Biochemical Pharmacology, Vol. 23, pp. 1227-1230. Pergamon Press, 1974. Printed in Great Britain

INHIBITION OF CHOLINE KINASE BY SELECTIVELY CYTOTOXIC PURINYL-6-HISTAMINE

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(*Received* 25 *November* 1973)

The search for chemical compounds which may be used as chemotherapeutic agents in cancer treatment has mainly been directed towards drugs which have an inhibitory action on nucleic acids or protein synthesis. The disadvantageous inhibition of physiologically proliferating tissues and the mutagenic and immunosuppressive effects of such cytostatic agents are well known (1). Purinyl-6-histamine (PH) which is cytotoxic against tumour cells in vitro ($0.4\text{-}0.9 \times 10^{-3}$M) has little or no effect on normal cells in this concentration (2-5), and inhibition of nucleic acids or protein synthesis is not an early effect of this drug (6). Morphological studies (5) have shown that membranes of tumour cells are changed by the drug whereas membranes of normal cells are less affected. Preliminary observations dealing with the inhibition of a choline kinase preparation and of the incorporation of choline-methyl-^{3}H into cells by PH are presented.

Materials and Methods:

Choline kinase was isolated from brewers' yeast according to the procedure described by McCaman et al. (7). Incubation mixtures for the estimation of K_i contained 100 mM 2-amino-2-methyl-1,3-propanediol, 10 mM $MgCl_2$, 10 mM ATP, 51.4 µg of choline-methyl-^{14}C (1 mCi/mmole) and inhibitor as indicated in the graph in a total volume of 100 µl. Cholinephosphate formation during incubation at 37° was estimated within 30 minutes by the reineckate

method (7). Incubation mixtures for the estimation of the maximal velocity were the same. Substrate and inhibitor concentrations are indicated in the graph. Incorporation of choline-methyl-^{3}H (17 Ci/mmole) into ascites cells was estimated by a modified filter technique (8). Incubation mixtures contained 1.5 x 10^7 cells, 5 µCi of choline-^{3}H and the amount of inhibitor as indicated in the Table in a total volume of 0.8 ml of HBSS (9). Incubations were performed at 37° for 60 min. Aliquots were taken and analyzed for acid insoluble radioactivity. All scintillation countings were carried out in a Tri Carb liquid scintillation spectrometer (Packard Instruments), model 3380, attached to an analyzer for absolute radioactivity countings, model 544. Dpm analyses (choline/cholinephosphate-ratio) were carried out in homogeneous solution (Protosol, NEN-Chemicals) according to the instructions of the producer of the scintillation spectrometer. Cpm analyses (incorporation of choline-methyl-^{3}H into cells) were performed as described previously (6).

Results:

Fig. 1 indicates that a preparation of choline kinase isolated from yeast is inhibited by PH and that this inhibition is of the competitive type (Fig. 2). This effect raised the question whether the choline kinase which is present in tumour cells is also susceptible to inhibition by PH. As a consequence of choline kinase inhibition on the cellular level, one should expect an inhibition of the incorporation of choline-methyl-^{3}H into the acid insoluble material of cells. This inhibition is demonstrated in Table 1.

Discussion:

The data presented demonstrate that PH inhibits a choline kinase preparation isolated from yeast. Furthermore, the incorporation of choline into the cold acid precipitable material of Ehrlich ascites cells is inhibited by PH. It is known that choline is predominantly incorporated into the lecithin of cells (10) and we conclude from our preliminary results that the inhibition of choline incorporation by PH reflects an inhibition of cellular lecithin synthesis on the choline kinase step. Further investi-

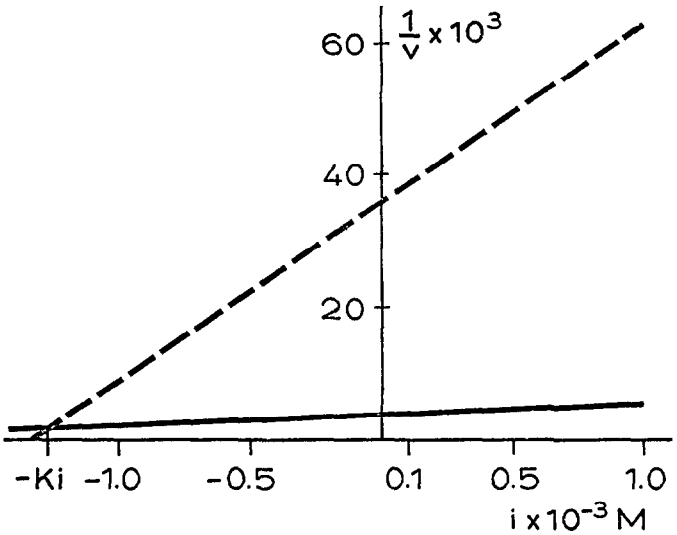

Figure 1. Estimation of K_i for the inhibition of choline kinase by purinyl-6-histamine.

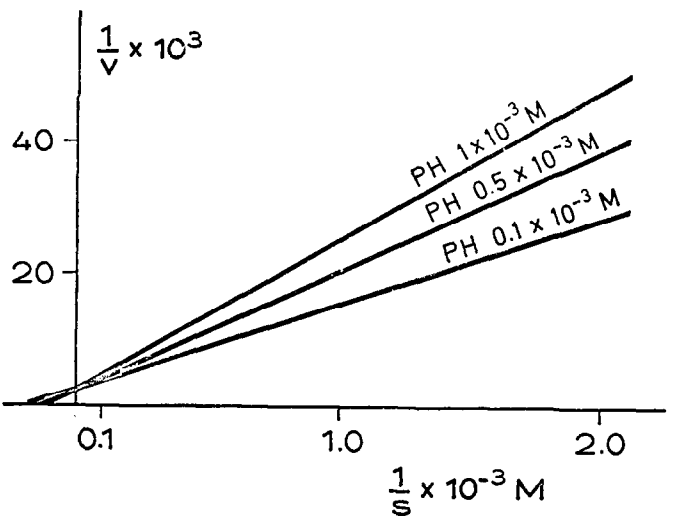

Figure 2. Lineweaver-Burk plot of the inhibition of choline kinase by purinyl-6-histamine.

gations will show whether or not this inhibition is relevant to the mechanism of action of PH.

Table 1. Inhibition of incorporation of choline-methyl-^{3}H into the cold acid insoluble material of Ehrlich ascites cells by purinyl-6-histamine

PH concentration	Cpm x $10^{-3}/10^6$ cells	% of Controls
Controls	30.1 ± 5.8	-
0.625 x 10^{-3} M	15.0 ± 1.6	50
1.250 x 10^{-3} M	13.3 ± 2.1	44
2.500 x 10^{-3} M	9.8 ± 3.8	33

Mean values of six identical experiments ± S.D.

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