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THYMIDINE INHIBITS THE INCORPORATION OF 5-FLUORO-2'-DEOXYURIDINE TO DNA OF MOUSE MAMMARY TUMOUR

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SUMMARY: 5-Fluoro-2'-deoxyuridine is incorporated into DNA of mouse breast tumour *in vivo*. The incorporation is inhibited by thymidine. Part of the fluorodeoxyuridine is cleaved to fluorouracil and is incorporated into RNA. This incorporation is enhanced by thymidine. The result suggests that the major mechanism of action of the fluorouracil is due to its incorporation into RNA.

5-fluorouracil (FUra) is a potent anticancer agent extensively used in the clinics. It has been demonstrated that one of the predominant mechanism of action of this reagent is due to its incorporation into RNA [1-5]. The incorporation into RNA is augmented by thymidine leading to the enhancement of its biological activity [1-5]. FUra is also incorporated into DNA as fluorodeoxyuridine monophosphate (FdUMP) [6-12]. The biological consequence of its incorporation into DNA is not clear. It is also not clear whether the biological activity of FUra in the presence of thymidine i.e., enhancement of its anticancer activity, has anything to do with its incorporation to DNA. In this communication it is shown that the incorporation of 5-FdUMP to DNA is inhibited by thymidine, while the incorporation of the label to RNA is augmented.

MATERIALS AND METHODS

CD8 mice bearing 1st generation subcutaneous breast tumour transplants (about 1 gm) were used in this study. The mice were injected with 15 mg of thymidine or saline 30 minutes before administration of radioactivity. The animals were given 250 μCi of (6-^{3}H) 5-fluoro-2'-deoxyuridine (New England Nuclear) and 250 μCi of [^{32}P] Na_2HPO_4 intraperitoneally. The *in vivo* labelling was carried out for 60 minutes. The animals were sacrificed and the tumour tissue was excised and processed

Abbreviations used: FUra, 5-Fluorouracil; FdUR, 5-Fluoro-2'-deoxyuridine; FdUMP, 5-Fluoro-2'-deoxyuridine-5'-monophosphate.

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for radioactivity in nucleic acids [2]. The nucleic acid samples were run on Cs_2SO_4 density gradient to confirm the presence of label in DNA and RNA regions of the gradient. Equal volume of nucleic acid samples were mixed with saturated Cs_2SO_4 in 5 mM EDTA and were run in a 50 Ti rotor at 45,000 rpm for 60 hrs at 20°C. The gradients were collected into 30 equal fractions. Density of every 5th fraction was taken and absorbance of each fraction at 260 nm was recorded after diluting the sample in water (1 : 10). Fifty microgram of yeast total RNA was added to each fraction and the samples were precipitated with 10% cold TCA. The precipitates were filtered through nitrocellulose membranes, washed six times with 5 ml of 5% TCA and dried under infrared lamp. The samples were counted in NEN formula 949 scintilation fluid for estimation of [^{3}H] and [^{32}P] radioactivity. DNA estimation was done by Burton's diphenylamine reaction [13].

RESULTS AND DISCUSSION

The incorporation of [^{32}P] phosphate and [^{3}H] FdUR into TCA precipitable, alkali labile and alkali resistant nucleicacids (RNA and DNA) is presented in Table 1. The Cs_2SO_4 gradient profile of labelled nucleic acid is presented in Figure 1. It is clear from the table and the figure that FdUR is incorporated into DNA and RNA. The incorporation of the label into RNA is due to the conversion of FdUR to FUra by nucleoside phosphorylase [14] and subsequent utilisation of FUra for RNA synthesis. The thymidine treatment almost completely inhibits the incorporation of [^{3}H] FdUR to DNA, while augmenting the incorporation of tritium into RNA. The enhancement of incorporation into RNA is in agreement with earlier reports [1-4]. Under the conditions of the present experiment,

Table 1

Incorporation of [^{3}H] fluorodeoxyuridine and [^{32}P] phosphate to nucleic acids of mouse mammary tumour in presence and absence of thymidine

Treatment	CPM in DNA		Ratio	CPM in RNA		Ratio
	[^{3}H]	[^{32}P]	[^{3}H]/[^{32}P]	[^{3}H]	[^{32}P]	[^{3}H]/[^{32}P]
Saline	7503	32429	0.219	6011	221837	0.027
Thymidine	692	50482	0.014	14446	117220	0.123
	Fold inhibition		15.64	Fold stimulation		4.55

Fold stimulation or fold inhibition is calculated from [^{3}H]/[^{32}P] ratio thus measuring the synthesis of [^{3}H] nucleic acids against newly synthesized [^{32}P] nucleic acids. Specific activity of [6-^{3}H]-5-fluorodeoxyuridine was 5.8 ci/mmole. CPM is normalised for mg DNA of tumour.

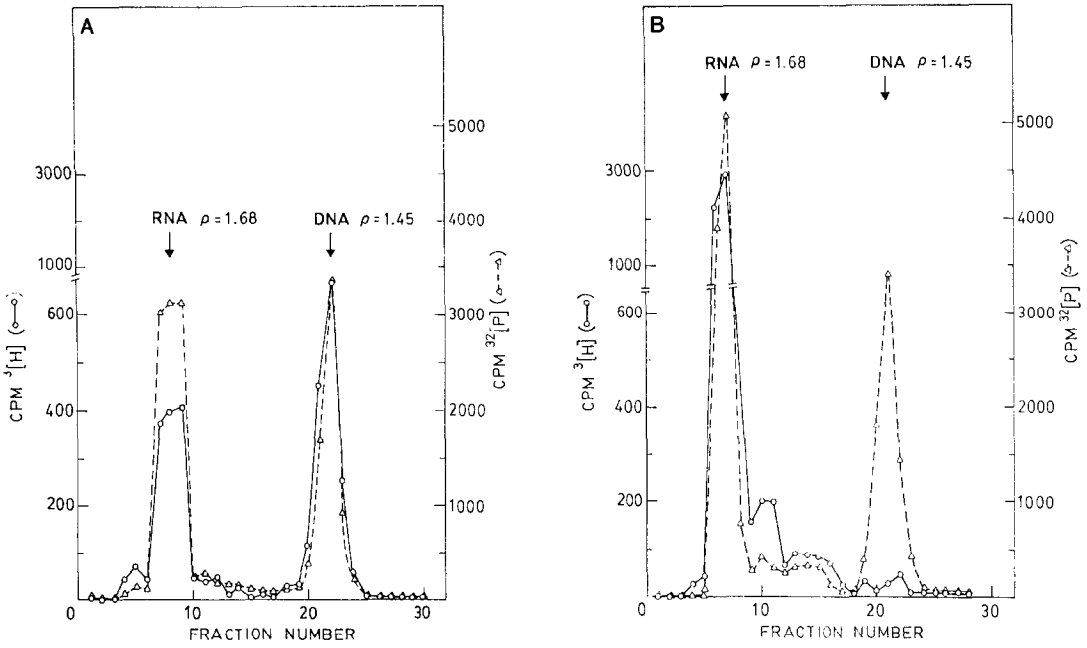

Fig. 1. CS_2SO_4 equilibrium density gradient profiles of nucleic acids of breast tumours of CD8 mice. The nucleic acids were labelled in vivo with [6-^{3}H] 5-fluoro-2'-deoxyuridine and [^{32}P] Na_2HPO_4.

A. Nucleic acids from saline treated animals.

B. Nucleic acids from animals treated with thymidine (500 mg/kg) half hour before labelling.

[^{32}P] incorporation into DNA was increased due to thymidine treatment (Table 1). Thymidine is expected to overcome the effect of the inhibition of the activity of thymidylate synthetase by FdUMP. The decreased ratio of [^{3}H] to [^{32}P] in DNA and the reverse in RNA on thymidine treatment indicates that there is augmentation of fluoropyrididine incorporation into RNA and inhibition of its incorporation into DNA. There are two possible reasons for the inhibition of incorporation of 5-fluorodeoxyuridine to DNA. Firstly thymidine could compete with fluorodeoxyuridine for the enzyme thymidine kinase, and possibly for deoxynucleotide kinases, and secondly TTP would compete with fluorodeoxy UTP for incorporation to DNA.

Earlier work has shown that thymidine treatment increases the chemotherapeutic effect of FUra [1-3, 5, 15]. The present data suggest that the incorporation of FUra into DNA could not have been the cause for the augmentation of its chemotherapeutic activity. The co-administration of thymidine also eliminates the possibility of inhibition of thymidylate synthetase as a cause for the biological activity of the drug. Therefore it is clear that incorporation of FUra to RNA is the major

reason for its chemotherapeutic activity, at least when it is given along with thymidine.

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