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Effect of 5-fluorouracil on Interleukin-2 expression

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Summary: The effect of 5-fluorouracil on the expression of IL-2 gene and the production of IL-2 protein has been studied using spleenic lymphocytes of rats. The IL-2 messenger RNA has been quantitated by dot-blots and IL-2 has been assayed by Con A-blast assay. The results show that there is up to 3.75 fold increase in the production of IL-2 message in 5-fluorouracil treated lymphocytes over Con A treated controls. There is also a concomitant increase in IL-2 protein production upon 5-fluorouracil treatment in rat lymphocytes.

The 5-fluorouracil (FUra) is an anticancer drug routinely used either singly or in combination with other anticancer agents in the treatment of various types of cancers (1). Three possible mechanisms of action for FUra have been proposed. First, the conversion of FUra to FdUMP, an irreversible inhibitor of thymidylate synthetase, results in the inhibition of DNA synthesis (2). Second, FUra residues get incorporated into DNA leading to fragmentation of DNA (3). Lastly, FUra via its conversion to FUTP is incorporated into RNA resulting in the altered functions of RNA (4-6).

In previous studies, we had observed that cytotoxic T lymphocytes (CTL) derived from FUra treated rats showed enhanced specific lysis towards target Yoshida ascities sarcoma cells compared to that of CTL's derived from control animals (unpublished observation). This indicates that perhaps FUra could alter the cytokine balance in the system. It has also been shown that the treatment of advanced colorectal cancer patients with FUra/folinic acid resulted in the generation of lymphokine-activated killer (LAK) cells from the peripheral blood mononuclear cells, showing that the treatment was not suppressive of IL-2 related lymphocyte responses (7). As a first step towards the elucidation of the effect of FUra on cytokine network, the effect of FUra on IL-2 has been studied.

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Materials and Methods

Isolation and culturing of lymphocytes: 2-4 month old Wistar rats were obtained from Central Animal Facility. Rats were killed by cervical dislocation, the spleens were aseptically removed and single cell suspension was obtained by straining through a wire mesh into modified RPMI 1640 (High Media, India) containing 2 mM glutamine, 10 mM HEPES, 5 x 10^{-5}M 2-mercaptoethanol, 50 units of penicillin/ml, 50 ug of streptomycin/ml and 10% fetal calf serum. The RBCs were lysed by Tris buffered ammonium chloride (Tris 10 mM, pH 7.2 and NH_4Cl 0.85%) and cells were washed twice with RPMI 1640 complete medium. The viability of the cells was greater than 85% as judged by trypan blue dye exclusion.

Preparation and analysis of RNA: Rat spleenocytes were cultured at a cell density of 10^7/ml (10^8 total cells) for 36 hours in 150 cm^2 flasks with Con A (5 µg/ml) and various concentrations of FUra ranging from 500 ng to 10 µg/ml. The lymphocytes were collected by centrifugation and washed with ice cold PBS pH 7.4. The total RNA was isolated by modified hot phenol-SDS method and stored in ethanol at -20°C (8).

For dot blot analysis, the total RNA samples (10 ug) from rat spleenocytes were spotted on 20 x SSC presoaked nitrocellulose filters using minifold filtration apparatus. The filters were baked at 80°C and prehybridised at 42°C for 4 hours. The blots were hybridised with human IL-2 cDNA probe (kind gift of Dr. T. Taniguchi, Osaka University, Japan). The cDNA probe was labelled by nick translation with [α - ^{32}P]dATP. Hybridization was carried out at 42°C for 36 hours in 50% formamide, 5 x SSPE, 5 x Denhardt's solution, 0.1% SDS and 200 ug/ml of Salmon sperm DNA. After hybridization, the blots were washed in 2 x SSC and 1 x SSC with 0.1% SDS. The filters were exposed at -70°C to Kodak X-ray films with intensifying screens. Quantitative analysis of dot blots were performed by measuring the spot intensities of autoradiogram using a scanning densitometer.

Interleukin-2 assay: The interleukin-2 activity was determined by T cell growth factor assay which measures the [^{3}H]-thymidine incorporation into Con A blasts (9). To prepare the blasts, spleenocytes of Balb/c mice were stimulated with 2.5 ug of Con A at a cell density of 5 x 10^6/ml in modified RPMI 1640 complete medium for 4 days. The lymphoblasts were washed with medium containing 40 mM α-methyl mannoside. 2 X 10^5 lymphoblasts per well were seeded into 96 well microtitre plates and incubated for 24 hours in modified RPMI 1640 complete medium, 10 mM α-methyl mannoside and $\log_2$ dilutions of samples to be tested in a final volume of 0.2 ml. The cells were pulsed with [^{3}H]-thymidine (1 µCi/ml) and TCA precipitable counts were monitored. The IL-2 activity is expressed in terms of units/ml with reference to standard human recombinant IL-2 (Sigma, U.S.A.)

Results and Discussion

In order to study the effect of FUra on IL-2 production, FUra treated spleenocyte RNA was probed with nick translated human cDNA for IL-2. Our initial results showed that FUra stimulated IL-2 mRNA expression at lower concentrations (0.5 µg to 5 µg/ml), while inhibiting the same at higher concentration (10 µg/ml) in Con A treated rat spleenocytes (Fig. 1). However the IL-2 mRNA content in cells

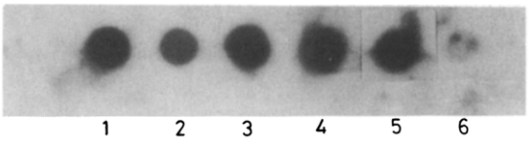


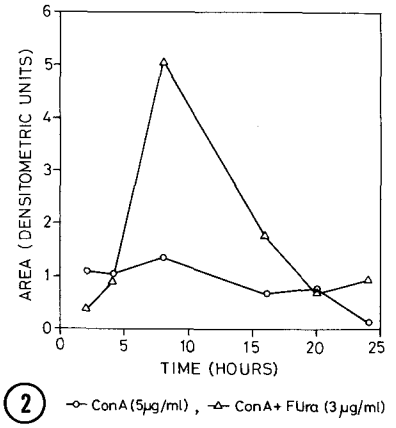
FIGURE 1. Effect of FUra on Interleukin-2 mRNA levels.

Rat spleenocytes were cultured with Con A and various concentrations of Fura, 36 hours later the levels of IL-2 mRNA were quantitated by RNA dot blots as described in materials and methods. Spot 1, Control; Spot 2, Con A (5 µg/ml); Spot 3-6, Con A (5 µg/ml) and FUra at 0.5, 3, 5, and 10 µg/ml, respectively.

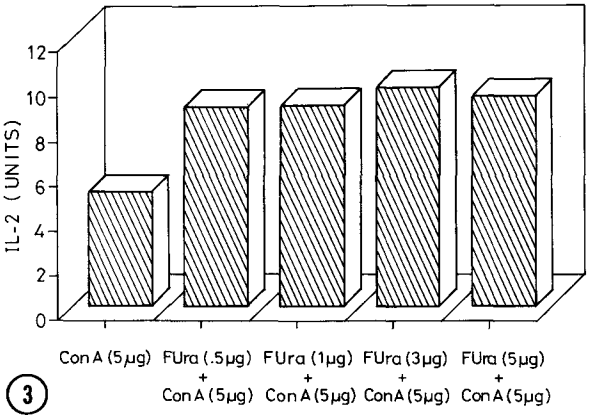
has increased only upto 38%. A time course study revealed that the IL-2 message can be increased by upto 3.75 fold on 8 hour treatment with drug. The effect declines on longer treatment (Fig. 2).

IL-2 synthesis by FUra treated rat spleenocytes was monitored by measuring the IL-2 biological activity in culture supernatants using Con A blast assay. The biological activity of IL-2 increases from 5 units to 10 units/ml as shown in Fig. 3.

The IL-2 RNA has a very short half life. It has been shown that an (A + U) rich untranslated region in various cytokine mRNA's including the IL-2 mRNA, imparts instability to mRNA (10, 11). In the 3' untranslated region of cytokine mRNA's (IL-1, IL-2, TNF, IFN's



(2)



(3)

FIGURE 2. Effect of FUra on the induction of IL-2 mRNA in rat spleenocytes.

The rat spleenocytes were cultured in the presence of Con A and FUra as described in materials and methods. At various time points, the levels of IL-2 mRNA were measured by densitometric readings of the RNA dot blots.

FIGURE 3. Effect of FUra on the IL-2 production.

The Interleukin-2 production by Con A and FUra treated rat spleenocytes was measured by Con A-blast assay as described in materials and methods.

etc), an 8 nucleotide consensus sequence **UUAUUUAU** has been identified, which is recognised by labile RNase/repressor (12). Therefore it is possible that substitution of 'U' with 'FUra' in this regulatory segment of IL-2 mRNA might result in its stabilization.

The IL-2 gene expression is controlled over a wide range by a labile repressor that disappears rapidly when the protein synthesis is blocked by inhibitors such as Cycloheximide (12), T-2 toxin, pactamycin and sparsomycin resulting in the superinduction of IL-2 mRNA. FUra decreases general protein synthesis by about 45.3% (unpublished observation). This might result in the decrease synthesis of labile repressor resulting in enhanced levels of IL-2 mRNA in FUra treated lymphocytes. Thus there are two possible reasons by which FUra could increase IL-2 production namely stabilizing the mRNA and inhibiting the synthesis of the repressor. The exact mechanism however remains to be worked out.

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