomegalovirus; HPMPA, (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine; araCDP and araCTP, 1-β-D-arabinofuranosylcytosine di- and triphosphate; PBS, phosphate buffered saline; TCA, trichloroacetic acid; TBHS, tetrabutylammonium hydrogen sulfate; EDTA, ethylenediaminetetraacetic acid; PNMP kinase, pyrimidine nucleoside monophosphate kinase; ddCDP and ddCTP, 2',3'-dideoxycytidine di- and triphosphate.

a "new metabolite", which was tentatively assigned the structure of HPMPCp-choline (CDP-choline analogue) (ref.[9]).

In this work we present our data on HPMPC metabolism in human embryonic lung fibroblasts which are permissive for the growth of HCMV, with an emphasis on the structure, kinetics of formation and persistence of individual HPMPC metabolites in the cell including the identification of cellular enzymes which catalyze their synthesis.

## EXPERIMENTAL

### Materials

HPMPC, HPMPCp, HPMPCpp and araCTP were synthesized as described previously[12,13]. HPMPCp-choline was prepared by the condensation of protected HPMPC with choline phosphate followed by deprotection (Liboska et al., unpublished data).

CDP-choline, choline phosphate, trioctylamine and alkaline phosphatase (*E. coli*, type III) were obtained from Sigma, freon 113 and CTP were delivered by Serva, phosphodiesterase I and Sepharose 6B by Pharmacia. [5-$^3$H]HPMPC and [U-$^{14}$C]cytidine were purchased from the Institute for Research, Production and Use of Radioisotopes, Prague, [methyl-$^{14}$C]choline and [5-$^3$H]CTP from New England Nuclear. All other chemicals used were of analytical grade.

Diploid human embryonic lung cells (LEP) were a kind gift from the Institute of Sera and Vaccines, Prague.

### Metabolic Studies in Tissue Cultures

LEP cells were grown in a volume of 5 ml of EPL medium (Institute of Sera and Vaccines, Prague) in Petri dishes (diameter 60 mm). 48 h after seeding (500 000 cells per dish) [5-$^3$H]HPMPC (specific activity 7 Ci mmol$^{-1}$) was added to a concentration of 1 μmol l$^{-1}$ of the medium. At given time intervals the culture was trypsinized, the cells were washed twice with PBS (0 °C) by centrifugation; thereafter they were frozen and thawn in ethanol–dry ice bath and extracted by 5% TCA. Following centrifugation (15 000 g, 5 min) TCA was removed by shaking up with an equal volume of freon 113–trioctylamine mixture (4 : 1, v/v) (ref.[10]). Both phases were separated by centrifugation and the aqueous phase was analysed with ion-pair HPLC using a column 250 x 4.6 mm RPS SGX 7 μm (Tessek, Czechoslovakia). Elution buffer A contained 50 mmol l$^{-1}$ $KH_2PO_4$ and 3 mmol l$^{-1}$ TBHS, pH 6.8. Buffer B had the same composition but contained acetonitrile (20%, v/v). The separation was carried out during first 5 min isocratically (95% A : 5% B) and during further 15 min with linear gradient up to 30% A : 70% B and with flow-rate 1 ml min$^{-1}$ (system I). The radioactivity of 0.5 ml fractions was assayed following the addition of scintilation coctail Bray. For the additional HPLC analysis of TCA extract the axial compression column Whatman Partisil SAX 100 x 3 mm was employed; the elution was carried out with a linear concentration gradient 0.05 – 0.6M-$KH_2PO_4$ (pH 4.3), 60 min, flow-rate 0.5 ml min$^{-1}$ (system II).

The acid-insoluble fraction was washed 3 times on a nitrocellulose filter BA 85 (Schleicher & Schuell) with 5 ml aliquots of 5% TCA and the radioactivity was determined using a toluene based scintilation solution.

The tissue culture medium was analyzed following TCA extraction in a similar way as cell extracts. [$^{14}$C]Cytidine (specific activity 350 mCi mmol$^{-1}$) was added to the medium at a concentration of 6 μCi per dish.

In case of double-labelling experiment the cells were grown in EPL choline-free medium in the presence of [$^3$H]HPMPC (100 μmol l$^{-1}$, specific activity 0.15 Ci mmol$^{-1}$ and of [$^{14}$C]choline (specific activity 53 mCi mmol$^{-1}$) at a concentration of 2 μCi per ml of medium, (final concentration, 4 mg l$^{-1}$ choline). The TCA

extracts were analyzed with HPLC using Whatman Partisil SAX column and isocratic elution with 0.01M-$KH_2PO_4$, pH 4.3 and flow-rate 0.5 ml $min^{-1}$ (system III). The radioactivity of $^3H$ and $^{14}C$ was measured simultaneously in each fraction (0.25 ml).

The electrophoretic separations were done using Whatman 3MM paper either in 1M acetic acid (40 V $cm^{-1}$, 90 min) (system IV) or in 50mM-$Na_2B_4O_7$, pH 9.2 (40 V $cm^{-1}$, 80 min) (system V). The paper was cut (0.5 cm strips) and the radioactivity was determined in a toluene based scintilation solution.

Enzyme Assays

TCA extract (200 μl) in 25mM Tris-HCl buffer (pH 8.0) was treated with alkaline phosphatase (0.3 U). The reaction mixture for phosphodiesterase digestion (200 μl) in 100mM Tris-HCl, pH 8.9 and 20mM-$MgCl_2$ contained 0.5 U of the enzyme. Incubations were performed at 37 °C for 60 min (phosphatase) and 120 min (phosphodiesterase). The incubation was stopped by addition of methanol and the samples were concentrated in vacuum and further analyzed by HPLC.

*Formation of CDP-choline, HPMPCp-choline and araCDP-choline.* CTP:phosphorylcholine cytidylyltransferase (EC 2.7.7.15) isolated from the rat liver[14] had specific activity 76 nmol $min^{-1}$ $mg^{-1}$; the reaction mixture (100 μl) contained 25 μg of enzyme protein, 50 $mmol^{-1}$ Tris-HCl, pH 7.4, 10 mmol $l^{-1}$ $MgCl_2$, 2 mmol $l^{-1}$ EDTA disodium salt, 2 mmol $l^{-1}$ [$^3H$]CTP (specific activity 1 mCi mmol $l^{-1}$) and 0.2 mmol $l^{-1}$ equimolar mixture of phosphatidylcholine and oleic acid (prepared as it was published previously[15]). The reaction mixture was incubated at 37 °C for different time periods and the reaction was terminated in boiling water for 2 min. A 30 μl aliquot supplemented with an appropriate amount of carrier standard was separated electrophoretically using Whatman 3MM paper in 0.2M triethylammonium bicarbonate, pH 9.1 (40 V $cm^{-1}$, 60 min) (system VI). The spot corresponding to CDP-choline was cut out and used for the radioactivity estimation in toluene based scintilation solution. Analogous reaction mixtures where CTP was substituted by HPMPCpp or araCTP, were analyzed using system III.

*Phosphorylation of HPMPC.* Pyrimidine nucleoside monophosphate kinase (EC 2.7.4.14; CMP-UMP-dCMP kinase, further PNMP kinase) was prepared from rat liver according to Maness and Orengo[16]. The resulting preparation had a specific activity of 7.5 μmol $min^{-1}$ $mg^{-1}$. The reaction mixture (100 μl) contained: 50 mmol $l^{-1}$ Tris-acetate, pH 7.5, 15 mmol $l^{-1}$ $MgCl_2$, 2.5 mmol $l^{-1}$ CMP or HPMPC (10 – 100 μmol $l^{-1}$), 12 mmol $l^{-1}$ ATP and 3.3 μg of enzyme; incubation, 30 min at 37 °C for CMP or 30 – 200 min for HPMPC. The reaction products were identified by electrophoresis in system IV and by HPLC (system II). For kinetic experiments [5-$^3H$]HPMPC at identical concentrations (total radioactivity 0.18 μCi in 100 μl of reaction mixture) was used. The radioactivity of the product was determined in spots after electrophoresis of reaction mixture aliquots.

## RESULTS AND DISCUSSION

### *Metabolism of HPMPC in LEP Cells*

LEP cells were incubated for 36 h in the presence of 1 μmol $l^{-1}$ [$^3H$]HPMPC. At this concentration, there is still an effective inhibition of HCMV multiplication in the infected LEP cells[8]. The analysis of TCA extract using system I revealed besides HPMPC three labelled metabolites in the cellular pool (Fig. 1). On comparing the retention times with those of standard materials they were identified as HPMPCp-choline, HPMPCp and HPMPCpp. This assignment was confirmed by digesting the whole TCA extract by alkaline phosphatase which resulted in the disappearance of the peaks

corresponding to phosphorylated products and the increase of the HPMPC peak, while the peak corresponding to HPMPCp-choline remained unchanged.

To confirm the identity of these metabolites (and to exclude the formation of further ones) the TCA extract was analyzed stepwise with HPLC using system II and paper electrophoresis in systems IV and V. The position of standards corresponded in all cases to radioactivity of the peaks. No further metabolites were detected.

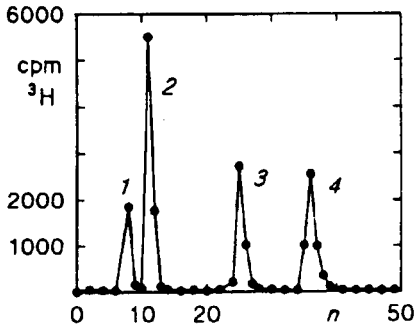


FIG. 1

Identification of HPMPC metabolites (TCA-extract) by HPLC (system I). *1* HPMPCp-choline, *2* HPMPC, *3* HPMPCp, *4* HPMPCpp, *n* fraction number

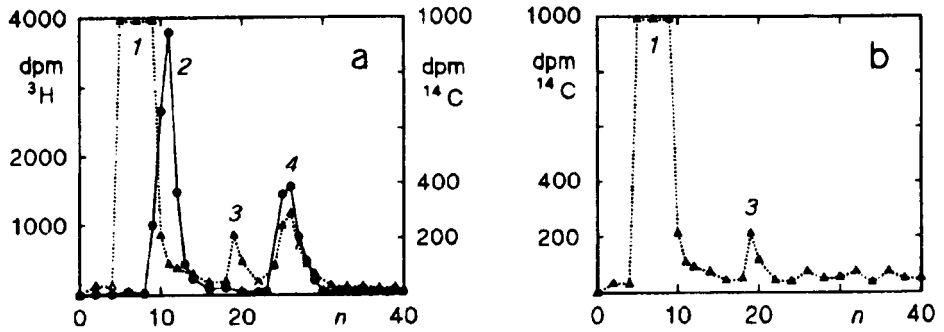


FIG. 2

Double-labelling of HPMPCp-choline in the cellular pool (system III). *1* Choline, *2* HPMPC, *3* CDP-choline, *4* HPMPCp-choline; Δ $^{14}$C, ● $^3$H, *n* fraction number. **a** After the incorporation of [$^3$H]HPMPC and [$^{14}$C]choline; **b** control experiment: [$^{14}$C]choline labelling only

*Confirmation of HPMPCp-Choline Identity*

In spite of the above evidence, the data hitherto presented afford only a partial proof of the CDP-choline analogue formation. Therefore, we have directly followed the incorporation of choline into the metabolite (HPMPCp-choline) by double-labelling experiment. The same procedure was applied earlier to identify analogous metabolites formed from araCTP and ddCTP: araCDP-choline (ref.[17]) and ddCDP-choline (ref.[18]). The LEP cell culture was incubated in the medium containing [$^3$H]HPMPC and [$^{14}$C]choline; in the control experiment, only [$^{14}$C]choline was added to the medium. The TCA extracts were digested with alkaline phosphatase to decompose choline phosphate into which the majority of labelled choline was incorporated. The HPLC analysis confirmed that the peaks of $^3$H and $^{14}$C radioactivity coincided with the position of HPMPCp-choline (Fig. 2a,b), indicating thus clearly the presence of both precursors in the molecule of presumed HPMPCp-choline. No [$^{14}$C]HPMPCp-choline peak could be detected in the parallel control experiment. Comparison of the results demonstrates that the presence of HPMPC metabolites in no way influences intracellular CDP-choline level (Fig. 2a,b).

*Isolation of $^3$H and $^{14}$C Labelled HPMPCp-Choline*

TCA extract from $10^7$ cells growing for 70 h in the presence of labelled precursors was concentrated and then electrophoretically purified at pH 9.3 (system V) with the addition of HPMPCp-choline carrier at a suitable concentration. The spot of the product was eluted, and desalted on reverse phase column in water acidified with acetic acid to pH 3. An aliquot was then analyzed using system III before and after digestion with phosphodiesterase. The substance was degraded to $^3$H peak corresponding by its retention to HPMPC and $^{14}$C peak identical with choline phosphate.

*Kinetics of Metabolite Formation*

The antiviral effect of the drugs of HPMPC type depends on the level of their active metabolites in the cellular pool which is determined both by outward and inward cellular transport as well as by the rate of metabolic transformations. Therefore, we investigated the time dependence of the cellular transport of HPMPC and of the formation of individual metabolites during the period of 2 – 100 h. The maximum intracellular concentration of the drug is reached at about 16 h following its addition to the medium (Fig. 3); the equilibrium concentration of HPMPC is reached after approx. 100 h. Additional incubation does not change the intracellular level of HPMPC metabolites any more. Similar sharp maximum followed by a decrease of the drug concentration has been observed in case of arabinofuranosylcytosine[17] and 2',3'-dideoxycytidine[19]. Minor radioactivity which accounts only for 1 – 2% of the radioactivity in cell pool (Fig. 3)

was present also in acid-insoluble fraction, which might reflect HPMPC incorporated into nucleic acids. Maximum incorporation is preceded by maximum content of HPMPC metabolites in cellular pool since the doubling time of the cell during this phase of growth is already relatively long.

The character of HPMPC uptake and the formation of its phosphorylated derivatives roughly copy the time course of the total influx of the drug (Fig. 4). HPMPC is phosphorylated to HPMPCpp at a relatively high rate (HPMPCpp accounts for 45% of total pool radioactivity after 2 h), the maximum phosphorylation is reached after 10 h. The high level of HPMPCpp probably causes the effective inhibition of viral DNA synthesis inhibition in cells infected with HCMV (ref.[6]).

The formation of HPMPCp-choline follows different pattern; a slow steady increase of this metabolite has been observed during the whole time period under study. The mutual liaison of the kinetics of HPMPCpp and HPMPCp-choline formation is similar to that observed for araCTP and araCDP-choline formation in RPMI 6410 myeloblasts[17] and AMT myeloblasts[20]. The synthesis of HPMPCpp clearly precedes that of HPMPCp-choline thus accentuating the participation of CTP:phosphorylcholine cytidylyltransferase in HPMPCp-choline formation. At the end of the cultivation, HPMPCp-choline is a dominant metabolite. The levels of all metabolites remain constant when the cultivation in the presence of the drug is prolonged beyond 100 h.

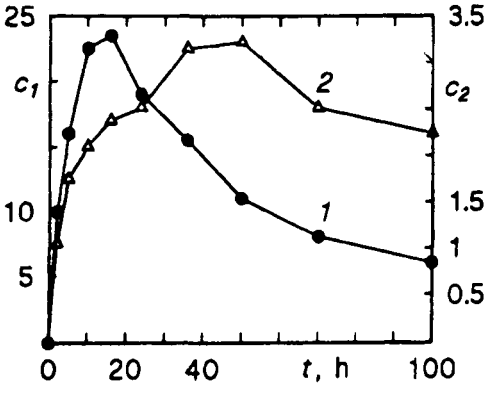


FIG. 3

Uptake of [$^3$H]HPMPC into LEP cells. The concentration of HPMPC in *1* TCA-soluble (cellular pool) ($c_1$, pmoles/$10^6$ cells) and in *2* TCA-insoluble fraction ($c_2$, pmoles/$10^6$ cells)

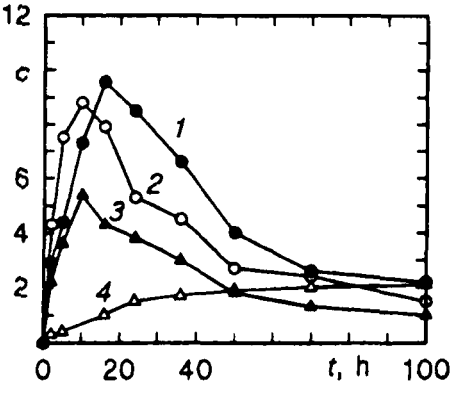


FIG. 4

Time course of HPMPC metabolites formation in the cellular pool. *1* HPMPC, *2* HPMPCpp, *3* HPMPCp and *4* HPMPCp-choline; *c* concentration of HPMPC metabolites (pmoles/$10^6$ cells)

*Persistence of Metabolites in Cellular Pool*

An antiviral effect of HPMPC was noted also in cells which were preincubated with the drug prior to HCMV infection[7,8]. Therefore, we have studied the decrease of the cellular concentration of metabolites following removal of HPMPC from the medium. The

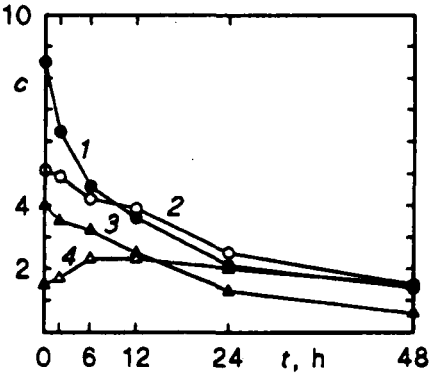


FIG. 5

Time course of decrease of HPMPC metabolites levels in cellular pool. LEP cells were incubated 24 h in the presence of [$^3$H]HPMPC (1 μmol l$^{-1}$), washed and than transferred to the fresh medium ($t$ = 0). *1* HPMPC, *2* HPMPCpp, *3* HPMPCp, *4* HPMPCp-choline; *c* concentration of HPMPC metabolites (pmoles/10$^6$ cells)

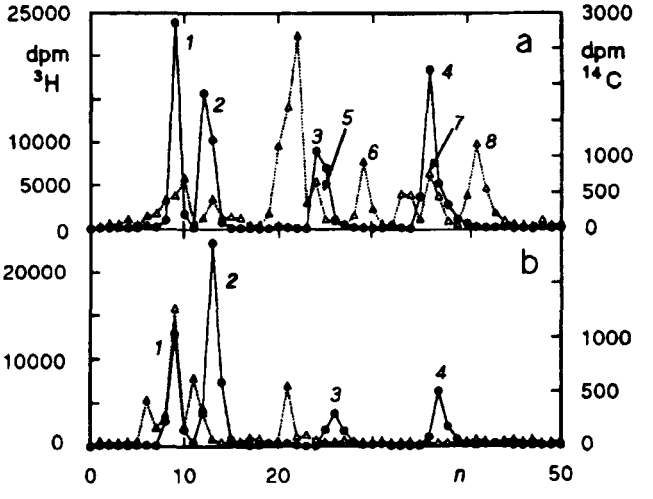


FIG. 6

HPLC analysis (system I) of [$^3$H]HPMPC and [$^{14}$C]cytidine metabolites effluxed a from the pool of LEP cells, b to the medium. LEP cells were incubated 24 h in the presence of [$^3$H]HPMPC and [$^{14}$C]cytidine, washed, transferred to the fresh medium and incubated for additional 24 h. ● $^3$H, Δ $^{14}$C, *1* HPMPCp-choline, *2* HPMPC, *3* HPMPCp, *4* HPMPCpp, *5* CDP, *6* UDP, *7* CTP, *8* UTP, *n* fraction number

cells were incubated for 24 h in the presence of 1 μM [$^3$H]HPMPC; thereafter, the incubation was continued in a fresh medium in absence of the drug. The decreased level of individual metabolites is summarized in Fig. 5. The efflux of HPMPC into fresh medium is about twice faster compared to the efflux into HPMPC-containing medium. The presence of 1 μmol l$^{-1}$ HPMPC in medium shows no substantial influence on rate of decrease of HPMPCp and HPMPCpp intracellular level. The synthesis of HPMPCp-choline continues for additional 12 h after HPMPC was removed from medium and then a small levelling off takes place.

The analysis of the TCA-treated extract of the media used for cultivation surprisingly revealed the presence of all above metabolites already after 2 h cultivation in fresh medium. Compounds as polar as HPMPCp and HPMPCpp would be expected not to permeate the cell membrane. After 24 h in the fresh medium, HPMPC amounted to 50% of total $^3$H activity in the medium, while HPMPCp-choline 27%, HPMPCp 10% and HPMPCpp 13%. However, since HPMPCp and HPMPCpp are probably dephosphorylated during the incubation, the above data do not reflect the relative efflux of singular compouds. Nonetheless, no HPMPC-metabolites have been detected on incubation of the medium containing [$^3$H]HPMPC in the absence of cells. To exclude the possibility that the appearance of such metabolites is caused by rupture or partial lysis of the cultured cells or by altered permeability of their cell membranes, we have carried out the incubation of the cells in the presence of [$^3$H]HPMPC and [$^{14}$C]cytidine. After 24 h the medium was substituted by a fresh one and the cells were incubated for additional 24 h. The analysis of TCA-treated extracts from cells and medium revealed that while phosphorylated HPMPC derivatives as well as cytidine and uridine phosphates (CMP, CDP, CTP, UMP, UDP, UTP) are present in the cellular pool, the medium contains only HPMPC metabolites (Fig. 6a,b). This observation suggests an existence of some selective mechanism which mediates the efflux of HPMPC metabolites from the cells. Such a mechanism may be connected to exocytosis, a process reversible to endocytosis, which is very likely involved in the transport of nucleotide phosphonate analogues into cells[21].

The incorporation of [$^3$H]HPMPC into cell acid-insoluble fraction increases at a somewhat lower rate for further 20 h following the removal of HPMPC from medium (data not given).

*Dose Dependent Synthesis of Metabolites*

The levels of the above four components in the cellular pool were determined after 24 h of incubation with 1 – 100μM HPMPC. Intracellular levels of HPMPC including its metabolites depend on the initial external concentration of HPMPC in the medium within the whole range of concentrations. At higher concentrations, HPMPCp-choline is the main metabolite. No additional metabolite was detected even at extreme concentration of HPMPC in the medium.

*Phosphorylation of HPMPC*

The synthesis of HPMPCp is a limiting step for intracellular HPMPC phosphorylation. PNMP kinase is the only known enzyme which catalyses the phosphorylation of CMP, UMP and dCMP in the presence of ATP (ref.[16]). This enzyme has a crucial position participating in the catalysis of both de novo and "salvage pathway" (ref.[16]). Our present results show that PNMP kinase from rat liver catalyses the formation of HPMPCp (analogue of CDP or dCDP). In this instance, HPMPC is a less efficient substrate than CMP. The ratio $K_m$(CMP) : $K_m$(HPMPC) = 0.1, where $K_m$(HPMPC) = 0.3 mmol $l^{-1}$ (Fig. 7), indicates that the affinity of enzyme to HPMPC is by one order of magnitude lower than that to CMP and is thus comparable to dCMP (ref.[16]). The time course of phosphorylation of HPMPC to HPMPCp has a long-term linear character. The initial reaction rate is lower by nearly three orders of magnitude than that for CMP reaction. HPMPCp synthesis is completely blocked by the addition of excess CMP to the reaction mixture (data not shown). The identity of HPMPCp was confirmed by comparison with standard materials on HPLC (system II) (Fig. 8a,b) and paper electrophoresis in system IV.

*Enzymatic Synthesis of HPMPCp-Choline*

The formation of HPMPCp-choline in the cultured cells occurs only after the high intracellular level of HPMPCpp has been achieved. Obviously the synthesis of this

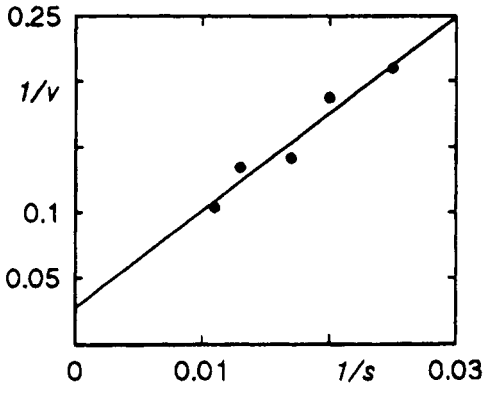


FIG. 7
Lineweaver–Burk plots of HPMPC phosphorylation catalyzed by rat liver PNMP kinase. $v$ (nmol $l^{-1}$ $min^{-1}$ $\mu g^{-1}$), $s$ ($\mu$mol $l^{-1}$)

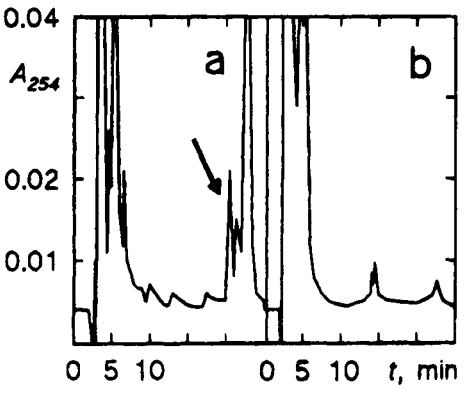


FIG. 8
HPLC analysis of product of HPMPC phosphorylation catalyzed by PNMP kinase (system II). a Reaction mixture; arrow indicates HPMPCp formed. b Reaction mixture after alkaline phosphatase treatment

metabolite is catalyzed by CTP:phosphorylcholine cytidylyltransferase which enables the formation of CDP-choline from CTP and choline phosphate[22]. This enzyme also catalyzes the formation of araCDP-choline from araCTP and choline phosphate[17].

We have found that HPMPCpp is a weak substrate for cytidylyltransferase from rat liver; during the formation of CDP-choline (under comparable conditions, i.e., at 2mM substrate) the conversion during the linear part of reaction course is 400-times higher. The conversion of araCTP into araCDP-choline was 20-fold increased under identical conditions (Fig. 9).

*Inhibition of Cytidylyltransferase*

On examining the inhibitory activity of HPMPCpp upon CDP-choline synthesis catalyzed by cytidylyltransferase, only 50% inhibition of the enzyme activity has been obtained at a substrate-inhibitor 1 : 1 ratio. Neither HPMPC nor HPMPCp have any inhibitory effect upon this reaction. We have also studied the possible feed-back inhibition of the enzyme by HPMPCp-choline (product analogue). In this case, the synthesis of CDP-choline from CTP and choline phosphate was investigated in the presence of HPMPCp-choline or CDP-choline (0.05 – 2mM). No inhibition was noted with HPMPCp-choline while CDP-choline was somewhat inhibitory.

The present data apparently indicate certain discrepancy between the enzymatic and cellular studies. While the enzymatic synthesis of CDP-choline is much more efficient than that of HPMPCp-choline, the level of intracellular CDP-choline in the cells is low. The cellular level of HPMPCp-choline is steadily increasing and at its maximum (100 h of incubation time) exceeds that of CDP-choline by 100%. One of the reasons may be the difference between the activity of isolated enzyme in vitro and enzyme present in intact cells. The main regulatory mechanism of the cellular cytidylyltransferase activity

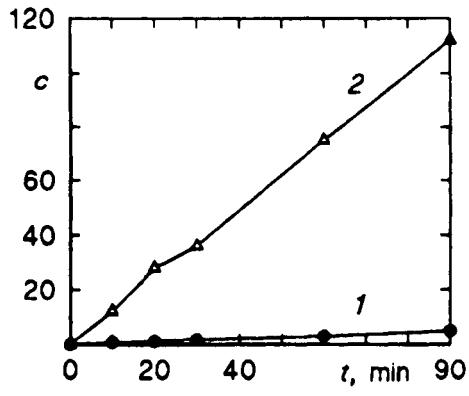


FIG. 9

Time course of HPMPCp-choline and araCDP-choline synthesis catalyzed by cytidylyltransferase. *1* HPMPCp-choline, *2* araCDP-choline, *c* ($\mu$mol $l^{-1}$).

is the translocation from the cytosol (inactive form) to the membrane (active form) (refs[23,24]). The association with the membrane structures is given by various factors, e.g., by reversible protein phosphorylation[25], by the presence of fatty acids[26,27] or diacylglycerol[28]. During the isolation procedure the cytosolic form is obtained. It must be activated by the addition of lipids.

The low intracellular concentration of CDP-choline as has been shown in different tissues[29,30] and cell lines[31,32] is a general phenomenon. This may be explained by the fact that CDP-choline is a substrate for CDP-choline:1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) (ref.[33]) which transfers choline phosphate from CDP-choline to diacylglycerol leading to the formation of phosphatidylcholine.

## CONCLUSIONS

As shown previously in vitro and in vivo, HPMPC manifests potent antiviral effects on HCMV multiplication[4,8]. This virus (as well as other members of *Herpesviridae*) belongs among enveloped viruses whose infective particles are surrounded by a bilayer of phospholipids with anchored surface glycoproteins. However, a number of HCMV infective particles shed by the cell contain two distinct phospholipide envelopes[34] originating either in the inner nuclear membrane[35] or in endoplasmatic reticula[36]. Thus, the drug interference with cellular phospholipid synthesis might have important role in the antiviral action. HPMPC which has an outspoken activity against HCMV infection, forms a CDP-choline analogue as one of the potential active antimetabolites. Further work is necessary to elucidate the relation of virostatic effect of HPMPC and HPMPCp-choline formation to phospholipid metabolism as well as phospholipid composition of viral envelope.

*This work was supported by Bristol-Myers Squibb Co. and by Grants No. 45 508 and No. 45 519 of Grant Agency of Czechoslovak Academy of Sciences.*

## REFERENCES

1. De Clercq E., Sakuma T., Baba M., Pauwels R., Balzarini J., Rosenberg I., Holý A.: Antiviral Res. *8*, 261 (1987).
2. Bronson J. J., Ghazzouli I., Hitchcock M. J. M., Webb II R. R., Martin C.: J. Med. Chem. *32*, 1457 (1989).
3. Bronson J. J., Ghazzouli I., Hitchcock M. J. M., Webb II R. R., Kern E. R., Martin J. C. in: *Nucleotide Analogues as Antiviral Agents* (J. C. Martin, Ed.), ACS Symp. Series 401, 88, (1989).
4. Snoeck R., Sakuma T., De Clercq E., Rosenberg I., Holý A.: Antimicrob. Agents Chemother. *32*, 1839 (1988).
5. Kern E. R., Vogt P.E.: *Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy (Houston, TX)*, No. 751. American Society for Microbiology, Washington DC 1989.
6. Neyts J., Snoeck R., Schols D., Balzarini J., De Clercq E.: Virology *179*, 41 (1990).
7. Neyts J., Snoeck R., Balzarini J., De Clercq E.: Antiviral Res. *16*, 41 (1991).

8. Otová B., Votruba I., Holý A.: Acta Virol., in press.
9. Bronson J. J., Ho H. T., DeBoeck H., Woods K., Ghazzouli I., Yang H., Klunk L. J., Russell J. W., Whiterock V. J., Datema R. I., Martin J. C., Hitchcock M. J. M.: Antiviral Res., Suppl. I., poster No. 136, 111 (1990).
10. Votruba I., Bernaerts R., Sakuma T., De Clercq E., Merta A., Rosenberg I., Holý A.: Mol. Pharmacol. *32*, 524 (1987).
11. Merta A., Veselý J., Votruba I., Rosenberg I., Holý A.: Neoplasma *37*, 111 (1990).
12. Holý A., Rosenberg I., Dvořáková H.: Collect. Czech. Chem. Commun. *54*, 2470 (1989).
13. Moffatt J. G.: Can. J. Chem. *42*, 599 (1964).
14. Choy P. C., Lim P. H., Vance D. E.: J. Biol. Chem. *252*, 7673 (1977).
15. Feldman D. A., Rounsifer M. E., Weinhold P. A.: Biochim. Biophys. Acta *429*, 429 (1985).
16. Maness P., Orengo A.: Biochemistry *14*, 1485 (1975).
17. Lauzon G. J.,Paran J. H., Paterson A. R. P.: Cancer Res. *38*, 1723 (1978).
18. Cooney D. A., Dalal M., Hiroaki M., McMahon J. B., Nadkarni M., Balzarini J., Broder S., Johns D. G.: Biochem. Pharmacol. *35*, 2065 (1986).
19. Balzarini J., Pauwels R., Baba M., Herdewijn P., De Clercq E., Broder S., Johns D. G.: Biochem. Pharmacol. *37*, 897 (1988).
20. Lauzon G. J., Paterson A. R. P., Belch A. W.: Cancer Res. *38*, 1730 (1978).
21. Palú G., Stefanelli S., Rassu M., Parolin C., Balzarini J., De Clercq E.: Antiviral Res. *16*, 115 (1991).
22. Kennedy E. P., Weiss S. B.: J. Biol. Chem. *222*, 193 (1956).
23. Sleight R., Kent C.: J. Biol. Chem. *258*, 831 (1983).
24. Tercé F., Record M., Ribbes G., Chap H., Douste-Blazy L.: J. Biol. Chem. *263*, 3142 (1988).
25. Watkins J. D., Kent C.: J. Biol. Chem. *266*, 21113 (1991).
26. Pelech S. L., Cook H. W., Paddon H. B., Vance D. E.: Biochim. Biophys. Acta *795*, 433 (1984).
27. Tronchere H., Tercé F., Record M., Ribbes G., Chap H.: Biochem. Biophys. Res. Commun. *176*, 157 (1991).
28. Choy P. C., Farren S. B., Vance D. E.: Can. J. Biochem. *57*, 605 (1979).
29. Possmayer F., Casola P. G., Chan F., MacDonald P., Ormseth M. A., Wong T., Harding P. G. R., Tokmakian S.: Biochim. Biophys. Acta *664*, 10 (1981).
30. Zelinski T. A., Savard J. D., Man R. Y. K., Choy P. C.: J. Biol. Chem. *255*, 11423 (1980).
31. Whitehead W. F., Trip E., Vance D. E.: Can. J. Biochem. *59*, 38 (1981).
32. Vance D. E., Trip E., Paddon H. B.: J. Biol. Chem. *255*, 1064 (1980).
33. Pelech S. L., Vance D. E.: Biochim. Biophys. Acta *779*, 217 (1984).
34. Farrar G. H., Oram J. D.: J. Gen. Virol. *65*, 1991 (1984).
35. Smith J. D., De Harven E.: J. Virol. *12*, 919 (1973).
36. Severi B., Landini M. P., Musiashi M., Zerbini M.: Microbiologica *2*, 265 (1979).

Translated by J. Veselý.