Comparative Study of 5-Fluorouracil and Its Derivative, N\textsubscript{1}-(2'-'-Tetrahydrofuryl)-5-Fluorouracil on Endogenous Colony Forming Units in Spleens of Mice

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The effect of intraperitoneal injection of N\textsubscript{1}-(2'-tetrahydrofuryl)-5-fluorouracil (FT-207) which is metabolized to 5-fluorouracil (5-FU) in vivo, on endogenous colony forming units in the spleen (endogenous CFU-s) was observed and compared with that of 5-FU. The 8 \times 10^{-4} \text{ m eqv} of FT-207 injected in mice 20 minutes before X-rays irradiation of 450 rads suppressed the endogenous CFU-s count to about 60\% and about 5.5 \times 10^{-3} \text{ m eqv} of 5-FU had the same effect. The suppression of FT-207 continued over 120 minutes and increased gradually. The effect of 5-FU was strong shortly after the injection but faded out for 120 minutes. FT-207 had almost no effect on the recovery constant (Blair) of endogenous CFU-s for 3 days, whereas 5-FU reduced seriously, especially for the first 2 days. The differences in the effects of both compounds could not be explained on the base of the only differences in the metabolism of both compounds.

INTRODUCTION

A compound, N\textsubscript{1}-(2'-tetrahydrofuryl)-5-fluorouracil (FT-207) was synthesized by Hiller et al.\textsuperscript{3} in 1967, as an anticancer compound. The molecular weight is 200.17, a

\begin{align*}
\text{FT-207} & \quad \text{5-FU} \\
\text{N}_{1}(2'-\text{tetrahydrofuryl})-5\text{-fluorouracil} & \quad 5\text{-fluorouracil}
\end{align*}

![Fig. 1. Chemical structures of FT-207 and 5-FU.](image-url)
molecular formula is C₄H₉O₂N₂F and the compound is a derivative of 5-fluorouracil (5-FU). The structures of FT-207 and 5-FU are shown in Figure 1.

A furan removes from FT-207 in vivo and FT-207 is converted to 5-FU as an anticancer compound mainly in the liver, which is essentially converted to 5-fluorouridine monophosphate (FdUMP) as a cytotoxic metabolite. For this reason, FT-207 is a masked form of 5-FU and the main anticancer activity of FT-207 in vivo is explained as that of 5-FU. Since 5-FU produced through the metabolic passway of FT-207 is much than that reduced through its own metabolic passway mainly in the intestine, the liver, the spleen and the kidney, 5-FU is supplied continuously and increases gradually in the body after the administration of FT-207.

Clinical trials have shown that FT-207 is an effective anticancer compound which causes hardly any damage to the peripheral blood system of patients. Single or fractionated intraperitoneal injections of FT-207 in rats affected the peripheral blood cells much less than single or fractionated injections of 5-FU, while it is reported that the repeated injections of 5-FU reduced remarkably the peripheral blood cell count. FT-207 was also reported to have less effect on colony forming units and their differentiation in mice. The slight effect of FT-207 on peripheral blood cells and hemopoiesis is one of beneficial properties of FT-207 as an anticancer compound.

The present report deals with the analysis of the effect of FT-207 on endogenous CFU-s as a hemopoietic stem cell system in mice, especially on the recovery processes of CFU-s after irradiation, in comparison with the effect of 5-FU, from which FT-207 is derived.

MATERIALS AND METHODS

Chemical compounds: FT-207 and 5-FU were obtained from Taiho Pharmaceutical Co. Ltd. and the purity of both compounds was confirmed by fluorophotometry. The doses of FT-207 and 5-FU were 10⁻²–10⁻³ m eqv per mouse. Both compounds were dissolved in physiological saline in a bath at 20°C for 24 hours. One ml of solution was injected intraperitoneally. One ml of physiological saline was injected in the control mice.

Mice: A closed colony of male mice dd/YF was used. The mice weighed 23.8±1.2 g (mean±SD) at the start of experiment. They were fed a standard diet with free access to drinking water and kept in plastic cages, each housing five animals at a temperature of 20°–22°C. Mice were housed one week prior to the start of experiment so that they could adapt to the conditions in the animal cages. The experiments started at the age of 65-70 days old. Food and water were replenished daily at 10:00 a.m.. Fifteen to 20 mice were used in each points of experiment.

Irradiation: A X-ray apparatus for animal experiment was used. The physical factors were 190 kvp, 24 mA with 0.5 mm Cu and 1.0 mm Al of added filters. The dose rate was 50 rads per minute at 55 cm. The dose and dose rate were controlled
by use of Victoreen Radocon Dosimeter during irradiation.

Counting endogenous CFU-s: The techniques of counting were described in the previous reports. The properties of endogenous CFU-s as a stem cell in hemopoiesis were analyzed in detail by the author and reported in a previous report.

Calculation of the recovery constant \( \beta \): The \( \beta \) value can be calculated according to Blair's equation, \( I_t = I_0 e^{-\beta t} \), where \( I_0 \) is the initial injury, \( I_t \) is the residual injury at the \( t \) days after irradiation to induce \( I_0 \). Usually, \( I \) is expressed as radiation dose required to induce a critical injury \( I \) and the radiation dose is obtained by use of divided irradiation method. In the present report, this method was used and the initial dose was 350 rads and a present critical level was 700 rads to give 0.84 (mean) of endogenous CFU-s count. The second doses were 350-600 rads with dose interval of 50 rads. The detail in calculation of \( \beta \) was presented in the previous report.

RESULTS

Effect of FT-207 and 5-FU depended on the injected

Mice were irradiated with 450 rads, 20 minutes after the injection of both compounds in doses of \( 10^{-6}-10^{-3} \) m eqv per mouse. The endogenous CFU-s count in the control mice were 7.4±1.7 (mean±SD). The count decreased in mice injected with 5-FU in the range of \( 0.6 \times 10^{-4}-5 \times 10^{-4} \) m eqv. The reduction in the count depended on the dose. In the case of FT-207, the counts were slightly smaller than the control in the range of \( 2 \times 10^{-4}-2 \times 10^{-3} \) m eqv. The results are shown in Figure 2. The
curves in Figures 2 and 3 were drawn by hand, because it is less meaningful statistically to draw the curves using a computer in this kind of experiment.

**Effect of time interval between injection and irradiation**

Mice were irradiated with 450 rads 5-120 minutes after injection of FT-207 (10^{-3} m eqv) or 5-FU (10^{-4} m eqv). The effect of 5-FU was strongest 5 minutes after injection and faded out when the time interval reached 120 minutes but the effect of FT-207 increased gradually with increasing the time interval. 5-FU was more effective than FT-207 when the time interval was less than 20 minutes but when the time interval was 120 minutes, FT-207 was more effective than 5-FU. The results are shown in Figure 3.

![Graph showing effects of FT-207 and 5-FU on endogenous CFU-s](image)

**Fig. 3.** Effects of FT-207 and 5-FU on endogenous CFU-s depended on time interval between injection and irradiation.

- Dose of FT-207: 10^{-3} m eqv per mouse.
- Dose of 5-FU: 10^{-4} m eqv per mouse.
- Radiation dose: 450 rads.
- Vertical bar: SD.

**Effect of both compounds on the recovery constant β of endogenous CFU-s**

One ml of physiological saline, FT-207 (10^{-3} m eqv) or 5-FU (10^{-4} m eqv) was injected immediately after the initial irradiation. The time intervals between the initial and second irradiation were one, two and three days. The results were listed in Table 1. The endogenous CFU-s count to reduce for 2 days in mice injected with 5-FU and the recovery constant β could not show for these time intervals. FT-207 did not reduce the β value comparing that of control mice.
DISCUSSION

Endogenous CFU-s which can be observed in the spleen of irradiated mice represents a kind of stem cells in the hemopoiesis of mice and the effects of chemical compounds on some part of hemopoietic processes can be expressed in terms of their effects on the endogenous CFU-s count. These points have been discussed in the previous report. In the present report, the effects of FT-207 and 5-FU on hemopoiesis are compared, each other.

Both 5-FU and FT-207 sensitized the endogenous CFU-s to radiation as shown in Figures 2 and 3. But the dose of FT-207 needed to cause about 60% reduction of the endogenous CFU-s count is roughly 14.5 times higher than that of 5-FU, that is, $8 \times 10^{-4} : 5.5 \times 10^{-5}$. This ratio presents that the effect of FT-207 is extremely smaller than that of 5-FU. As shown in Fig. 3, the effect of FT-207 increased with increasing the time interval after injection and continued over 120 minutes. On the contrary, the effect of 5-FU faded out after 120 minutes. The difference in effects of both compounds seems to be based on the difference in the metabolism in vivo, since 5-FU is converted with comparatively high rate through the metabolic passway but the conversion from FT-207 to 5-FU continues in comparatively long term. According to the data using rabbits injected intravenously with FT-207, the concentration of 5-FU in blood was almost constant for 8 hours, while the concentration of 5-FU in rabbits injected with 5-FU was actually zero 2 hours after injection.

There is one problem here, though the quantitative changes of both compounds through the metabolic passways could explain the present data in Figures 2 and 3. If 5-FU affects only on cells in cycle as shown in many in vitro experiments, the serious reduction of the count shown in figures can not be explained, since the proliferation size of the endogenous CFU-s is less than 25%. The transfer of the endogenous CFU-s from the rest phase to the cycle phase after irradiation can not be observed shortly after irradiation when the compounds remain in the amount to enough to have these activity. 5-FU affects on cells in the rest phase to some extend. This is one of causes to induce such serious reduction.

There are various unphysiological processes including the disturbances of various metabolic passways between the treatment and the endpoints in all in vivo experi-

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Table 1

<table>
<thead>
<tr>
<th>period of observation (day)</th>
<th>$\beta$</th>
<th>control</th>
<th>FT-207 treated</th>
<th>5-FU treated</th>
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<tr>
<td>1</td>
<td>0.197</td>
<td>0.281</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.117</td>
<td>0.120</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.168</td>
<td>0.195</td>
<td>0.160</td>
<td>-</td>
</tr>
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</table>
ments. For this reason, the effects of both compounds can occur through not only the processes affecting directly on the cells which are observed in the in vitro experiment but also various other processes. This could be one of causes to induce the serious reduction.

The present data is different from those on leukemic cells in vivo, where the effect of 5-FU continued for a long term.\textsuperscript{19} In the causes to induce such difference, the difference in the proliferation size of both cells could be included, since the difference in proliferation size seems to be large in both populations.

The effects of both compounds on the recovery constant are interesting. The recovery constant indicates the rate of reduction of injury per day in terms of the radiation dose.\textsuperscript{14,16} The $\beta$ value of control mice on the first day was similar to the previously reported value.\textsuperscript{11} The reason that the $\beta$ value on the second day in both control and FT-207 treated mice were smaller than those on the first day, is unclear. A kind of synchronous change in radiosensitivity of the endogenous CFU-s to the second irradiation in mice for 48 hours after the first irradiation, as observed in comparatively short period,\textsuperscript{13} FT-207 has almost no effect on the recovery constant $\beta$ during the period of observation, as shown in Table 1. However, 5-FU has a marked effect during the same period, especially the first and second days. The results were similar to that of leukemic cells.\textsuperscript{18} As the overall metabolism of 5-FU in the irradiated body is practically same as that in the unirradiated body\textsuperscript{20} and the population size of cycling CFU-s immediately after irradiation does not increase,\textsuperscript{15} that is, there is the difference in the population size of both kinds of cell (leukemic cells and endogenous CFU-s) in cycle, the similarity might be based on some mechanisms concerned with the first stage of radiation damage.

In the observation of effects of both compounds injected in mice before irradiation, the contribution of effects of compounds administered immediately after irradiation to the former is considered. The present data show that the observed effect of 5-FU injected shortly before irradiation (Fig. 3) is contributed by the effect of 5-FU injected immediately after irradiation (Table 1), but the effects of FT-207 injected shortly before irradiation is not contributed by the effect of FT-207 injected immediately after irradiation. The difference seems to be hardly explained on the base of the only difference in the metabolic passway of both compounds (Fig. 3, Table 1).

The relationship between the recovery constant and the recovery from damages after irradiation should be noted. In the present report, the fractionation of irradiation with the time interval of more than 24 hours and the modification of the conditions after irradiation are combined. The recovery constant could be an overall expression of recovery processes including the recovery from sublethal damage and the recovery from potentially lethal damage. The injections of both compounds were carried out within five minutes after irradiation and within 15 minutes after the start of irradiation. For this reason, the effect of 5-FU on the recovery constant occurred shortly after irradiation but the effect of FT-207 did not occur in a short time after irradiation.
The $\beta$ value of FT-207 treated mice seems to be slightly larger than that of control mice. Though the difference does not seem to be significant, the continuously supplied 5-FU as a metabolite of FT-207 might stimulate the recovery of potentially lethal damage as reported by Hahn et al. \cite{11}.

The present data could be partially explained with the papers reported by many authors but not a few part of the present data remains to be explained, though both compounds, especially FT-207 is already used in clinic as an effective anticancer compound. The problems on the metabolisms of both compounds, their toxicities to various tissues and organs including hemopoietic system, target sites of both compounds on endogenous CFU-s, the effects of continuous exposure of both compounds with comparatively low dose on hemopoietic system and so on, should be analyzed in future.

REFERENCES


