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5-FLUOROPYRIMIDIN-2-ONE, A NEW METAPHASE ARRESTING AGENT

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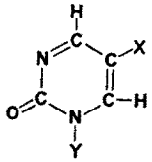
Abstract—5-Fluoropyrimidin-2-one arrests reversibly the Chang strain of human liver cells in the metaphase. The effect is dependent on the presence of the halogen in the molecule and seems to be due to the substance *per se*.

PREVIOUS experiments,[1,2] have shown that ribosylpyrimidin-2-one (1A) and deoxyribosyl-5-fluoropyrimidin-2-one (1B) inhibit synthesis of DNA in *Escherichia coli*, whereas the parent substances, pyrimidin-2-one (1C) and 5-fluoropyrimidin-2-one (1D) do not.



1A: X = H, Y = ribosyl
1B: X = F, Y = deoxyribosyl
1C: X = H, Y = H
1D: X = F, Y = H

In order to obtain further information about the biological properties of these substances, the effects on mammalian cells were studied. Unexpectedly, it was found that 5-fluoropyrimidin-2-one had a metaphase arresting effect. The present report describes experiments which demonstrate this.

MATERIALS AND METHODS

In all experiments monolayer cultures of the Chang strain of human liver cells were used. The cells were grown in E2a medium[3] and the effect of the substances on the mitotic index was studied by a method previously described.[4]

Ribosylpyrimidin-2-one, pyrimidin-2-one and deoxyribosyl-5-fluoropyrimidin-2-one were prepared as previously described.[1,2] 5-fluoropyrimidin-2-one was prepared from 5-fluorouracil, as described by Undheim and Gacek.[5] In order to remove contaminating 5-fluorouracil, the material was purified twice by sublimation in vacuum.

RESULTS AND DISCUSSIONS

When cells were grown in the presence of 1·75 mM (0·2 mg/ml) of 5-fluoropyrimidin-2-one (1D) an almost constant increase in the mitotic index occurred during a 12 hr period (Fig. 1, curve A). The control cells exhibited during the same period a constant mitotic index (curve B). Since in the cells treated with 5-fluoropyrimidin-2-one usually

no anaphases or telophases were found, while a normal and constant level of prophases and an increasing number of metaphases were present (see Table 1); the experiments indicate that the cells in the presence of 5-fluoropyrimidin-2-one enter mitosis at a normal rate and are arrested at the metaphase level.

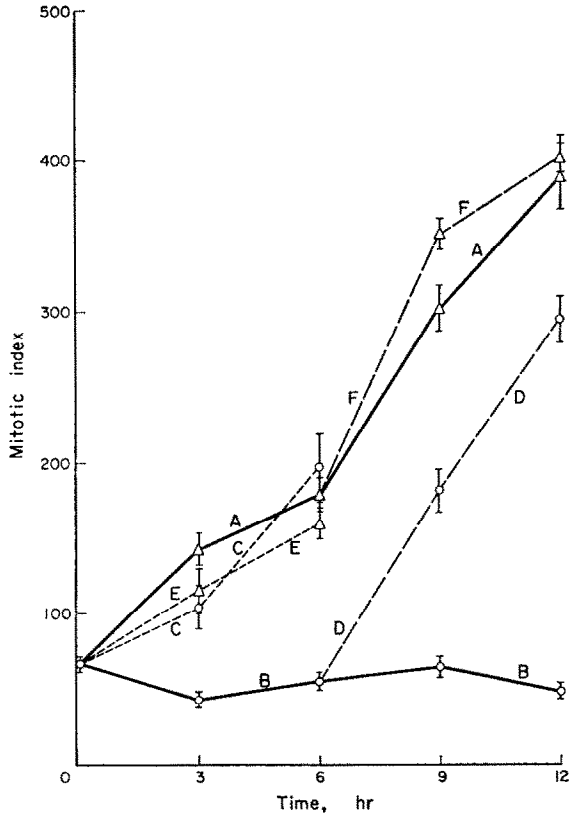


FIG. 1. Effect of 5-fluoropyrimidin-2-one on the mitotic index of monolayer cultures of Chang liver cells. Exponentially growing cells were added 5-fluoropyrimidin-2-one (1·75 mM) at zero time and the mitotic index determined during a 12 hr period at intervals of 3 hr. Every point represents the average mitotic index of five parallel cultures. Curve A: Cells treated with 5-fluoropyrimidin-2-one. Curve B: Control cells (no additions). Curves C and D: Cells added colcemid (0·04 μg/ml) at zero time (Curve C) and at 6 hr (Curve D). Curves E and F: 5-Fluoropyrimidin-2-one treated cells added colcemid at zero time (Curve E) and at 6 hr (Curve F).

To compare the effect of 5-fluoropyrimidin-2-one with a known metaphase inhibitor such as colcemid, several experiments were carried out. When colcemid was added at zero time (Fig. 1, curve C), there was no significant difference in the mitotic index after 6 hr when compared to the 5-fluoropyrimidin-2-one treated cells (curve A). The same increase in mitotic index was also obtained when both inhibitors were added together at zero time (curve E). If the cells had been treated with 5-fluoropyrimidin-2-one for 6 hr, addition of colcemid did not significantly alter the increase in mitotic index found for cells containing only 5-fluoropyrimidin-2-one. Thus, 5-fluoropyrimidin-2-one has a colcemid like effect.

TABLE 1. FREQUENCIES OF MITOTIC STAGES AFTER ADDITION OF 1·75 mM 5-FLUOROPYRIMIDIN-2-ONE TO CHANG CELLS

Hr after addition	Number of cells per 1000 cells			
	Prophase	Metaphase	Anaphase	Telophase
0	9·4	38·0	4·2	15·6
3	6·2 (3·0)	136·6 (25·0)	0 (4·2)	0 (10·8)
6	4·4 (5·8)	174·6 (33·0)	0 (2·8)	0 (13·4)
9	6·4 (7·2)	297·0 (31·6)	0 (4·6)	0 (20·4)
12	6·2 (5·3)	384·2 (26·5)	0 (3·0)	0 (13·4)

Controls in parentheses.

Figure 2 demonstrates the relationship between the concentration of 5-fluoropyrimidin-2-one and the metaphase arresting effect. It is seen that 0·87 mM resulted in some accumulation of cells in the metaphase whereas 1·75 mM gave optimal effect. Larger concentrations gave no further detectable increase in the mitotic index within the 12 hr period. The concentrations of 5-fluoropyrimidin-2-one required to arrest the cells in the metaphase is in contrast to colcemid considerably higher.

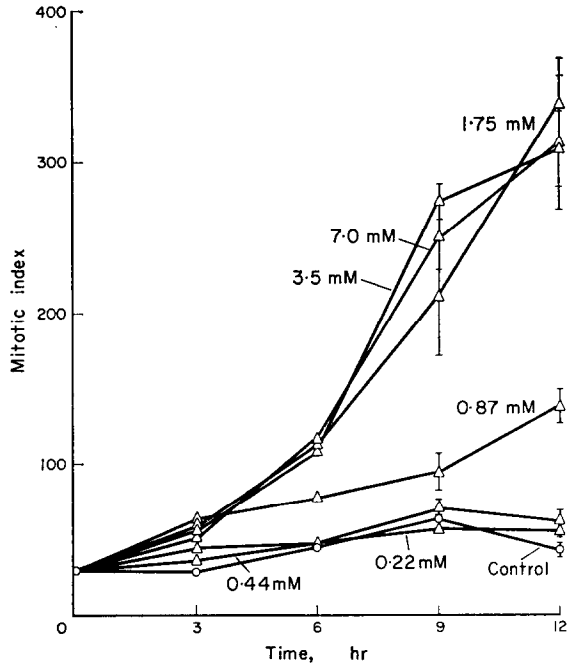


FIG. 2. Effects of different concentrations of 5-fluoropyrimidin-2-one on the mitotic index of Chang liver cells. 5-Fluoropyrimidin-2-one, in concentrations as indicated on the figure, was added at zero time and the mitotic index determined at intervals of 3 hr.

The presence of a halogen atom in the molecule is necessary for the metaphase arresting effect since pyrimidin-2-one (1C) in concentrations up to 8 mM had no effect on the mitotic index.

Experiments were then carried out to decide whether the metaphase arresting effect of 5-fluoropyrimidin-2-one was reversible. Cells grown for 6 hr in the presence of 5-fluoropyrimidin-2-one were incubated with fresh medium containing no inhibitor and the mitotic index determined after 45 min. The index was significantly reduced (from 191·2 to 138·0) and the number of telophases per 1000 cells had increased from zero to about 5-fold (73·5) that of the control cells (14·2). It is seen in Fig. 3 that when the mitotic index was determined 1·5, 3 and 6 hr after the removal of the inhibitor, it had dropped to that of the control cells. The number of metaphases, anaphases and telophases were also the same as for the control cells. Hence, the metaphase arresting effect of 5-fluoropyrimidin-2-one at the concentration used is fully reversible.

Previous work with *E. coli*[1,2] has shown that the ribosylpyrimidin-2-one and the deoxyribosyl-5-fluoropyrimidin-2-one inhibited DNA synthesis and it was thought of interest to examine the effect of these substances on the Chang cells. It was found that either substance had no metaphase arresting effect but inhibited the cells prior to mitosis. The results with the ribosylpyrimidin-2-one are shown in Fig. 4. It is seen that the substance at a concentration of 0·85 mM (0·2 mg/ml) reduced significantly

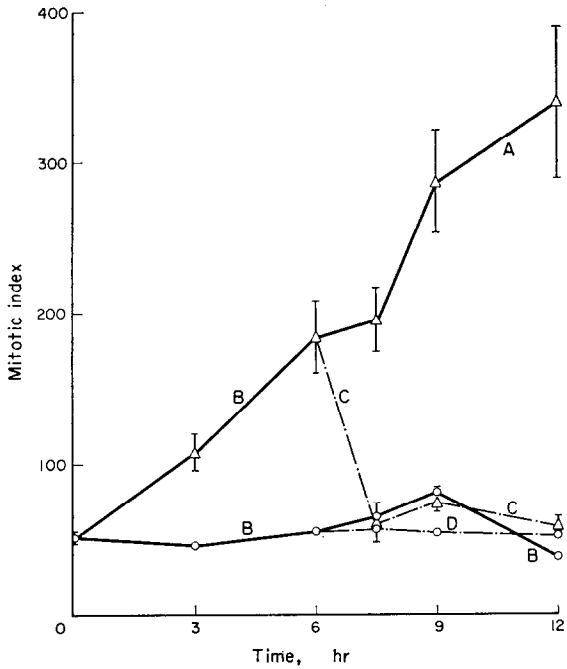


FIG. 3. Response in the mitotic index upon removal of 5-fluoropyrimidin-2-one from cultures of Chang liver cells. Cells were grown in presence of 5-fluoropyrimidin-2-one (1·75 mM). After 6 hr, the medium was removed and replaced with fresh medium containing no inhibitor. To assure a complete removal of the inhibitor, this medium was again changed with a fresh one after 15 min incubation. The mitotic index was then determined 1·5 and 3 and 6 hr after the first washing. Curve A: Cells treated with 5-fluoropyrimidin-2-one. Curve B: Control cells. Curve C: Cells washed after treatment with 5-fluoropyrimidin-2-one for 6 hr. Curve D: Control cells washed in the same manner as those in Curve C.

the accumulation of metaphases caused in the presence of colcemid (curves B and E). When the cells were incubated in the presence of ribosylpyrimidin-2-one for 6 hr and colcemid then added, a similar reduction was found (curves C and F). Since a reduced accumulation of metaphase cells was found already 3 hr after the addition of the ribosylpyrimidin-2-one (curve E) the effect may not be on DNA synthesis as was the case in *E. coli.*

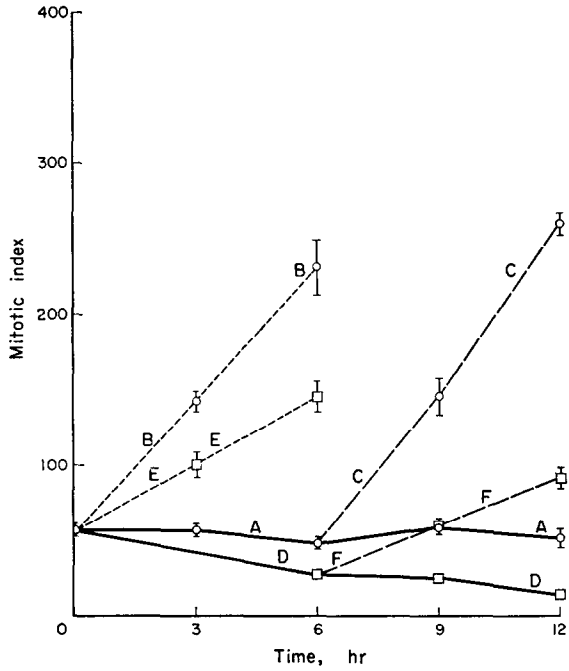


FIG. 4. Effect of ribosylpyrimidin-2-one on the accumulation of metaphase cells in cultures of Chang liver cells treated with colcemid. Exponentially growing cells were added ribosylpyrimidin-2-one (0·85 mM) at zero time. Colcemid (0·04 μg/ml) was then added either at zero time or at 6 hr. The mitotic index was determined at intervals of 3 hr during a 12 hr period. Curve A: Control cells. Curves B and C: Control cells added colcemid at zero time (Curve B) or at 6 hr (Curve C). Curve D: Cells treated with ribosylpyrimidin-2-one. Curves E and F: Ribosylpyrimidin-2-one treated cells added colcemid at zero time (Curve E) and at 6 hr (Curve F).

The metaphase arresting effect of 5-fluoropyrimidin-2-one could be due to the substance *per se* or to a metabolite.

From the experiments described above the conversion of 5-fluoropyrimidin-2-one to the deoxyriboside can be excluded.

Since the riboside of pyrimidin-2-one exhibited inhibitory properties while the pyrimidin-2-one itself had no effect, the cells used do not seem to be able to convert pyrimidin-2-one to ribosides. Hence, the conversion of 5-fluoropyrimidin-2-one to the riboside does not seem very likely.

Since the Chang liver cells did not contain aldehyde oxydase (EC 1.2.3.1, aldehyde: oxygen oxido reductase) (Grimmer and Øyen unpublished results) which is known to convert 5-fluoropyrimidin-2-one to 5-fluorouracil)[6] and since the effect of deoxyribo-

syl-5-fluoropyrimidin-2-one and ribosylpyrimidin-2-one is quite different from that of 5-fluoropyrimidin-2-one, we favour the view that the metaphase arresting effect is caused by the substance itself.

The present results which indicate that no conversion of 5-fluoropyrimidin-2-one to nucleosides occur in the Chang cells, are in agreement with previous experience which has shown that the pyrimidin-2-ones are not substrates for the nucleoside phosphorylases (EC 2.4.2.4, thymidine:orthophosphate deoxyribosyl transferase and EC 2.4.2.3, urisine:orthophosphate ribosyltransferase).[1,7] Thus 5-fluoropyrimidin-2-one differs in this respect to the well known fluoropyrimidines such as 5-fluorouracil and trifluoromethyluracil which exert their biological effect after being converted to the nucleosides.[8]

Several known metaphase arresting agents are complex molecules such as colcemid and vinca alcaloids. The 5-fluoropyrimidin-2-one is a molecule with a simple structure. It is also interesting that a pyrimidin derivative possesses metaphase arresting activity. The detailed mode of action of this substance is presently unknown.

Since the metaphase arresting effect of 5-fluoropyrimidin-2-one is reversible, it might be interesting to use it in attempts to obtain synchronously dividing cells.

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