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PRELIMINARY COMMUNICATION

PREVENTION OF ANTITUMOR EFFECT OF 5-FLUOROURACIL BY HYPOXANTHINE

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5-Fluorouracil (FU) has been widely used in the clinical treatment of gastro-intestinal cancer [1]. The attempts to reverse the growth inhibition by FU were carried out by the addition of related metabolites in mammalian cells [2-4], bacteria [5] and fungus [6]. In mammalian cells, however, almost all the reports failed in a complete reversal of FU inhibition by normal metabolites.

We attempted to find the most effective compound to reverse FU inhibition using L5178Y a mouse leukemic cell line. As a result, hypoxanthine and adenine completely reversed FU cytotoxicity. The results suggest that hypoxanthine produces a block in the pathway leading to 5-fluorouridine monophosphate (FUMP) by phosphoribosyltransferase.

Approximately 5×10^4 cells were cultured in 1.0 ml of RPMI 1640 medium supplemented with 10% calf serum at 37°C with a CO_2 incubator; the number of cells was counted with a microcellcounter (Model CC-108, Toa Medical Electronic Co., Kobe, Japan). In reversal studies, FU and its nucleosides were used under the equi-effective concentration of IC_{90} (the concentration caused 90% growth inhibition of the control) and a normal nucleic acid metabolite at various concentrations was also added to the culture. IC_{90}s after 48-hours cultivation were 3.8×10^{-6} for FU, 7.6×10^{-8} for 5-fluorouridine (FUR) and 4.1×10^{-9} M for 5-fluoro-2'-deoxyuridine (FUdR), respectively.

As a result of reversal studies, hypoxanthine (20 μg/ml) was found to reverse the growth inhibition by FU to 96% of control which was completely overcome at a higher concentration as shown in Fig. 1. Adenine also reversed FU cytotoxicity as did hypoxanthine, but guanine did not. The growth inhibition by FU was reversed only to 20-40% by both thymidine and deoxyuridine, but not by pyrimidine analogues such as uracil [2, 4], uridine [4], thymine [2] and

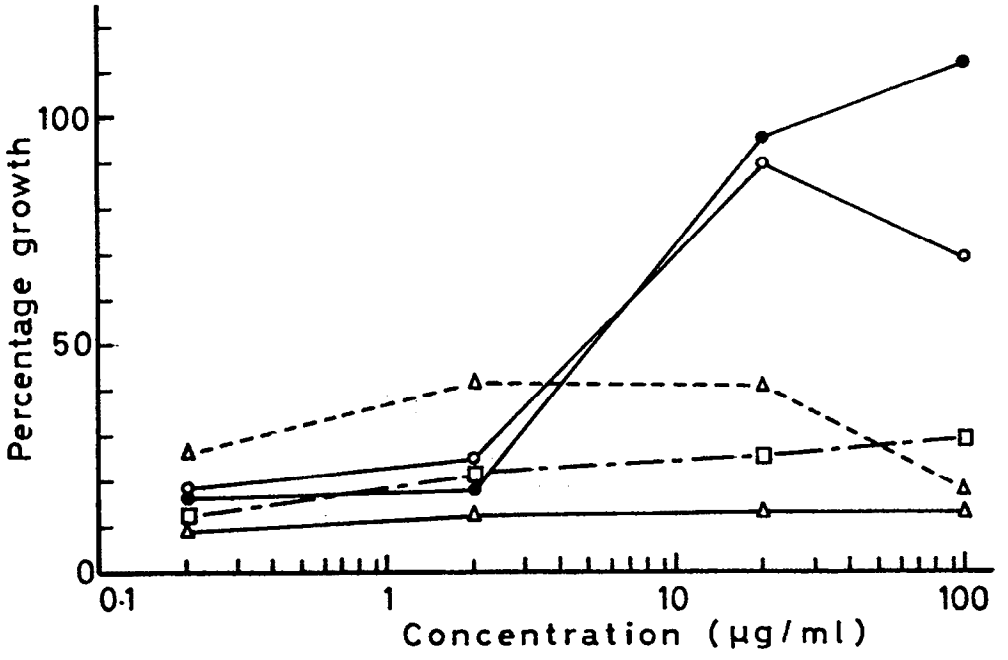

Fig. 1. Effects of several compounds on growth inhibition of L5178Y cells by FU (3.8×10^{-6} M). L5178Y cells (5×10^{4} cells/ml) were grown for 48 hours in a medium with the following compounds added at various concentrations. ●——● FU + hypoxanthine; o——o FU + adenine; Δ-------Δ FU + thymidine; □-·—·-□ FU + deoxyuridine; Δ——Δ FU + guanine.

orotic acid [4]. Hypoxanthine was not effective on the cytotoxicity of either FUR or FUdR. FUR cytotoxicity was completely overcome by uridine [2] and FUdR by both thymidine [2, 3] and deoxyuridine [2].

The change of generation time of L5178Y cells was examined under the above conditions. As shown in Fig. 2, FU (3.8×10^{-6} M) increased the generation time of L5178Y cells from 10.6 to 42.4 hours in the exponential growth phase for 48 hours after zero time. By further addition of thymidine (20 μg/ml) to the culture, the generation time was reduced to 19.0 hours. Furthermore, by adding hypoxanthine and adenine (20 μg/ml), the generation time was further shortened to 11.6 and 12.9 hours respectively, approaching that of control.

To clarify the differences between hypoxanthine and thymidine with respect to reversal of FU cytotoxicity, effects of both compounds on the cell growth after exposure to FU were examined. As shown in Fig. 3, toxicity in a short-term exposure (3 hours) to FU at a higher concentration (7.7×10^{-5} M) was not reversed by continuous exposure to hypoxanthine (20 μg/ml) after cell washing,

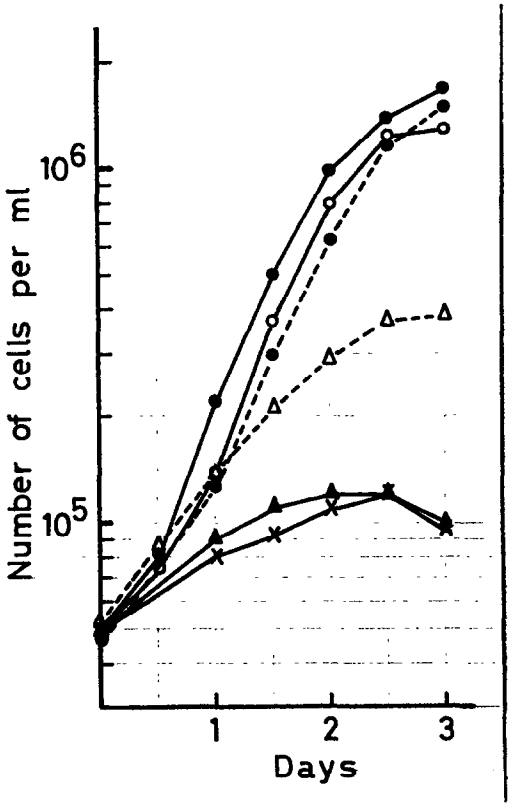

Fig. 2

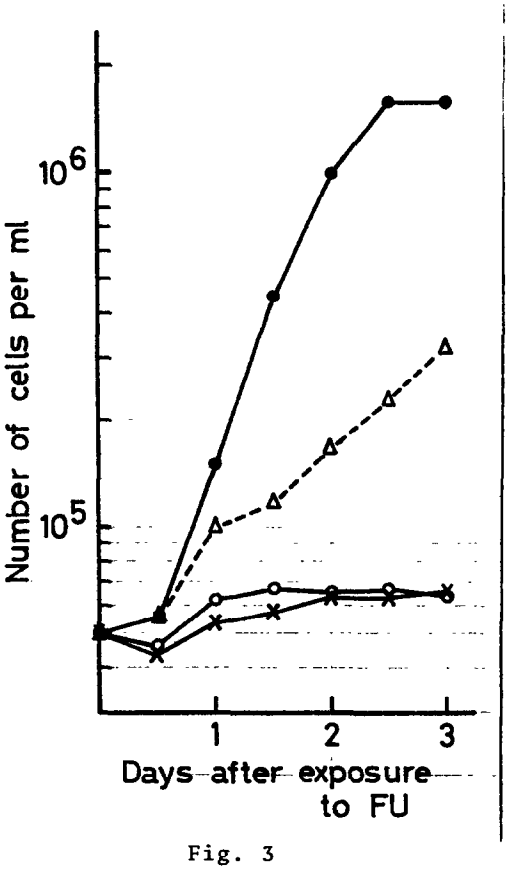

Fig. 3

Fig. 2. Effects of hypoxanthine, adenine, guanine and thymidine on growth inhibition by FU (3.8×10^{-6} M). All values are mean of duplicate cultures. ●——● control; o——o FU + 20 μg/ml hypoxanthine; ●------● FU + 20 μg/ml adenine; Δ----- Δ FU + 20 μg/ml thymidine; ▲——▲ FU + 20 μg/ml guanine; ✕——✕ FU only.

Fig. 3. Effects of hypoxanthine and thymidine on growth inhibition after exposure to FU (7.7×10^{-5} M) for 3 hours. ●——● no treatment; Δ-----Δ 20 μg/ml thymidine; o——o 20 μg/ml hypoxanthine; ✕——✕ no addition after FU.

but thymidine (20 μg/ml) reversed the FU toxicity and its extent was about equivalent to that observed in simultaneous incubation with FU [4]. This result suggests that the thymidine mode of protection against FU toxicity is different from that of hypoxanthine and that hypoxanthine inhibits FU activation in a co-existing state with FU.

In L5178Y cells reported here, the following inference concerning FU metabolism is suggested; i. e., FU is not metabolized to FUR and transformed directly to the ribonucleotide; 5-fluorouridine monophosphate (FUMP) by phos-

phoribosyltransferase [7, 8], because the inhibitory effect of FU was not reversed by uridine, but uridine completely overcame FUR cytotoxicity. On the other hand, FU was completely reversed by hypoxanthine which showed no effect on FUR cytotoxicity. From these results, hypoxanthine and adenine might bring about a phosphoribosylpyrophosphate(PRPP)-less state in cells with the result that FU might not be metabolized to FUMP.

Our findings were unexpected, and in the L1210/C cell line, we also found that hypoxanthine completely reversed the growth inhibition by FU. Further analysis of the reverse effect by hypoxanthine and adenine is now in progress.

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