A COMPARATIVE STUDY ON THE CARDIOTOXICITY OF 5-FLUOROURACIL AND FTORAFUR IN RABBITS

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Abstract—Intravenous administration of 5-FU 15 mg/kg significantly lowered the blood pressure 17 hours after, while ftorafur 20 mg/kg did not alter the pressure. Ftorafur 60 mg/kg significantly lowered the pressure 17 hours after the drug administration. In a large number of rabbits treated with 5-FU 15 mg/kg or ftorafur 60 mg/kg, the ventricular fibrillation spontaneously produced within 17 hours after the treatment. Adrenaline 5-50 μg/kg was injected into an ear vein in a stepwise manner, to assess whether or not the drug would induce a ventricular fibrillation. Pretreatment with 5-FU 15 mg/kg „sensitized” the ventricle to adrenaline-induced ventricular fibrillation, while ftorafur 20 and 60 mg/kg did not. The time required to produce ventricular fibrillation after a large dose (200 μg/kg) of ouabain was significantly shorter in 5-FU 15 mg/kg and ftorafur 60 mg/kg treated (17 hours before) groups than in the control group, while in the ftorafur 20 mg/kg treated group, the time was much the same as seen in the control group. These findings indicate that the cardiotoxicity of ftorafur in rabbits may be weaker than that of 5-FU.

In preliminary work, we found that i.v. administration of 15 mg/kg of 5-fluorouracil (5-FU) frequently produced sudden death in consequence of ventricular fibrillation in conscious rabbits (1). The ventricular fibrillation usually occurred within 24 hr after the administration. Intravenous administration of adrenaline frequently produced ventricular fibrillation in the 5-FU treated rabbits. Also, the time required to produce ventricular fibrillation by continuous infusion of ouabain was markedly shortened in the 5-FU treated rabbits (2). In the present work, a comparison of the effects of 5-FU and ftorafur was made with regard to the cardiotoxicity in rabbits.

MATERIALS AND METHODS

Albino rabbits of either sex, weighing 2.0–3.0 kg, were fed with a standard cube diet (CE-2, Central Laboratory of Experimental Animals, Japan) and water was provided ad libitum. 5-FU or ftorafur was injected into the ear vein. The administered doses were 15 and 30 mg/kg of 5-FU, and 20, 40, 60 and 90 mg/kg of ftorafur. The administered doses of 5-FU and ftorafur were chosen with reference to the doses used in clinical practice, in which 12–15 mg/kg and 20 mg/kg of 5-FU and ftorafur have been i.v. administered to patients, respectively. The survivors at 17 hr after the injection were anesthetized with pentobarbital sodium (35 mg/kg, i.m.) and fixed in the supine position.

The blood pressure of right common carotid artery and II-lead ECG (time constant 2 sec) were recorded using a polygraph (Nihon Kohden RM 150) and monitor oscilloscope (Nihon Kohden VC-6). Adrenaline in the
doses of 5, 10, 20, 30, 40 and 50 μg/kg was infused in a stepwise manner into an ear vein to assess whether fatal ventricular fibrillation would occur. The injection time was about 10 sec and the intervals between administrations were about 20 min. Also, a large dose (200 μg/kg; the injection time was about 10 sec) of ouabain was infused into the jugular vein and the time required to produce ventricular fibrillation was compared among the control, 5-FU and ftorafur treated groups. The obtained results were analyzed by Student’s t-test.

Drugs used were 5-fluorouracil (5-FU, Kyōwa Hakko Co.), ftorafur (N1-(2’-tetrahydrofuryl)-5-fluorouracil, Taihō Yakuhin Co.), adrenaline hydrochloride (Sankyo Co.) and ouabain (g-strophanthin, Merck). 5-FU and ftorafur were dissolved in tris-aminomethane solution (84.7 mg/ml) and adjusted to pH 8.2–8.6.

RESULTS

1. Acute toxicity: The rate of death at 17 hr after intravenous administration of various doses of 5-FU, ftorafur and tris-aminomethane solution (equi-volume used in 90 mg/kg of ftorafur) is shown in Table 1. When the rabbits were about to die, they suddenly struggled and appeared to be in shock. At this time, the chest of the animals was quickly opened and ventricular fibrillation was observed. In the 5-FU 30 mg/kg treated group, ventricular fibrillation occurred in all six animals. The attack was frequently observed several hr after the injection. The intravenous administration of 20 or 40 mg/kg of ftorafur produced no remarkable change of the behavior. However, the intravenous administration of 60 mg/kg of ftorafur was lethal in 6/21 rabbits, due to the sudden appearance of ventricular fibrillation. The intravenous administration of 90 mg/kg of ftorafur was lethal in 6/8 rabbits. Ventricular fibrillation occurred in 4 but the other 2 died after repeated occurrence of clonic convulsion. The intravenous administration of tris-aminomethane solution (equi-volume of the solution of ftorafur 90 mg/kg), which used as vehicle had little effect on the animal behavior.

2. Effects of 5-FU and ftorafur on adrenaline-induced ventricular fibrillation: The blood pressure of the animal group treated with 5-FU (15 mg/kg, 17 hr before) was significantly lower than that of the control group. The blood pressure of animal group treated with ftorafur 20 mg/kg (17 hr before) was much the same with that of the control group. However, the blood pressure of the animal group treated with ftorafur 60 mg/kg (17 hr before) was significantly

| Table 1. Comparison of the rate of death* from intravenous treatment of 5-FU and ftorafur |
|---------------------------------|----------------|-----------|
| Treatment                      | Death**       |           |
| 5-FU                           |               |           |
| 15 mg/kg                       | 6/24          |           |
| 30 mg/kg                       | 6/6           |           |
| Ftorafur                       |               |           |
| 20 mg/kg                       | 0/6           |           |
| 40 mg/kg                       | 0/6           |           |
| 60 mg/kg                       | 6/21          |           |
| 90 mg/kg                       | 6/8           |           |
| Control (equi-volume of the solution with ftorafur 90 mg/kg) | 0/6 |           |
| * within 17 hr after the treatment |
| ** Number of rabbits died/Number of rabbits used |
lower than that of the control group. A tall, flat or reversed T-wave was often observed 17 hr after injection of 5-FU 15 mg/kg. On the other hand, ftorafur 20 mg/kg produced no remarkable change of ECG, but in a dose of 60 mg/kg produced similar ECG changes to those induced by 5-FU 15 mg/kg in some cases (Fig. 1). In two animals treated with ftorafur 60 mg/kg, ventricular fibrillation spontaneously occurred after the fixation in supine position and under anesthesia.

Adrenaline, 5, 10, 20, 30, 40 and 50 μg/kg was successively injected, in a stepwise manner, into the ear vein in these groups. Out of six animals treated with 5-FU 15 mg/kg, 1, 1, 2, and 2 animals died due to the ventricular fibrillation induced by the injection of 5, 20, 30 and 50 μg/kg of adrenaline, respectively. The ventricular fibrillation induced by adrenaline appeared during periods when the blood pressure was elevated, in some cases, but, in others, this fibrillation appeared after the pressure had returned to the previous level. On the other hand, the adrenaline challenge did not produce ventricular fibrillation in the ftorafur 20 mg/kg treated group. However, in 1/9 treated with ftorafur 60 mg/kg, adrenaline 30 μg/kg produced ventricular fibrillation. In two survivors treated with ftorafur 90 mg/kg ventricular fibrillation did not occur with the adrenaline challenge. The vehicle, which was equi-volume with the solution of ftorafur 90 mg/kg, had no effect on the blood pressure.
Also, the adrenaline challenge did not produce ventricular fibrillation in the vehicle treated group (Table 2). The pressor response to adrenaline was suppressed in 5-FU 15 mg/kg and ftorafur 60 mg/kg treated groups (Fig. 2).

3. Effects of 5-FU and ftorafur on ouabain-induced ventricular fibrillation: Intravenous administration of a large dose (200 μg/kg) of ouabain produced a transient bradycardia, ventricular arrhythmia and ventricular fibrillation, in that order. The time required to produce the ventricular fibrillation after the ouabain injection was remarkably short in 5-FU 15 mg/kg treated group, compared with that of the control group. On the other hand, the difference in the time required to produce the ventricular fibrillation between the control group and ftorafur 20 mg/kg treated group was not statistically significant. However, increase in the dose of ftorafur to 60 mg/kg significantly shortened the time to produce fibrillation. The blood pressure assessed just before the ouabain injection was also markedly low in 5-FU 15 mg/kg treated and ftorafur 60 mg/kg treated groups (Table 3).

### Table 2. Incidence of ventricular fibrillation induced by i.v. administration of adrenaline in 5-FU and ftorafur treated rabbits

| Adrenaline (μg/kg, i.v.) | Control | 5-FU (15 mg/kg, 17hr) | ftorafur (20 mg/kg, 17hr) | ftorafur (60 mg/kg, 17hr) |
|--------------------------|---------|-----------------------|---------------------------|---------------------------|
| 5                        | 0/6     | 1/6                   | 0/6                       | 0/9                       |
| 10                       | 0/6     | 0/5                   | 0/6                       | 0/9                       |
| 20                       | 0/6     | 1/5                   | 0/6                       | 0/9                       |
| 30                       | 0/6     | 2/4                   | 0/6                       | 1/9                       |
| 40                       | 0/6     | 0/2                   | 0/6                       | 0/8                       |
| 50                       | 0/6     | 2/2                   | 0/6                       | 0/8                       |
| **Total**                | 0/6     | 6/6                   | 0/6                       | 1/9                       |

Blood pressure (mmHg): 5-FU: 120.0±7.1, 60.0±9.9**, 115.8±5.4, 88.3±7.8**

5-FU: (5-fluorouracil), ftorafur: (N1-(2'-tetrahydrofuryl)-5-fluorouracil). *Number of rabbits with evidence of induced fibrillation/Number of rabbits challenged. Firstly, 5 μg/kg of adrenaline was injected in each six controls, 5-FU 15 mg/kg and ftorafur 20 mg/kg treated or in nine ftorafur 60 mg/kg treated rabbits, and then 10, 20, 30, 40 and 50 μg/kg of adrenaline were injected in a stepwise manner in the remaining, in which fatal ventricular fibrillation did not occur. **Significant difference from the mean of control (P<0.01).
DISCUSSION

Since Levy (3) reported that chloroform inhalation produced a "sensitization" of the myocardium to adrenaline-induced ventricular fibrillation, many compounds, such as unsubstituted or halogenated hydrocarbons were found to have such "sensitizing" property (4). For example, acute administration of alpha-phenoxy-alpha-dimethylaminomethyl propiophenone hydrochloride (U-0882) (5) or 2,4,5-triphenyl-2-imidazoline hydrochloride (amarine) (6) "sensitizes" the ventricle to adrenaline-induced fibrillation. These sensitizations including those of hydrocarbons were usually elicited 15 to 120 sec after the drug administration. However, the "sensitization" induced by 5-FU 15 mg/kg required more than several hr. Thus, 5-FU differs from other well-known "sensitizing" agents. Marshall and Lewis (7) also reported that 4,4'- (isopropylidenedithio) bis [2,6-ditert-butylenol] (probufol) "sensitized" the ventricle to adrenaline-induced fibrillation only after a long-term repeated administration (a few or several weeks) of the drug. 5-FU shortened the time required to initiate the ventricular fibrillation induced by i.v. administration of a large dose of ouabain. Although ouabain has various effects on the myocardium such as inhibition of Na+, K+-ATPase, one of the reasons for the synergistic action may be due to the "sensitization" of the ventricle induced by 5-FU to endogenous catecholamine as the sympathetic nervous system plays an important role in the arrhythmogenic action of digitalis drugs (8). Toxic doses of ouabain could protect against U-0882-adrenaline fibrillation but not against hydrocarbon-adrenaline fibrillation (5). Thus, 5-FU-adrenaline fibrillation also differs from U-0882-adrenaline fibrillation.

The present study demonstrated that the cardiac toxicity of ftorafur was remarkably weaker than that of 5-FU. The spontaneous ventricular fibrillation often appeared in ftorafur 60 mg/kg treated rabbits, but the drug did not "sensitize" the ventricle to adrenaline-induced fibrillation. However, the ECG pattern elicited by a large dose (60 mg/kg) of ftorafur resembled that of 5-FU. The hypotension and suppression of pressor response the adrenaline were observed in the 5-FU 15 mg/kg and ftorafur 60 mg/kg treated groups. Namely 5-FU "sensitized" the myocardium to adrenaline-induced ventricular fibrillation, but rather suppressed the pressor response to adrenaline. 5-FU may be act on not only the cardiac, but also the vascular system. In respect of the pressor response to adrenaline, probucol showed neither an antagonism nor an enhancement (7). Chloroform, which sensitizes the myocardium to adrenaline-induced ventricular fibrillation, depressed the contractility of cardiac muscle (4). Further study should elucidate the relationship between the antitumor and the "sensitizing" properties of

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Table 3. Times required to produce ventricular fibrillation by ouabain (200 µg/kg, i.v.) in 5-FU and ftorafur treated rabbit

|                | Blood pressure (mean±S.E. mmHg) | Time to produce V.F. (mean±S.E. sec) |
|----------------|---------------------------------|-------------------------------------|
| Control        | 117.5±3.4                       | 282.5±47.2                          |
| 5-FU 15 mg/kg  | 67.5±6.3**                      | 68.8±11.8**                         |
| Ftorafur 20 mg/kg | 128.3±7.8                       | 231.7±21.1                          |
| 60 mg/kg       | 85.8±6.6**                      | 119.5±37.1                          |

Ouabain was injected into jugular vein 17 hr after treatment of each drug. Each group comprised 8 rabbits. Significant difference from the mean of control (*P<0.02, **P<0.01).
5-FU. The reason for the marked species difference with regard to the cardiac toxicity of 5-FU (9) is unknown.

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