STUDIES ON DISTRIBUTION OF ORAL 5-FLUOROURACIL FOR TREATING GASTRIC CANCER

2) COMPARISON OF THREE ORAL FORMULATIONS

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In vivo metabolism was studied on three kinds of formulations, dry syrup, tablet and emulsion of 5-FU in patients with malignant tumor and normal dogs. As a result, the following conclusions were obtained :

i) The 5-FU concentration in blood of the peripheral vein in human reached the peak levels at 15 minutes after administration of dry syrup and tablet, and thereafter it showed a pattern to decrease rapidly, however in case of emulsion the concentration reached a peak level which was about 1/4 of the peak levels of the above-mentioned two formulations at 30 minutes after administration and thereafter tended to decrease gradually.

ii) The 5-FU concentrations in the gastric mucosa in normal dogs showed the peak levels at 20 minutes after administration of dry syrup, at 40 to 90 minutes after tablet, and at 40 minutes after emulsion, all of which gradually decreased throughout 6 hours, and were well absorbed and remained in the tissues.

iii) The 5-FU concentration in the blood of the portal vein in normal dogs was higher than that of the peripheral vein with all three formulations.

iv) In case of emulsion, the 5-FU concentrations in the lymph of the thoracic duct in normal dogs were able to be determined at each time of blood collection throughout 180 minutes after administration (peak : at 30 minutes after administration), but the concentrations were out of the measurable limit all the time with dry syrup and tablet. This result proved that emulsion is easily directed to the lymph.

INTRODUCTION

Oral antineoplastic agents among the cancer chemotherapies for advanced gastric cancer are expected because they not only enable to visit to hospital as outpatients, but also contact directly to the cancer lesion of the stomach[1~3]. Presently three formulations, dry syrup, tablet and emulsion have been prepared in order to enhance of anti-tumor effect and to decrease side effects. Thus, in order to examine the characteristics of each formulation, comparative studies have been conducted on kinetics of dry syrup, tablet and emulsion in the blood and stomach of humans and dogs.

I. MATERIALS AND METHODS

1. Determination of 5-FU concentration in venous blood of patients with malignant tumor

a. Subject

The study with tablet was conducted on 6 in-patients with gastrointestinal cancer and that with emulsion was conducted on 6 in-patients without passage disturbance, hepatic metastasis or renal and cardiac diseases.

b. Method of administration

Either 200 mg of 5-FU tablet or 5-FU emulsion was given to the same patient at 30 minutes after breakfast and the blood was collected from the peripheral vein.

2. Determination of 5-FU concentration in blood, lymph of the thoracic duct, gastric mucosa and various organs of normal dogs

a. Dogs

The study with tablet and emulsion was conducted in adult 6 mongrel dogs (11±3 kg, B. W.).

b. Method of administration

In case of 5-FU tablet, (1) 5-FU tablets (100 mg or 200 mg) were grasped by forceps for biopsy and inserted into the stomach with endoscopy under anesthesia to determine the 5-FU concentration in the blood and lymph of the thoracic duct, and (2) immediately after 5-FU (100 mg) was compulsively given by oral route, the animals were anesthetized to determine the 5-FU concentration in the gastric mucosa of the normal dog.

In case of emulsion, the emulsion containing 10 mg/kg or 20 mg/kg of 5-FU was infused into the stomach of the anesthetized normal dogs under endscopy.

c. Collection of the peripheral blood

About 3 ml of blood of the peripheral vein was collected from the femoral vein.

d. Collection of the portal blood

After abdominal incision, a catheter was inserted into the portal vein from the splenic vein and about 3 ml of blood of the portal vein was collected.

e. Collection of the lymph

A vinyl tube was introduced into the thoracic duct beneath the left clavicular bone and the lymph of the thoracic duct was collected.

f. Collection of gastric mucosa and various organs

The tissue specimen of the gastric mucosa were collected from 8 sites of the stomach by the procedures of the gastric biopsy. Three dogs were bled to death after 60 minutes, 150 minutes and 360 minutes of 5-FU administration respectively and small pieces of each organ were collected after autopsy.

3. Method of determination of 5-FU concentration

The 5-FU concentration was determined according to bioassay using *Staphylococcus aureus* 209 P described by FUJITA[4].

II. EXPERIMENTAL RESULTS

1. Time course variation of 5-FU concentration in human blood

The results are shown in Fig. 1. In case of tablet administration, the mean levels of 5-FU rose to 0.0120±0.0080 μg/ml after 5 minutes of administration, 0.0406±0.0197 after 10 minutes and 0.1442±0.0984 after 15 minutes, and thereafter tended to decrease : 0.0810±0.0253 after 30 minutes, 0.0375±0.0193 after 60 minutes and 0.0072±0.0050 after 120 minutes. In case of emulsion, the mean levels rose to 0.0064±0.0017 μg/ml after 5 minutes, 0.0096±0.0042 after 10 minutes, 0.0223±0.0103 after 15 minutes and 0.0388±0.0178 after 30 minutes, and afterwards tended to decrease : 0.0325±0.0162 after 60 minutes and 0.0064±0.0013 after 120 minutes.

Fig. 1 5-FU levels in peripheral plasma after oral administration of 5-FU in patients with gastrointestinal cancer (5-FU 200 mg/patient)

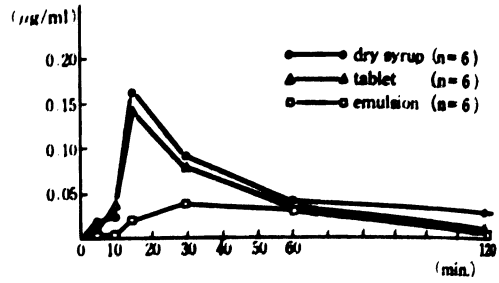
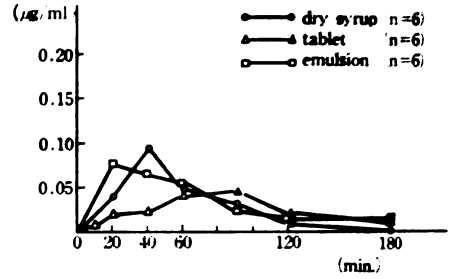


Fig. 2 5-FU levels in peripheral plasma after intragastric administration of 5-FU in dogs (5-FU 10 mg/kg/dog)



2. Time course variation of 5-FU concentration in normal dogs

a. Blood of the peripheral vein

The results are shown in Fig. 2. In case of tablet, the mean levels rose to 0.0097±0.0028 μg/ml after 10 minutes of administration, 0.0243±0.0115 after 20 minutes, and 0.0420±0.0242 after 60 minutes, reaching the maximal level of 0.0432±0.0365 after 90 minutes, and thereafter tended to decrease gradually : 0.0210±0.0110 after 120 minutes and 0.0142±0.0061 after 180 minutes.

Fig. 3 5-FU levels in portal plasma after intragastric administration of 5-FU in dogs (5-FU 10 mg/kg/dog)

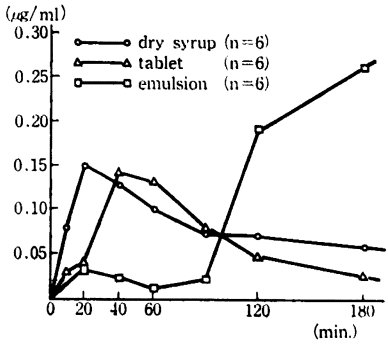
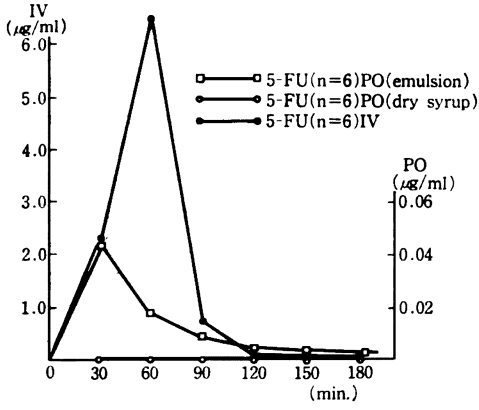


Fig. 4 Lymph 5-FU levels of thoracic duct after administration of 5-FU in dogs (5-FU 20 mg/kg/dog)

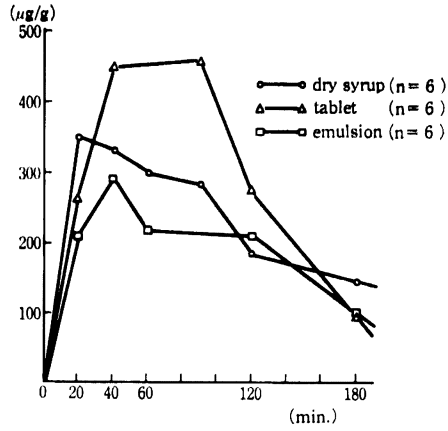


In case of emulsion, the 5-FU concentration reached to the maximal level of 0.0772±0.0200 μg/ml after 20 minutes of administration, and thereafter tended to decrease : 0.0651±0.0247 after 40 minutes, 0.0543±0.0306 after 60 minutes, 0.0235±0.0128 after 90 minutes, 0.0182±0.0075 after 120 minutes and 0.0153±0.0065 after 180 minutes.

b. Blood of the portal vein

The results are shown in Fig. 3. In case of tablet, the mean levels rose to 0.0325±0.0155 μg/ml after 10 minutes and 0.0440±0.0150 after 20 minutes and reached the maximal level of 0.143±0.0324 after 40 minutes, and thereafter tended to decrease : 0.1355±0.0378 after 60 minutes, 0.0823±0.0269 after 90 minutes, 0.0510±0.0241 after 120 minutes and 0.0297±0.0133 after 180 minutes. In case of emulsion, the concentration reached the maximal level of 0.3286±0.1664 μg/ml after 20 minutes, and thereafter decreased to 0.2327±0.1651 after 40 minutes and 0.1223±0.0931 after 60 minutes. However, the concentration began to rise again to 0.1325±0.0982 after 90 minutes, 0.1943±0.0692 after 120 minutes and 0.2645±0.1858 after 180 minutes.

Fig. 5 Gastric mucosal 5-FU levels after oral administration of 5-FU in dogs (5-FU 10 mg/kg/dog)



c. Lymph of the thoracic duct

Either 10 mg/kg of tablet and emulsion were administered by oral route, and the 5-FU concentrations in lymph of the thoracic duct were determined every 30 minutes up to 180 minutes after administration. However, all of the 5-FU concentrations were trace at all. Then, 20 mg/kg of emulsion was orally administered and the 5-FU concentration in lymph of the thoracic duct were determined at hourly intervals. The results are shown in Fig. 4.

The concentration attained to the peak level of 0.0460±0.0392 μg/ml after 30 minutes of administration and thereafter the level tended to decrease to 0.0196±0.0107 after 60 minutes, 0.0092±0.0035 after 90 minutes, 0.0050±0.0026 after 120 minutes, 0.0036±0.0023 after 150 minutes and 0.0024±0.0016 after 180 minutes.

d. Gastric mucosa

The results are shown in Fig. 5. In case of tablet, the mean levels of 5-FU were 263.9±161.1 μg/g after 20 minutes, 499.0±236.1 after 40 minutes and 459.2±230.1 after 90 minutes of

Table 1 5-FU concentration in various organs after oral administration of 5-FU tablet in dogs.
(5-FU 100mg/dog)

Viscera \ Time (min.)			60	180	360
Stomach	Corpus	superficial layer	15.60	14.27	11.50
		deep layer	1.08	0.85	0.48
	Antrum	superficial layer	1.39	0.96	0.64
		deep layer	0.21	0.10	0.20
Small intestine			0.40	0.40	0.40
Colon			0.06	0.06	0.03
Mesenteric lymph node			t	t	t
Brain			0.01	0.003	0.03
Lung			0.05	0.09	t
Liver			t	t	t
Pancreas			0.04	0.04	0.001
Kidney			t	t	t

t : trace (μg/g)

Table 2 5-FU concentration in various organs after intragastric administration of 5-FU emulsion in dogs.
(5-FU 100mg/dog)

Viscera \ Time (min.)			60	180	360
Stomach	Corpus	superficial layer	38.30	11.52	1.63
		deep layer	2.07	2.28	0.15
	Antrum	superficial layer	4.73	0.94	1.30
		deep layer	0.84	0.45	0.04
Small intestine			t	0.18	0.39
Colon			t	t	t
Mesenteric lymph node			t	t	0.06
Brain			0.01	0.01	t
Lung			t	t	t
Liver			t	t	t
Pancreas			t	t	t
Kidney			t	t	t

t : trace (μg/g)

administration, and thereafter tended to decrease : 276.1±150.4 after 120 minutes, and 105.2±40.0 after 180 minutes. In case of 10 mg/kg of emulsion, the 5-FU concentrations rose to 209.6±96.1 μg/g after 20 minutes and 290.7±165.7 after 40 minutes, and thereafter tended to decrease : 229.0±71.8 after 60 minutes, 221.9±86.5 after 120 minutes, 108.9±46.3 after 180 minutes, 68.5±23.5 after 300 minutes and 76.4±30.5 after 360 minutes.

e. 5-FU concentration in various organs on autopsy

The results measured the 5-FU concentration in the organs are shown in Table 1 and Table 2. In case of tablet, the mean levels in the superficial layer (mucosa and submucosa) of the corpus of stomach were 15.60±9.72 μg/g after 60 minutes, 14.27±9.01 after 180 minutes, and 11.50±7.34 after 360 minutes, and those in the deep layer (muscular layer and serosa) 1.08±0.75 μg/g after 60 minutes, 0.85±0.44 after 180 minutes and 0.48±0.25 after 360 minutes. The mean levels in the superficial layer of the antrum were 1.39±0.72 μg/g, 0.96±0.58 after 180 minutes and 0.64±0.28 after 360 minutes, and those in the deep layer of the antrum were 0.21±0.13 μg/g after 60 minutes, 0.10±0.04 after 180 minutes and 0.20±0.12 after 360 minutes. In case of emulsion, the mean levels of 5-FU in the superficial layer of the corpus were 38.30±20.46 μg/g after 60 minutes, 11.52±9.37 after 180 minutes and 1.63±0.81 after 360 minutes, and those in the deep layer of the corpus were 2.07±1.36 after 60 minutes, 2.28±1.24 after 180 minutes and 0.15±0.08 after 360 minutes. The mean levels in the superficial layer of the antrum were 4.73±2.98 μg/g after 60 minutes, 0.94±0.72 after 180 minutes, and 1.30±0.66 after 360 minutes, and those in the deep layer were 0.84±0.53 μg/g after 60 minutes, 0.45±0.19 after 180 minutes and 0.04±0.03 after 360 minutes.

III. DISCUSSION

We have had gradually increasing opportunities to contact to the reports that administration of fluorirated pyrimidine by oral route improved not only the metastatic lesion of advanced gastric cancer but also the gastric X-ray and endoscopic pictures of primary lesion of gastric cancer and resulted in disappearance of cancer[1-3]. We have also experienced a case in which gastric cancer type IIa of the early stage was disappeared by oral administration of 5-FU. Thus, we investigated on kinetics of 5-FU in the blood and stomach after administration of 3 formulations of 5-FU : dry syrup, tablet and emulsion. Oral administration of 5-FU tablet was tried by method of GOLD et al.[5] (1959) and STARLEY et al.[6] (1962), but

there has been no report that 5-FU tablet was effective for gastric cancer. SHIOSAKA et al.[7] (1968) reported that 7.5 to 15.0 mg/kg of 5-FU tablets were administered to 13 cases of malignant tumors (6 cases of gastric cancer, 3 cases of rectal cancer, etc.) by oral route and the reduction of abdominal tumors was observed in 7 of 13 cases, but no report has been found in the literatures for a long time. Since then 5-FU dry syrup is so difficult to be dissolved when administered by oral route that sometimes out-patients take it before dissolution, and the method of administration is so complicated that patients easily notice it as antineoplastic agents. Therefore, 5-FU dry syrup has some problem in practical use. Moreover, we thought that gastrointestinal side effects might occur more frequently because dry syrup would reach more rapidly to the small intestine and contact to the digestive organs. Accordingly, we tried to develop the tablet as a formulation to decrease side effects, to remain be adsorbed to the stomach and to exert the effect against gastric cancer. On the basis of our intention[8], 5-FU tablet prepared so as to destruct and diffuse rapidly in the stomach has been developed. According to the results of clinical studies conducted in Japan which was summed up by KURIHARA[9], the effects more than I-A on the basis of KARNOFSKY's criteria were found 18.7% (28 out of 150 cases) in dry syrup administration group, and 20.1% (28 out of 139 cases) in tablet administration group. The peak of the 5-FU concentration curve in the canine gastric mucosa appeared more later with tablet than with dry syrup, and the levels were slightly low in tablet. The levels even in the autopsied stomach after 6 hours of administration were about 2 times higher with tablet than with dry syrup, remaining in the stomach for a longer time. This suggests a possibility to contact to gastric cancer for a long time and to exert the antitumor effects against tumor. However, the 5-FU concentration in the blood of the peripheral and portal veins of normal dogs was relatively lower with tablet than with dry syrup and also the concentrations in the various organs were lower with tablet. Therefore, the formulation of tablet is a proper method of administration to directly approach the primary lesion of gastric cancer. According to the data of side effects collected from the clinical studies conducted in Japan[9], side effects occurred less frequently with tablet than dry syrup: diarrhea (11.6% with dry syrup, and 7.8% with tablet), leukopenia (2.4% and 0.6%), decrease in WBC (10.1% and 4.4%), and thrombocytopenia (4.2% and 3.5%). This result demonstrates that the purpose to decrease side effects was obtained by changing the formulation. In the experiment using dogs, 5-FU was detected in the canine autopsied brain when administered orally in the formulations of dry syrup[10], tablet and emulsion, but 5-FU by intravenous route was not detected. This face seems to be related to dizziness[11~12] which rarely occurred by administration of dry syrup, and therefore, a careful attention should be required to use tablet or emulsion in clinical practice.

The oral formulation of emulsion was clinically used for the first time by TAKAHASHI et al.[12~18] perceiving easy direction to the lymph, high adsorbability to the tissue and long-term deposition of fat emulsion, and being characterized by the fact that the high level of 5-FU is found in the lymphatic glands as well as in the gastric wall. It has been confirmed in rats[13~17] and dogs[19] that when emulsion was orally administered, the peak level of 5-FU in the thoracic duct was higher and maintained a high-level for a long time as compared with that of 5-FU solution. Furthermore, in our experiment using dogs, the 5-FU concentration in the lymph of the thoracic duct was not detected at all up 3 hours after administration of tablet, whereas when administered in the formulation of emulsion, the peak level was found at 30 minutes after administration and thereafter decreased gradually, being detected throughout 3 hours after administration. The 5-FU concentration in the mesenteric glands was not detected until 3 hours after administration and after 6 hours 0.03 to 0.11 μg/g level was detected in all the 3 dogs. However, the experiment to follow longer time than 6 hours was not conducted and the subsequent kinetics was unclear. On the contrary, the high levels of 5-FU were detected during 1 to 6 hours of the experimental measurable range by intravenous administration, and even after 6 hours after administration, 0.12 to 0.25 μg/g of 5-FU level were detected. It is well known that the high levels of 5-FU were kept for a longer

time by intravenous administration than by administration of 5-FU solution in the lymphatic glands neighboring the stomach extracted at the operation of human gastric cancer[19] and the rat mesenteric glands[20]. However, this study was conducted under anesthesia and if the experiment is performed for a longer time, the direction of 5-FU to the lymph will be confirmed more clearly. In case of emulsion, the higher 5-FU concentrations in the blood of the portal vein were maintained for a long time as compared with dry syrup or tablet, but the 5-FU concentrations in the blood of the peripheral vein were, on the contrary, lower than those of dry syrup or tablet. This fact suspected to have a correlation to adsorbability and depositability to the various organs including the liver, and in fact, there was a similar report[20]. In our experiment in which the 5-FU concentration in the gastric mucosa of the dogs under anesthesia was investigated, the concentration curves of 5-FU emulsion showed almost the same pattern of those of dry syrup. Accordingly the local effects on the tissue of gastric cancer were expected to be almost the same. TAKAHASHI et al.[18] reported that the macroscopic changes in X-ray examination, endoscopic examination and resected specimen findings were observed in 26 out of 44 cases (59%). Accordingly, the formulation of emulsion seems to be effective for gastric cancer under consideration of the directional property of emulsion to the lymph.

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胃癌治療を目的とした経口抗癌剤（5-FU）の生体内動態に関する研究

2) 経口3剤の比較検討

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5-FU ドライシロップ，錠剤，エマルジョンの3剤型について，悪性腫瘍患者，正常犬における生体内動態を検討した結果，次の結論を得た。

i）ヒトの末梢静脈血中濃度は，ドライシロップ，錠剤では投与後 15 分にピークを有し，急激に減少するパターンを示すが，エマルジョンの場合は，30 分後に前2剤の約 1/4 のピーク値を示した後，漸減する傾向を示した。

ii）正常犬の胃粘膜内 5-FU 濃度は，ドライシロップでは 20 分後，錠剤では 40 ないし 90 分後，エマルジョンでは 40 分後に各々ピークを示したが，いずれも測定6時間にわたって漸減し，組織への吸着性，滞留がよいことを認めた。

iii）正常犬の門脈内 5-FU 濃度は，検討した3剤とも末梢静脈血より高値をとって推移した。

iv）エマルジョンでは，正常犬の胸管リンパ液の 5-FU 濃度が，投与後3時間（ピークは 30 分後）にわたって，各採液時間に認めたが，ドライシロップ，錠剤は，測定限界外値に終始し，エマルジョンのリンパ指向性を証明できた。