The inhibitory effects of 5-fluorouracil on the metabolism of preribosomal and ribosomal RNA in L-1210 cells in vitro

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Summary. Addition of 5FU to the culture medium of mouse L-1210 cells resulted in inhibition of the maturation process of ribosomal RNA precursors in vitro. In the presence of 10^{-8}M 5FU for 2 h, the 45S preribosomal RNA was processed to 32S preribosomal RNA, but 28S rRNA was not produced. The processing to 18S rRNA was intact at this drug concentration. Higher concentrations of 5FU for a longer incubation period affected the RNA processing more severely. At 10^{-6}M of the drug for 24 h the processing to 18S rRNA was also inhibited, in addition to the processing to 28S rRNA and 32S preribosomal RNA. When the cells were labeled with 14C-UR for 2 h following 3H-5FU at 10^{-6}M for 24 h, the radioactivities of newly synthesized RNA labeled with 14C-UR accumulated in the region of 45S and 32S preribosomal RNA, and no processing to 28S RNA was observed. Radioactivity corresponding to 3H-5FU did not persist in the preribosomal RNA region, because further maturation proceeded in the condition of depletion of 5FU after the long incubation period. Thus, inhibition of the processing of preribosomal RNA to 28S rRNA was not brought about by the accumulation of 5FU-substituted 45S preribosomal RNA, but by some other, yet unknown, mechanism.

Introduction

Since 5-fluorouracil (5FU) was first introduced in 1957 [4], it has been one of the most commonly used anticancer drugs in the clinical treatment of certain types of cancer [1, 10, 11, 13]. The main mechanism of the effect has been considered to be inhibition of DNA synthesis by one of the metabolites, FdUMP, through its covalent binding to thymidilate synthetase in the presence of N-methylene-tetrahydrofolate [8, 9]. On the other hand, 5FU was shown to be incorporated to various classes of cellular RNA at a substantial rate and produce structurally and functionally defective RNAs [3, 5-7, 10, 12, 16]. A number of reports have shown that 5FU also inhibits the metabolism of RNA, especially the processing of rRNA [2, 17-19]. Wilkinson et al. reported that the inhibition of ribosomal RNA maturation by 5FU in Novikoff hepatoma cells appeared to depend upon the incorporation of the analog into 45S rRNA precursor [17]. In mammalian cells, mature 28S and 18S rRNA are produced in the nucleolus from a common precursor by a common complex maturation process [18, 20]. The initial rRNA transcript product is a 45S molecule, which contains the sequence of both 28S and 18S rRNA and additional sequences whose functions have not yet been defined [19]. The 45S molecule is methylated in both sugar and base moieties either during or soon after its synthesis. After methylation, the 45S molecule is converted to a 41S molecule, which also contains the sequence for both 28S and 18S rRNA. The 41S molecule is cleaved to a 32S molecule and a 20S molecule, which are the immediate precursors of mature 28S and 18S rRNA, respectively [14]. 5FU is metabolized, phosphorylated, converted to FUTP, and incorporated into 45S ribosomal precursor uracil, and this precursor appears to be processed finally to mature 28S and 18S ribosomal RNA.

However, it has been reported that maturation of 5FU-containing RNA is severely inhibited at the 45S and 32S regions causing congestion of preribosomal RNA to occur [17-19]. The present experiments were designed to investigate the effect of 5FU on the processing of preribosomal RNA, particularly that of 5FU incorporation into the precursor molecule.

Materials and methods

Cells and incubation medium. L-1210 cells maintained in BDF1 mice were obtained 3 days after inoculation and introduced into the RPMI 1640 incubation medium supplemented with 10% fetal bovine serum and 10 μM 2-mercaptoethanol. The cells were grown at 37°C in suspension culture under an atmosphere of 5% CO2; 95% air.

Incorporation of 5FU into nucleic acids. L-1210 cells were cultured by plating 1 x 10^6 cells into Falcon dishes 60 mm in diameter and containing 5 ml medium in the presence of 1 μCi 3H-UR in various concentrations of 5FU, or 10^{-6}M 3H-5FU for 24 h, followed by 1 μCi 14C-UR for an additional 2 h. After the incubation, the cells were collected by centrifugation at 500 rpm for 10 min, and washed...
twice with ice-cold saline. The cells thus treated were used for RNA extraction.

**Extraction of RNA.** RNA was extracted by sodium dodecyl sulfate methods directly from the labeled cells without prior preparation of the nuclear or nucleolar fraction as an RNA source [19]. Briefly, the cell pellet was suspended by vortexing in buffer containing 10 μM sodium acetate (pH 5.1), 0.14 M NaCl, 0.1 M ethylenediamine tetraacetic acid-sodium salt (EDTA-Na) and polyvinyl sulfate-K (20 μg/ml). Then 0.1 ml 3% sodium dodecyl sulfate was added to the cell mixture to give a final concentration of 0.3%. An equal volume of water-saturated phenol solution (phenol : water : m-cresol, 7 : 2 : 1,8-hydroxyquinoline 1 g/liter) was added, and the mixture was shaken vigorously at 60°C for 5 min.

After cooling in an ice bath, the phases were separated by centrifugation and the phenol phase was removed and discarded. The aqueous phase and interphase were extracted again with another equal volume of phenol solution. After the centrifugation, the aqueous phase was transferred to an another tube and extracted again with an equal volume of the phenol phase. RNA was precipitated from the final aqueous phase with 2 vol 95% ethanol containing 2% potassium acetate at -20°C overnight. Precipitated RNA was collected by centrifugation, washed twice with 75% ethanol containing 2% potassium acetate, and dissolved in distilled water.

**Sucrose density gradient centrifugation.** RNA precipitated from the aqueous phase was collected by centrifugation, washed with 95% ethanol, and dissolved in a small amount of 0.05 M sodium acetate solution at pH 5.1. RNA thus prepared was placed gently on a 15-ml, 5%-30% linear sucrose gradient containing 0.01 M sodium acetate, 0.1 M NaCl, and 1 mM EDTA, and centrifuged in a Hitachi RPS 25.3 rotor at 22000 rpm for 18 h at 4°C. The gradient was fractionated with the aid of an ISCO Model UA5 density gradient fractionator with monitoring absorbance at 254 nm. The radioactivity was determined for each fraction.

**Determination of radioactivity.** To each 0.6 ml sucrose density fraction an equal volume of distilled water was added, after which 10 ml ACS II aqueous counting scintillant (Amersham Corp., UK) was added in a scintillation vial. The radioactivity was counted by means of separation windows for each isotope in a Beckman LS 150 liquid scintillation spectrometer. Appropriate corrections were made for the contamination of 14C radioactivity in the 3H window. With this technique, counting efficiency was almost constant throughout the gradient for each isotope.

**Results**

The effects of 5FU on the processing of preribosomal RNA of L-1210 cells are shown in Figs. 1 and 2. As indicated in Fig. 1, when the cells were incubated with 3H-UR for 2 h it was evident that 45S preribosomal RNA was well processed to 28S and 18S rRNA. Although separation of either the nuclear or the nucleolar fraction was omitted before the RNA extraction, our extraction procedure is considered to be satisfactory for the analysis of RNA metabolism.

![Fig. 1a-c. Processing of preribosomal RNA of L-1210 cells labeled with 3H-UR for 2 h in the absence or presence of 5FU.](image-url)