ENHANCEMENT OF LYMPHATIC TRANSPORT OF N1-(2-TETRAHYDROFURYL)-5-FLUOROURACIL (FT-207) BY WATER-IN-OIL EMULSION IN POSTOPERATIVE ADJUVANT CHEMOTHERAPY FOR GASTRIC CANCER

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ABSTRACT

In 7 postoperative gastric cancer patients, the influence of fat emulsification of N1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207) on lymphatic transport was investigated.

The water in oil type of emulsion of FT-207 (FT-w/o) and enteric coated preparation (FT-G) in 1 gram each calculated in terms of FT-207 were administered orally, and lymph from the thoracic duct fistula prepared in advance and blood from peripheral vein were collected simultaneously with time after the administration to determine the concentrations of FT-207 and 5-fluorouracil.

In FT-w/o, FT-207 concentration showed significantly higher values than those in FT-G in 30-120 minutes after the administration, respectively, both in lymph and blood.

In FT-w/o, 5-FU concentration showed a significantly higher value than that in FT-G in lymph 30-240 minutes and in blood 30-480 minutes after the administration.

Thus, FT-w/o having the excellent activity in lymph and blood transition was considered useful as a adjuvant chemotherapeutic drug for postoperative treatment in gastric cancer.

INTRODUCTION

Late postoperative results for gastric cancer have improved remarkably with the recent spread of enlarged radical operation with extensive removal of regional lymph node. However, lymphatic recurrence after radical operations is still
frequently observed today to pose a major problem in the surgical treatment of gastric cancer. For prevention of such recurrence, it is necessary to administer adjuvant chemotherapy to keep the concentration of anti-cancer agents in the lymph and blood at high levels and to stamp out remaining cancerous tissues.

In order to enhance the transportability of anti-cancer agents to the lymph tissue, experiments on fat emulsification of Mitomycin C (MMC), Bleomycin (BLM), 5-fluorouracil (5-FU) and N1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207) have been conducted, and an enhanced transportability of anti-cancer drugs to lymph has been reported in the animal models. However, studies in clinical cases are indispensable since the enzyme system concerned with the in vivo metabolism of anti-cancer drugs is not uniform in various species of animals.

FT-207, a masked compound considered a precursor of 5-FU is anti-cancer agent which is converted into 5-FU through several in vivo metabolic routes. As compared with 5-FU, FT-207 is significantly superior in the anti-tumor effect and causes less delayed myelosuppression in adenocarcinoma, thus it is used widely in Japan as adjuvant chemotherapeutic agent in gastric cancer. However, we do not know any reports in which fat emulsion of this preparation is studied in clinical cases.

In the present study, a water in oil type emulsion (FT-w/o) of FT-207 were prepared for comparison with a commercially available enteric coated granule (FT-G) as to the transportability of the drugs to lymph and blood by oral administration.

SUBJECTS AND METHODS

The subjects were 7 cases of gastric cancer on which radical gastrectomy was performed. All were male, their ages ranging from 34 to 67 and averaging 53.

The emulsion of FT-207 was prepared in accordance with the method of Nakamoto et al.

Briefly, FT-w/o was prepared by adding 1 gram of FT-207 in 20 ml of water, 0.33 g of hydrogenated caster oil (HCO-60, Nikko Kagaku, Tokyo, Japan) and 1.38 g of sorbitan sesquioleate (SO-15, Nikko Kagaku, Tokyo, Japan) to 30 ml of sesame oil and subjecting the mixture to the ultrasonic treatment in an ultrasonic emulsifier (Model US-600, Nihon Seiki, Tokyo, Japan) at 60°C for 1-2 minutes.

FT-207 was administered as a single oral dose of 1,000 mg.

In order to study changes in the transportability of the drug to the lymph tissue and blood due to a change in the drug form of FT-207, lymph of the thoracic duct and blood from peripheral vein were collected simultaneously before administration and 30, 60, 120, 240, 360 and 480 minutes after administration.
The thoracic duct fistula was made to collect lymph as follows: after applying topical anesthesia to fovea superior of the left cravicle, incision was made along the upper margin of the clavicle to separate the cervical muscle. Adipose tissue located at the cranial to the subclavicular vein, ventral to the anterior scalenus muscle and around the internal jugular vein was removed with Virchow lymph node for histopathological examination. While raising the internal jugular vein, the junction with the subclavicular vein was detached posteriorly to expose the thoracic duct entering the bifurcation of the jugular and subclavicular vein. The thoracic duct was raised with silk sutures and detached from the surrounding tissue after that, a multi-purpose tube was inserted to use as the thoracic duct fistula. After collecting lymph from the thoracic duct fistula, heparin was injected into the multi-purpose tube which was then closed with a cap.

Concentration of FT-207 and its active substance 5-FU was determined as illustrated in Fig. 1.\textsuperscript{10} FT-207 was measured by high pressure liquid chromatography (HPLC method) and 5-FU by gas chromatography-mass fragmentography (CG-MP method).

Analytical procedure

<table>
<thead>
<tr>
<th>plasma 1.0 ml</th>
<th>+ distilled water 1.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>added internal standard (1,3-bis-\textsuperscript{15}N-5-FU, 0.1 μg)</td>
<td></td>
</tr>
<tr>
<td>adjusted to be pH 2.0-4.0 with HCl soin.</td>
<td></td>
</tr>
<tr>
<td>chloroform extraction. 20 ml × 2, room temp.</td>
<td></td>
</tr>
<tr>
<td>chloroform layer</td>
<td>aqueous layer</td>
</tr>
<tr>
<td>evaporated and dryness under N\textsubscript{2}</td>
<td>neutralized with NaOH soin.</td>
</tr>
<tr>
<td>resolved in 1,2-dichloroethane 100 μl</td>
<td>added 0.5 M NaH\textsubscript{2}PO\textsubscript{4} 0.2 ml</td>
</tr>
<tr>
<td>20 μl injected into HPLC</td>
<td>centrifugation. 10 min at 2000 \times g</td>
</tr>
<tr>
<td>FT-207</td>
<td>c. f. g. sup.</td>
</tr>
<tr>
<td>ethyl acetate extration. 40 ml × 1</td>
<td></td>
</tr>
<tr>
<td>ethyl acetate layer evaporated dryness under N\textsubscript{2} at 40°C</td>
<td></td>
</tr>
<tr>
<td>dryness over P\textsubscript{2}O\textsubscript{5} for overnight</td>
<td></td>
</tr>
<tr>
<td>resolved in BSTFA*/pyridine (1/3%) 100 μl</td>
<td></td>
</tr>
<tr>
<td>heated for 20 min at 70°C</td>
<td></td>
</tr>
<tr>
<td>1-3 μl injected into GC-MS</td>
<td></td>
</tr>
<tr>
<td>\textsuperscript{*} BSTFA: N. 0-bis (trimethylsilyl)-trifluoroacetamide.</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 Method of chemical assay of FT-207 and 5-FU.
RESULTS

FT-207 concentration in the thoracic duct lymph and plasma before and after administration of FT-207 are presented in Table 1.

Table 1
FT-207 concentration in thoracic duct lymph and plasma after administration of FT-207. FT207 was administered as a single oral dose of 1,000 mg

<table>
<thead>
<tr>
<th></th>
<th>time after administration (min)</th>
<th>before</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>lymph</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT-w/o</td>
<td>ND</td>
<td>24.7±4.2</td>
<td>28.5±4.9</td>
<td>34.6±6.6</td>
<td>39.0±7.5</td>
<td>35.6±5.6</td>
<td>34.5±5.8</td>
<td></td>
</tr>
<tr>
<td>FT-G</td>
<td>ND</td>
<td>13.0±2.6</td>
<td>12.4±2.2</td>
<td>12.7±2.2</td>
<td>21.6±7.0</td>
<td>20.8±5.9</td>
<td>19.7±5.3</td>
<td></td>
</tr>
<tr>
<td>plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT-w/o</td>
<td>ND</td>
<td>27.5±5.1</td>
<td>31.0±5.3</td>
<td>33.8±5.7</td>
<td>40.9±6.1</td>
<td>39.4±6.2</td>
<td>32.0±6.3</td>
<td></td>
</tr>
<tr>
<td>FT-G</td>
<td>ND</td>
<td>12.1±2.4</td>
<td>15.9±3.5</td>
<td>17.2±3.7</td>
<td>24.5±5.9</td>
<td>24.0±6.3</td>
<td>24.8±7.2</td>
<td></td>
</tr>
</tbody>
</table>

M ± SEM (µg/ml)
n=7
ND: not detectable

FT-207 concentration in these drug forms rose 30 minutes after administration both in thoracic duct lymph and plasma, gradually increased thereafter and reached a peak in 240–480 minutes.

FT-207 concentration was significantly high 30, 60 and 120 minutes after administration in both thoracic duct lymph and plasma in FT-w/o compared with that in FT-G (Fig. 2).

Comparison was made of these drug forms as to FT-207 concentration in thoracic duct lymph and plasma, but the difference was not significant at any point of time.

5-FU concentration in thoracic duct lymph and plasma before and after administration of FT-207 are presented in Table 2.

5-FU concentration in these drug forms rose 30 minutes after administration, and gradually increased thereafter to reach a peak after 120–480 minutes in both thoracic duct lymph and plasma. In FT-w/o, 5-FU concentration rose 30 minutes after administration at 0.063 ± 0.010 µg/ml in thoracic duct lymph and 0.060 ± 0.008 µg/ml in plasma, reached a peak 240 minutes after administration at 0.089 ± 0.016 µg/ml in thoracic duct lymph and 120 minutes after administration at 0.101 ± 0.014 µg/ml in plasma and maintained a level well above the minimum effective concentration being 5-FU (0.05–0.06 µg/g)11,12 at 0.075 ± 0.011 µg/ml in thoracic duct lymph and 0.082 ± 0.001 µg/ml in plasma even 480 minutes after
Fat Emulsification of FT-207

FT CONCENTRATION

Fig. 2 Change in FT-207 concentrations in plasma and lymph after administration of FT-w/o or FT-G. FT-207 concentrations in plasma and lymph 30-120 minutes after administration of FT-w/o were significantly high.

*P: P<5% between w/o and FT-G in plasma
**P: P<1% between w/o and FT-G in plasma
***P: P<0.1% between w/o and FT-G in plasma
*L: P<5% between w/o and FT-G in lymph
**L: P<1% between w/o and FT-G in lymph
***L: P<0.1% between w/o and FT-G in lymph

administration.

In FT-G, the 5-FU concentration rose 30 minutes after administration at 0.020 ± 0.004 µg/ml in thoracic duct lymph and 0.022 ± 0.005 µg/ml in plasma and exceeded the minimum effective concentration of 5-FU being 0.054 ± 0.010 µg/ml in thoracic duct lymph 360 minutes after administration and 0.052 ± 0.008 µg/ml in plasma 480 minutes after administration.

As for the difference in 5-FU concentration according to the drug form, FT-w/o showed significantly high 5-FU concentration 30-240 minutes after administration in thoracic duct lymph and 30-280 minutes after administration in plasma compared with FT-G (Fig. 3).

Comparison was made of the 5-FU concentrations in the thoracic duct lymph and plasma with each drug form, and no significant difference was observed at any point of time.

The concentration ratio of 5-FU and FT-207 in thoracic duct lymph and
Fig. 3 Changes in 5-FU concentrations in plasma and lymph after administration of FT-w/o or FT-G. In FT-w/o, 5-FU concentrations were significantly high 30–480 minutes after administration in plasma and 30–240 minutes after administration in lymph.

Fig. 4 Changes in the 5-FU/FT-207 concentration ratio in plasma and lymph after administration of FT-w/o and FT-G. No significant differences in the concentration ratios of 5-FU and FT-207 was observed in the these drugs.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>before</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>lymph</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT-w/o</td>
<td>ND</td>
<td>0.063±0.01</td>
<td>0.064±0.00</td>
<td>0.074±0.00</td>
<td>0.089±0.01</td>
<td>0.073±0.01</td>
<td>0.075±0.01</td>
</tr>
<tr>
<td>FT-G</td>
<td>ND</td>
<td>0.020±0.00</td>
<td>0.023±0.00</td>
<td>0.024±0.00</td>
<td>0.037±0.00</td>
<td>0.054±0.01</td>
<td>0.044±0.01</td>
</tr>
<tr>
<td>plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT-w/o</td>
<td>ND</td>
<td>0.060±0.00</td>
<td>0.070±0.00</td>
<td>0.101±0.01</td>
<td>0.089±0.01</td>
<td>0.083±0.01</td>
<td>0.081±0.00</td>
</tr>
<tr>
<td>FT-G</td>
<td>ND</td>
<td>0.021±0.00</td>
<td>0.026±0.00</td>
<td>0.036±0.00</td>
<td>0.041±0.00</td>
<td>0.042±0.00</td>
<td>0.051±0.00</td>
</tr>
</tbody>
</table>

M ± SEM (μg/ml)

n=7

plasma is illustrated in Fig. 4. FT-w/o showed slightly high values 30 minutes both in lymph and plasma after administration, but the difference was not significant. From 60 minutes after administration downward, each drug form maintained the value of 0.5% or less.

DISCUSSION

The tissue concentration of the anti-cancer drug is determined as a means to find the transportability of an anti-cancer drug to the tissue.

Where, however, this method is used in clinical cases, the tissue concentration of an anti-cancer drug at a certain point of time after administration of a single anti-cancer drug is all that can be found, and elucidating how the anti-cancer drug is transported to the tissue with time is not possible.

In view of the fact that 5-FU concentrations in thoracic duct lymph, gastric lymph node and stomach wall show quite similar changes after oral administration of 5-FU emulsion, we thought that the mode of an anti-cancer drug’s transport to the lymph tissue could be surmized by determining the concentrations of an anti-cancer drug in thoracic duct lymph with time.

This study has revealed that when FT-w/o is orally administered, significantly high FT-207 and 5-FU concentrations can be obtained not only in lymph but also in plasma from immediately after administration compared with those of FT-G.

The minimum effective concentration of 5-FU is reported to be 0.05–0.06 g/g. By oral administration of 5-FU emulsion, 5-FU concentration in the regional lymph node and stomach wall reaches 1.5–10 times that in thoracic duct
lymph. Therefore, the fact that 5-FU concentration in thoracic duct lymph maintained the value of 0.06 μg/ml or more for 30–480 minutes after administration of FT-w/o suggests that FT-w/o can be used as a very useful emulsion in the adjuvant chemotherapy for cancerous tissues remaining in the regional lymph node or lymph tissue in the residual stomach wall after operation for gastric cancer.

FT-w/o shows good absorption probably because this type of emulsion is easily converted to water in oil in water type emulsion in which FT-207 is contained in the innermost layer in intestinal fluid.

In order to carry out the effective anti-cancer therapy with few side effects, it is desirable to keep the concentration of anti-cancer drugs in plasma at low levels. According to our results, however, FT-207 and 5-FU concentrations in plasma after administration of FT-w/o rose as did not concentrations in thoracic duct lymph, showing no significant difference between the two. This result differs from results of an experiment by Nakamoto et al. in which FT-w/o was injected directly into the small intestine loop 20 cm anal to the Treitz's ligament to raise concentrations in thoracic duct lymph without raising plasma concentrations of FT-207 and 5-FU. This is probably due to a difference in species and the method of administration. However, a comparative study referring to literature cannot be made since clinical results concerning fat emulsion of FT-207 have not been reported yet.

The concentration ratio of 5-FU and FT-207 in plasma and lymph was determined in order to learn the efficiency of in vivo conversion from FT-207 to 5-FU. It showed low value of 0.5% or less in any drug form.

This value is almost consistent about 0.1% with FT-207 suppository by Eguchi et al. and 0.5–2% with intravenous injection by Fujita et al. So, the activation rate of FT-207 does not appear to be influenced by a difference in the drug form or administration route.

For the 5-FU concentration in tumor tissue to be raised further by administration of FT-207, an attempt to expedite the in vivo metabolism from FT-207 to 5-FU or to inhibit decomposition of 5-FU in tumor tissue would be necessary.

REFERENCES

Fat Emulsification of FT-207


