*Acta Pharmacologica Sinica* 1986 Jul; 7 (4) : 368-373

# Effect of cytarabine upon metabolism of phospholipids in leukemia L5178Y cells

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**ABSTRACT** The influence of cytarabine on phospholipids metabolism in murine leukemia L 5178 Y cells *in vitro* has been studied. Cytarabine increased the incorporation of [$^{32}$P]phosphoric acid into total phospholipids of cells treated with 0.01-0.1 m*M* by 2-5 folds. The change started at 8 h after incubation with cytarabine. The effect may be correlated to the cytotoxicity of the drug but not correlated to protein synthesis, protein content and cell size. Cytarabine did not change individual components of phospholipids, but the quantities of phosphatidylcholine, phosphatidylethenolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin were increased significantly.

**KEY WORDS** cytarabine; leukemia L5178Y; phospholipids; [$^{32}$P]phosphoric acid; [$^{3}$H]leucine; cytotoxicity; protein synthesis; cell size

Cytarabine (cytosine arabinoside, Ara-C) is one of the most effective agent in the treatment of acute nonlymphocytic leukemias in man[1]. Two main mechanisms responsible for its antitumor activity are involved. Firstly, cytarabine transported across the cell membrane and activated to the form of 5′triphosphate, ara-triphosphate, which directly inhibits DNA polymerase by competing with the binding of 2-deoxycytidine 5′triphosphate (dCTP) to this enzyme[2]. Secondly, incorporation of cytarabine into DNA, producing chain termination of polydeoxynucleotide elongation, leads to an inhibition of DNA synthesis. Cytarabine inhibited cytidylatedeoxycytidylate (Cyd-dCyd) deaminase[3] and increased the activity of Cyd-dCyd kinase[4].

Although the catabolism of cytarabine *in vivo* and *in vitro* and the basic mechanisms responsible for its cytotoxicity have been known for some years there are many recent investigations of their metabolism and possible mechanisms for producing lethal cellular events. Recent work from our laboratories demonstrated that the choline metabolism produced a significant change when cells were treated with cytarabine[5]. In this study we found a change of phospholipids metabolism in murine leukemia cells treated with cytarabine.

## MATERIALS AND METHODS

**Growth of cells** The murine leukemia L5178Y cell line originally came from the National Cancer Institute. The cells were propagated in suspension culture with Fischer's medium supplemented with 10% horse serum as described previously[6]. If not otherwise stated, in routine experiments logarithmically growing 5-10 × $10^3$ cell/ml were cultured in plastic culture flasks or

Received 1985 Jul 30 Revised 1985 Dec 9
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tubes and preincubated in a 5% $CO_2$–95% air humidified incubator at 37℃ for 24 h.

**Viability and size of cells** Total cell number was enumerated by a Coulter counter. The % of cells stained with trypan blue were scored. The numbers of live cell were calculated by total cell mumbers time % of unstained cells time 100. Clonogenic efficiency was measured by soft agar method as described previously[6]. Cell size was measured by Coulter Channelyzer C-1000 with Apple II PLUS computer record system (Coulter Electronics Ltd). In these experiments the median values were used since these data are less liable to errors at both extremities of the distributions. Each sample was measured for at least 1000 cells.

**Protein systhesis and content** $5\times10^4$ preincubated cells/ml were exposed to different concentrations of cytarabine. After 23.5 h of incubation, [$^3$H]leucine (370 kBq/ml) was added and the incubation continued for 0.5 h. Acid-precipitate materials were extracted by 10 ml of 10% trichloroacetic acid (TCA) and loaded onto DE 81 filter paper. The papers were washed with 10 ml of 5% TCA for 3 times and placed in vials containing 10 ml ACS scintillation fluid. The radioactivity was measured by a Beckman LS 1800 scintillation counter. The results were expressed as % of radioactivity of untreated cells.

Determination of protein content was performed on duplicate samples containing 10–100 μg protein using the chromometer method[7].

**Lipid extraction** Extraction of total lipids was carried out according to the method of our laboratory[8]. Cells labelled with [$^{32}$P] phosphoric acid ($^{32}$P) were harvested by centrifugation and washed 3 times with PBS. NaCl 1 *M* and methanol were added to the cell pellets. Lipids of cells were extracted twice by chloroform. Aliquots of the chloroform extract were counted to determine total radioactive precursor incorporated in the lipid.

Individual phospholipids were separated by 2-dimensional TLC plates (19×19 cm) of 0.25 mm thickness which were prepared with a Silica Gel H (Sigma) slurry. Aliquots of lipid extract were spotted onto the plates and developed in chloroform: methanol : aceticacid : water 75 : 48 : 12.5: 4.5 (vol:vol). Standard reference compounds including phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (Sph) and phosphatidylinositol (PI) or phosphatidylserine (PS) were spotted for identification of lipids. The lipid spots were stained with iodine vapor. The individual phospholipids were expressed as % of total lipids.

## RESULTS

**Influence of cytarabine on the incorporation of $^{32}$P into phospholipids** Cytarabine 0.01 and 0.1 m*M* produced a marked inhibition on cell growth. The % of

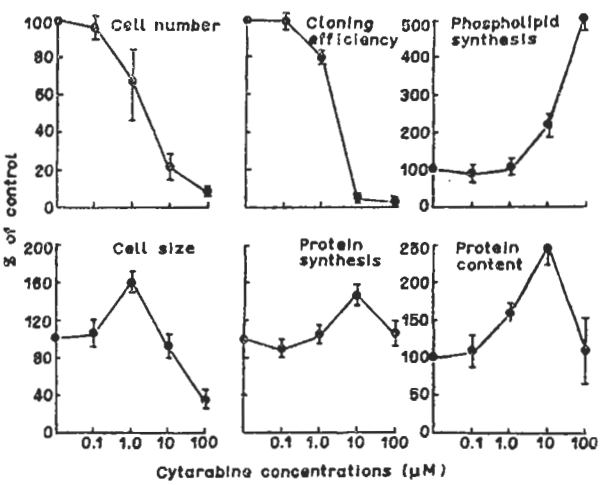


**Fig 1. Effects of cytarabine on phospholipid synthesis and some parameters of L 5178 Y cells. Cells growing in the exponential phase were exposed to cytarabine 0.1–100 μ*M*. After 4 h, [$^{32}$P] 370 kBq/ml were added to cell cultures and incubated for 20 h. Results are expressed as % of control culture of untreated cells which were normalized to 100%. $\bar{x}\pm$SD of 3 experiments in duplicates for each experiment.**

Tab 1. Ratios between phospholipids (PL) synthesis and some parameters of cells treated with cytarabine

| Concn ($\mu M$) | PL Synthesis/ Protein synthesis | PL synthesis/ Cell size | PL synthesis/ Protein content | Protein content/ Cell size | Protein content/ Protein synthesis |
|---|---|---|---|---|---|
| 0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 0.1 | 1.08 | 0.90 | 0.87 | 1.03 | 1.25 |
| 1.0 | 0.94 | 0.65 | 0.63 | 1.04 | 1.50 |
| 10.0 | 1.50 | 2.60 | 0.86 | 3.03 | 1.75 |
| 100.0 | 5.09 | 13.7 | 3.17 | 4.32 | 1.61 |

inhibition were 21 and 7 %, respectively, in comparison with control cultures (Fig 1). These concentrations of cytarabine also yielded a remarkable cytotoxicity in a soft agar culture system. As Fig 1 shows, cytarabine 0.01 and 0.1 m$M$ increased the incorporation of $^{32}P$ into the phospholipids of cells by 2- and 5-fold, respectively, when compared to nontreated cells. Cytarabine had no influence on the incorporation of [$^{3}H$]leucine into protein. The cell size was changed only at 0.1 m$M$. Protein content of cells was increased when they were exposed to 0.01 m$M$. When the phospholipids synthesis of cells rose continuously with cytarabine 0.1 m$M$, the protein content of cells was not increased. The ratios between phospholipids synthesis and some parameters of treated cells are shown in Table 1. The rate of phospholipids synthesis in cells treated with cytarabine 100 $\mu M$ was higher than that of protein synthesis. On the basis of cell volume, the phospholipids synthesis was increased 13.7 times when cells were exposed to cytarabine 0.1 m$M$. These experiments also implied the accumulation on protein in cells treated with cytarabine 0.001-0.1 m$M$.

**Composition change of phospholipids in cells treated with cytarabine** From Fig 2, it was found that PC composed approximately 50% of the total phospholipids in L 5178 Y cells. PI + PS and PE composed 10-20%, and Sph was only about 8%. When cells were exposed to 4 different concentrations of cytarabine the

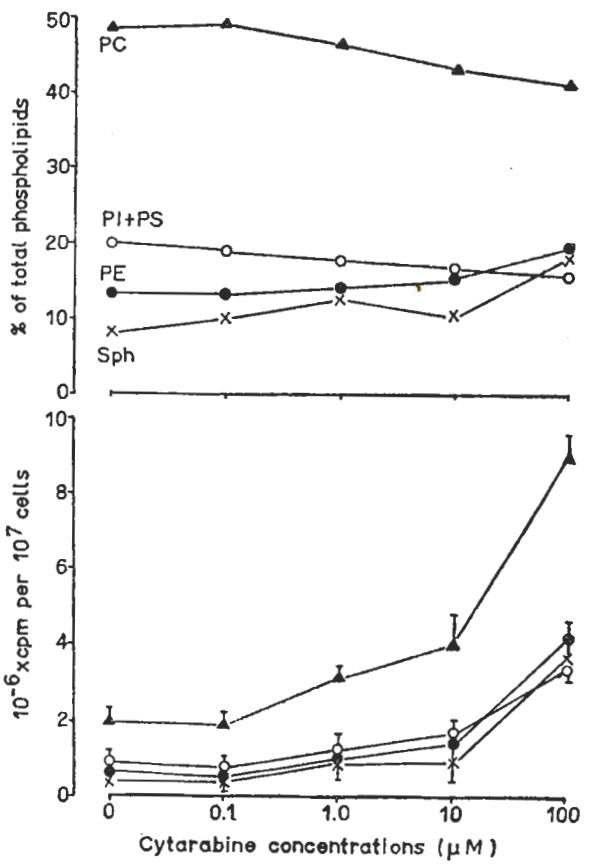


**Fig 2. Composition of phospholipids (phosphatidylethenolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylserine and sphingomyelin) in L 5178Y cells treated with cytarabine. After 4 h of incubation with cytarabine, cells were labeled with $^{32}P$ 370 kBq/ml for 20 h. The changes are expressed as % of total phospholipids or cpm/$10^{7}$ cells.**

PC and PI + PS were slightly decreased. In contrast, Sph and PE were increased to 117 and 44%, respectively. However, the quantity of each component was raised with the increasing cytarabine concentrations. At 0.1 m*M* of cytarabine the quantities of PC, PI + PS, PE and Sph were increased to 4.3, 4.1, 7.5, and 11.2 folds, respectively.

**Time course of the incorporation of $^{32}P$ into phospholipids of cells treated with cytarabine** After 24 h of preincubation, the cells were exposed to 0.01 m*M* cytarabine. While the control culture was treated with saline. After 24 h, $^{32}P$ was added to a final concentration of 370 kBq/ml of cells to cultures. At 1, 2, 4, 8 and 24 h intervals, an aliquots was collected from each culture. The phospholipids were extracted by chloroform. The radioactivity of each culture was measured. Results are shown in Fig 3.

The cells exposed to cytarabine 0.01 m*M* for 1,2 and 4 h showed no change in the incorporation of $^{32}P$ into phospholipids when compared with the control. The increase of radioactivity started at 8 h of incubation. After 8 and 24 h the radioactivity of cells was 2-fold of that in nontreated cells. The incorporation of $^{32}P$ into phospholipids was enhanced when cells were treated with cytarabine.

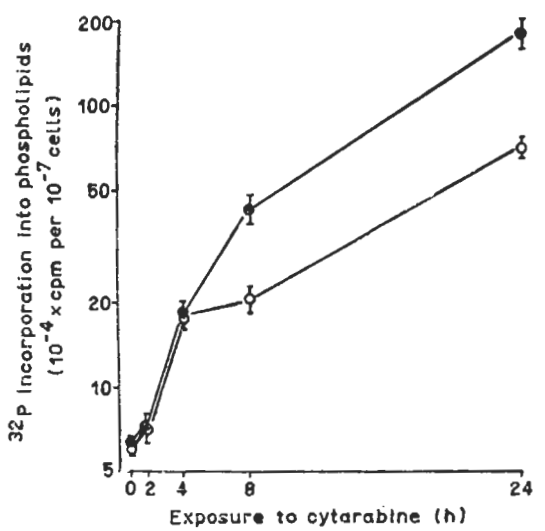


Fig 3. **Effect of duration of cytarabine treatment on the incorporation of $^{32}P$ into phospholipids in L 5178 Y cells. Cells were preincubated at 37℃ for 24 h. Cytarabine 10 μ*M* and $^{32}P$ 370 kBq/ml were than added to cultures. The cells were collected after 1, 4, 8 and 24 h. A culture of drug-free cells was exposed to $^{32}P$ alone as control.**

## DISCUSSION

The phospholipids are essential components of the cell membrane system. The permeability and electrochemical properties of cells may be connected to phospholipids. Although a few studies have demonstrated the effects of drugs on phospholipids metabolism, there has been little indication that the metabolism of phospholipids can be modified by the action of anticancer compounds[9]. Since cytarabine influence CDP-choline metabolism, it is interesting to study the effects of cytarabine on phospholipids metabolism in cells. In this study we found that the rate of phospholipids synthesis in L 5178 Y cells, a murine leukemic cells line, was changed by cytarabine. The incorporation of $^{32}P$ into phospholipids was increased 2 to 5 folds in comparison with untreated cells. These alterations seem to have a good correlation to the cytotoxicity of the drug, but appear to have no correlation to protein synthesis. The increase of protein content in the cells may be due to the loss of the ability of cell division when treated with cytarabine, which is a potential inhibitor of DNA polymerase and is also incorporated into DNA. Although the main lesions on cellular DNA produced by cytarabine are at the sites where singlestrand DNA breaks and base damage occurs, alterations in any of the quality and/or quantity of phospholipids may lead to a loss of growth control or prolific capacity of cells.

Phospholipid compositions of cells vary considerably with species and tissues. The major components include PC, PI, PS, PE

and Sph. When cells were labeled with $^{32}P$ for 4–24 h, the radioactivity measured in PC was increased 2 to 4 folds. The synthesis of PC in mamalian systems utilizes 1,2-diacylglycerol, CTP and phosphocholine. As is frequently the case, this anabolic reaction requires the participation of CTP: phosphocholine cytidyltransferase. Then the CDP-choline is catalyzed by cytidine diphosphocholine: 1,2-diacylglycerol choline-phosphotransferase to PC. It has been reported that a metabolite, ara-CDP-choline, was formed when cells were incubated with cytarabine[10] and increased ara-CDP-choline of L 5178 Y cells. These data implied that the mechanisms of cytarabine may be related to the phospholipids metabolism in cells.

**ACKNOWLEGMENTS** This work was supported in part by grant CA-12197 from the National Cancer Institute of NIH and grant CH-35 E from the American Cancer Society and the Gastonai Cancer Society.

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中国药理学报　1986年7月；7(4)：368-373

# 阿糖胞苷对小鼠白血病 L 5178 Y 细胞磷脂代谢的影响

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**提要**　阿糖胞苷是治疗非淋巴细胞性白血病最有效的抗癌药之一。本实验研究表明，0.01-0.1 mM 阿糖胞苷能增加 $^{32}P$ 参入 L5178Y 白血病细胞总磷脂 2—5 倍。作用起始于暴露药物后 8 h。阿糖胞苷促进磷脂代谢与药物的细胞毒作用相关，但与细胞的蛋白质合成、蛋白质总量以及细胞大小无关。阿糖胞苷对各磷脂成分的比例无明显影响，但能明显增加磷脂酰胆碱，磷脂酰乙醇胺，磷脂酰肌醇，磷脂酰丝氨酸和鞘磷脂的量。

**关键词**　阿糖胞苷；　白血病 L5178Y；　磷脂；[$^{32}P$] 磷酸；　[$^{3}H$]亮氨酸；　细胞毒；蛋白质合成；细胞大小

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