Investigation of Thymidylate Synthase Induction in Colorectal Tissues after Administration of Anticancer Drug, Fluorinated Pyrimidine Fluoride Derivatives

ORIGINAL ARTICLE Annals of Cancer Research and Therapy

Kitaro Futami • Sumitaka Arima^{*1)}

Patients with colorectal carcinoma were treated with UFT and Tegafur for 7 days repeated (600 mg per day), and 5-FU concentrations in tissues and inhibition rate of Thymidylate Synthase (TS) were compared. As a control, total amounts of TS were determined in tissues from patients who did not receive any drug. Total amounts of TS were compared between UFT or Tegafur group and control group.

Both 5-FU concentration and inhibition rate of TS in tumor tissues were significantly higher in UFT group than Tegafur group.

Total amounts of TS in tumor tissues, normal mucous membranes, and lymph nodes were significantly higher in patients treated with drugs than patients who did not receive any drug. Particularly, high level of TS was induced in patients treated with UFT. Relationship between total TS in tumor tissues and inhibition rate of TS were then studied. Inhibition rate of TS remained 50 to 60% regardless of total TS.

Ann Cancer Res Ther 2 (1) : 101-104, 1993 / Received 21 Dec 1992

Key words : TS induction, TS inhibition rate, Colorectal cancer, UFT

It is generally believed that 5-FU (one of pyrimidine fluoride derivatives) manifests antitumor activity primarily by inhibiting DNA replication by inhibiting Thymidylate Synthase (TS) by way of its active metabolite, 5-fluorodeoxyuridinemonophosphate (FdUMP)¹⁾. UFT is a masked compound of 5-FU²⁾, an exciting biochemical modulator, and one of the most widely used antitumor drugs for gastrointestinal carcinoma today. It has been reported that high levels of 5-FU concentrations in tumor tissues were achieved after the administration of UFT³⁾ and that TS was greatly inhibited from basic and clinical studies^{4, 5)}. Higher levels of TS were detected in tumor tissues than normal mucous membranes, and it has been pointed out that TS was induced in tissues after the administration of pyrimidine fluoride derivatives from basic science research^{6, 7)}. The authors investigated 5-FU concentration and inhibition rate of TS in tissues after the repeated administration of UFT and Tegafur in patients with colorectal carcinoma and monitored TS activity as compared with cases who did not receive any drug as a control.

Subjects and methods (Fig 1)

Forty-eight cases with colorectal carcinoma were studied. All cases had resection of colorectal carcinoma. Thirty-seven cases were treated with drugs, i.e. UFT or Tegafur was administered repeatedly and orally for 7 days (600 mg daily, 200 mg per administration, 3 times a

Fig.1 Materials and methods

Case with colorectal carcinoma : 48 cases

(Dose group : 37 cases. Control group : 11 cases)

day) before the surgeries. Tumor tissues, mucous membranes in normal areas, and regional lymph nodes were collected at the time of surgeries. Tissues were frozen and stored. 5-FU concentration, total amount of TS and inhibition rate of TS in various tissues were measured. Twenty cases received UFT, 17 cases received Tegafur, and these two groups were compared. Eleven cases did not receive any drug. Various tissues were collected from these 11 patients as well at the time of surgeries, and total amounts of TS were measured. Total amounts of TS were compared between dose group and control group. Statistical analysis was done by t-test, and statistical significance was determined by a risk rate less than 5%.

Procedures

5-FU concentrations in tissues were measured by Gas Chromatographic Mass Fragmentographic Method (GCMF) according to Marunaka⁸⁾.

TS was measured according to a modified method by Spears et al.⁹⁾, total amounts of TS & free TS were measured, and inhibition rate of TS (%) was calculated by the following formula.

^{*} Department of Surgery, Chikushi Hospital, Fukuoka University, 377-1 Zokumyoin, Chikushino City, Fukuoka 818, Japan

Fig. 2 5-FU concentrations after UFT or Tegafur administration

Fig.3 Inhibition rate of TS after UFT or Tegafur administration

$$\text{Inhibition rate of TS (\%)} = \frac{\text{TS total} - \text{TS free}}{\text{TS total}} \times 100$$

Results

Comparison of 5-FU concentrations among dose groups (Fig.2)

5-FU concentrations in tumor tissues were $0.208 \pm 0.143 \mu\text{g/g}$ among UFT group, and $0.077 \pm 0.027 \mu\text{g/g}$ among Tegafur group. UFT group showed significantly higher 5-FU concentration ($P < 0.05$). 5-FU concentrations in metastasized lymph nodes were $0.101 \pm 0.075 \mu\text{g/g}$ among UFT group, and $0.032 \pm 0.004 \mu\text{g/g}$ among Tegafur group. UFT group showed higher 5-FU concentration, but no statistical significance was detected. No difference was seen in normal mucous membranes and non-metastasized lymph nodes between two groups.

Comparison of inhibition rate of TS among dose groups (Fig.3)

Inhibition rates of TS in tumor tissues were 56.12% among UFT group, and 45.52% among Tegafur group. UFT group showed a significantly higher inhibition rate ($P < 0.05$). UFT group showed a higher inhibition rate of TS in metastasized lymph nodes (57.45%), but no statistical significance was detected. Inhibition rates of TS in normal mucous membranes were 39.0% among UFT group, 38.3% among Tegafur group, and no

Fig. 4 Total amounts of TS in tumor tissues

Fig.5 Total amounts of TS in normal mucous membranes

statistical significance was detected.

Comparison of total TS in tumor tissues (Fig.4)

Total TS in tumor tissues were $16.35 \pm 13.15 \text{ pmol/g}$ among UFT group, $11.90 \pm 9.84 \text{ pmol/g}$ among Tegafur group, and $4.95 \pm 5.96 \text{ pmol/g}$ among control group. Therefore, UFT showed approximately 3.3 times higher total TS than control group. Tegafur group showed approximately 2.4 times higher total TS than control group, and both UFT group and Tegafur group showed statistically higher total TS.

Comparison of total TS in normal mucous membranes (Fig.5)

Total TS in normal mucous membranes were $4.80 \pm 3.05 \text{ pmol/g}$ among UFT group, $3.52 \pm 1.63 \text{ pmol/g}$ among Tegafur group, and $1.52 \pm 1.35 \text{ pmol/g}$ among control group. Therefore, both UFT and Tegafur groups showed significantly higher total TS than control group.

Comparison of total TS in lymph nodes (Fig.6)

Total TS in lymph nodes were $13.61 \pm 11.0 \text{ pmol/g}$ among UFT group, $6.43 \pm 3.86 \text{ pmol/g}$ among Tegafur group, and $2.43 \pm 3.70 \text{ pmol/g}$ among control group. Therefore, both UFT and Tegafur groups showed higher

Fig. 6 Total amounts of TS in lymph nodes

Fig.7 Relationship between total amounts of TS and inhibition rate of TS in tumor tissues

total TS than control group, but statistical significance was not detected.

Relationship between total TS and inhibition rate of TS in tumor tissues (Fig.7)

Inhibition rate of TS was 50 to 60% regardless of total TS.

Discussion

Principle chemotherapeutic drugs against gastrointestinal carcinoma are pyrimidine fluoride derivatives, and UFT is one of the most widely used drugs in this country. Colorectal carcinoma is not as sensitive to chemotherapy as gastric carcinoma. Therefore, various chemotherapeutic drugs have been searched for presently. The authors investigated 5-FU concentrations and inhibition rates of TS in tissues after administration of UFT in patients with colorectal carcinoma, and reported that higher 5-FU concentrations and significantly higher inhibition rate of TS were achieved after administration of UFT as compared with administration of Tegafur¹¹⁾. This study was conducted, based on the previous results. From the standpoint of responsiveness to drugs, UFT may have a component which is responsible for more potent antitumor activity against colorectal carcinoma than Tegafur.

Additionally, Takashima et al.¹²⁾ compared Tegafur and UFT as supplementary chemotherapeutic drugs after resection of colorectal carcinoma and reported that higher 5-year survival rate was achieved among UFT group. In the near future, UFT will be a main chemotherapeutic drug against colorectal carcinoma.

Generally, primary mechanism of antitumor activity of pyrimidine fluoride at clinical doses is believed to be by inhibition of DNA replication⁶⁾. Details of the mechanism can be summarized as follows. 5-FU is taken up in tumor tissues, 5-FU is converted to FdUMP in tumor cells, FdUMP-TS-methylene hydrofolate (Me-THF) binds, and then ternary complex is formed. TS activity will be inhibited by this ternary complex, DNA replication will be inhibited, and eventually anti-tumor effects will be manifested¹³⁾. What kinds of effects may pyrimidine fluoride have on TS in tissues? It has been reported that TS was induced in tumor tissues after the administration of pyrimidine fluoride from basic science studies⁶⁾, but no paper has been published regarding clinical aspects. Therefore, we investigated TS activity clinically after the administration of UFT or Tegafur in patients with colorectal carcinoma, and found that TS was highly induced by UFT of Tegafur. In tumor tissues, TS was induced approximately 3.3 times higher in UFT group, and approximately 2.4 times higher in Tegafur group than control group. In normal mucous membranes, TS was induced approximately 3.2 times higher in UFT group, and approximately 2.3 times higher in Tegafur group than control group. The mechanism of TS induction by pyrimidine fluoride may be due to simple compensatory reactions in vivo, but details are unclear. Washtien¹⁴⁾ speculated that TS gene was amplified, but further basic studies may be necessary. Additionally, induction of TS and increase of total TS may not necessarily cause higher inhibition rate of TS, since our present data showed that inhibition rate of TS remained 50 to 60% regardless of total TS. This level of inhibition may be the limitation by administration of clinical doses (400 to 600 mg/day) of UFT alone. Recently, many papers have been published regarding enhanced antitumor effects by combination of 5-FU&CDDP¹⁵⁻¹⁷⁾ or Leucovorin¹⁸⁻¹⁹⁾. Shirasaka et al.²⁰⁾ elucidated mechanism of synergistic effect by 5-FU and CDDP from basic studies. Briefly, they reported the following findings. CDDP manifests antitumor effects by inhibiting transport of methionine into cells. Since folic acid metabolism is coupled with methionine metabolism, folic acid metabolism is also influenced by CDDP during transport process. Namely, reduced folic acids such as Me-THF, intermediary metabolites of folic acids, accumulate in tissues, which supplement Me-THF that was used up for ternary complex formation.

Therefore, ternary complex can be formed easily, which may in turn amplify antitumor effects of 5-FU. Our clinical studies did not correlate increase of total TS with inhibition rate of TS. It could be due to depletion of Me-THF, but in order to prove this theory, determination of TS as well as Me-THF may be required²¹⁾. Suga et al.²²⁾ performed combination therapy consisting of UFT and CDDP in patients with gastric cancer, and found that combination therapy was very effective in case of gastric cancer. Colorectal carcinoma is dependent on TS and so it may respond to inhibition of TS sensitively⁷⁾. Therefore, UFT may be an effective chemotherapeutic drug for the treatment of colorectal carcinoma.

Conclusion

5-FU concentration and inhibition rate of TS in tissues were compared after repeated administration of UFT or Tegafur in patients with colorectal carcinoma. As a control, we tested cases who never received any drug. Total TS in tissues was monitored after the administration of UFT or Tegafur.

- 1) In tumor tissues, significantly higher 5-FU concentration and inhibition rate of TS were detected among UFT group than Tegafur group.
- 2) Significantly higher total TS was detected after the administration of UFT or Tegafur. Particularly, the effect was more remarkable among UFT group.
- 3) In tumor tissues, There was no correlation between total TS and inhibition rate of TS. Inhibitory rate of TS and inhibition rate of TS. Inhibitory rate of TS was 50 to 60% regardless of total TS.

References

- 1) Cohen, S S, Flaks, JG., Barner, M D et al. : The mode of action of 5-fluorouracil and its derivatives. *Proc. Natl. Acad. Sci. USA*, 44 : 1004-1012, 1958.
- 2) Fujii, S, Ikenaka, K, Shirasaka, T et al. : Effect of Uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. *Gann*, 69 : 763-772, 1978).
- 3) Arima, S, Futami, K, Shigeta, M et al. : 5-FU concentration in the blood and tissue of patients with gastric and colorectal cancer after administration of UFT or Tegafur. *Jpn. J. Cancer Chemother*, 13 : 2618-2624, 1986.
- 4) Suzuki, M, Sekiguchi, I, Tamada, T et al. : Level of inhibition of thymidylate synthase activity and 5-fluorouracil in tumor tissues after administration of UFT or Tegafur. *J. Jpn. Soc. Cancer Ther*, 24 : 1399-1404, 1989.
- 5) Sakamoto, S, Kudo, H, Kuwa, K et al. : Effects of neo-adjuvant chemotherapy with UFT (A combination of tegafur and Uracil) on DNA-synthesizing enzyme activities in human colorectal carcinomas. *J. Jpn. Soc. Cancer, Ther*, 24 : 94-98, 1989.
- 6) Nakamura, H, Wang, Y Q, Miyauchi, S et al. : Studies on the mechanism of antitumor activity of 5-FU and its derivatives-Relationship between the inhibition of tumor growth and the inhibition of thymidylate synthase in vivo. *Jpn. J. Cancer Chemother*, 11 : 1049-1056, 1984.
- 7) Walberg, W H, Moris, J. : Thymidylate synthase activity and fluorouracil sensitivity of human colonic cancer and normal mucosal tissues preparations. *Cancer*, 47 : 1313-1317, 1981.
- 8) Marunaka, T, Umeno, Y, Yoshida, K et al. : High-pressure liquid chromatographic determination of futraful [1-(tetrahydro-2-furonyl)-5-fluorouracil] and GLC-Mass spectrometric determination of 5-fluorouracil and uracil in biological materials after oral administration of uracil plus futraful. *J. Pharm. Sci*, 69 : 1296-1300, 1980.
- 9) Spears, C P, Shabinian, A H, Moran, RG et al. : In vitro kinetics of thymidylate synthase inhibition in 5-fluorouracil sensitive and resistant murine colon adenocarcinoma. *Cancer Res*, 42 : 450-456, 1982.
- 10) Takeda, S, Uchida, J, Yamada, Y et al. : The significance of measuring inhibition of thymidylate synthase activity as a parameter for antitumor activity of 5-fluorouracil derivatives. *Jpn. J. Cancer Chemother*, 15 : 2125-2130, 1988.
- 11) Futami, K, Arima, S, Yoshimura, S et al. : Drug concentration in cancerous large bowel tissue and thymidylate synthase inhibition rate after administration of tegafur and UFT. *Jpn. J. Cancer Chemother*, 18 : 215-220, 1991.
- 12) Takashima, S, Tomita, F, Saito, H et al. : Clinical effect of postoperative adjuvant chemotherapy for advanced colorectal cancer-Comparisons of tegafur and UFT-. *Jpn. J. Cancer Chemother*, 18 : 2315-2323, 1991.
- 13) Oie, S, Okabe, H, Takeda, S et al. : Analysis of the mechanism of increased antitumor activity of UFT after combined treatment with CDDP. *Jpn. J. Cancer Chemother*, 17 : 1321-1326, 1990.
- 14) Washtien, WL : Increased levels of thymidylate synthase in cells exposed to 5-fluorouracil. *Mol. Pharm*, 25 : 171-177, 1983.
- 15) Cantrell J E, Hart, R D, Tayler, R F et al. : Pilot trial of prolonged continuous infusion 5-FU and weekly cisplatin in advanced colorectal cancer. *Cancer Treat. Rep*, 71 : 615-618, 1987.
- 16) Cohn, J, Tester, W, Deszi, A et al. : Continuous infusion 5-fluorouracil and weekly low dose cisplatin in metastatic gastrointestinal malignancies. *Proc. Am. Soc. Clin. Oncol*, 7 : 115, 1988.
- 17) LoRusso, P, Pazdur, R, Redman, B. G et al. : Low-dose continuous infusion 5-fluorouracil and cisplatin : Phase - evaluation in advanced colorectal carcinoma. *Am. J. Clin. Oncol*, 12 : 486-490, 1989.
- 18) Petrelli, N. P, Herrera, L, Rustum, Y et al. : A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J. Clin. Oncol*, 5 : 1559-1565, 1987.
- 19) Sasaki, T, Ota, K, Sakata, S et al. : High-dose leucovorin and 5-fluorouracil in advanced gastric and colorectal cancer-High dose leucovorin and 5-FU study group-. *Jpn. J. Cancer Chemother*, 17 : 2361-2368, 1990.
- 20) Shirasaka, T, Shimamoto, Y, Kinoshita, H et al. : Mechanism for synergistic antitumor effect in the combination of 5-fluorouracil with cisplatin in vivo tumor models : from the view of biochemical modulation of 5-fluorouracil. *Jpn. J. Cancer Chemother*, 18 : 403-409, 1991.
- 21) Spears, C P, Gustarsson, B G, Berne, M et al. : Mechanism of innate resistance to thymidylate synthase inhibition after 5-fluorouracil. *Cancer Res*, 48 : 5894-5900, 1988.
- 22) Suga, S, Kimura, R, Nagai, H et al. : An evaluation of gastric cancer given UFT/CDDP chemotherapy. *Jpn. J. Cancer Clin*, 37 : 829-839, 1991.

Offprint request to : K Futami, Department of Surgery, Chikushi Hospital, Fukuoka University, 377-1 Zokumyoin, Chikushino City, Fukuoka 818, Japan

HLA Antigens and Postoperative Adjuvant Immunotherapy in Gastric Cancer ; a Multidimensional Data Analysis

p 95~99

Kyoji Ogoshi

癌治療を行う前に、効果のある、患者個人個人に最適な治療法の予測が可能であれば、癌患者のquality of life (QOL) の向上に役立つものと考えられる。そこで、本研究では、個人を同定出来るHLA抗原を用いて、個人個人の治療法が予測できないかどうか検討を行った。

【対象と方法】 対象患者の術前にmicrocytotoxicity assayを用いてHLA-A, -B, -C抗原を測定した。HLA抗原の分類は、胃癌切除例429例を対象とし、数値化Ⅲ類^{11, 12)}を用いて四つの型に分類した。予後因子の検討は、胃切除術単独90例、胃切除術+化学療法98例、胃切除術+PSK併用免疫化学療法133例を対象として、Coxの比例ハザードモデルを用いて解析した。

【結果】 ① 数値化Ⅲ類により、1型はA-3, Aw-19, Aw-33, B-12, B-44, Bw-56, B-35抗原、2型はAw-31, B-5, B-51, B-13, Cw-4抗原、3型はA-2, A-9, Bw-52, B-7, B-15, Bw-62, B-16, B-39, B-40, Bw-60, Bw-48, Cw-7抗原、4型はA-10, A-11, Bw-22, Bw-54, Bw-55, Bw-61, Bw-46, Bw-59, Cw-1, Cw-3抗原に分類された(図1)。

② 単一予後因子としての年齢は49歳以下の患者が最も予後良好で、60歳以上の患者との間、進行度では、pTNM stage 1Aの患者がもっとも予後良好で、stage 2, 3A, 3B, 4の患者との間、治療法別では、PSK併用免疫化学療法群がもっとも予後良好で、胃切除群との間、HLA分類型は、3型が最も予後良好で、1型との間に有意差を認めた(表2)。

③ 多因子での検討では、HLA抗原型4型でPSK併用免疫化学療法施行群が最も予後良好で、1型、2型、3型の胃切除群、1型の化学療法施行群に比し有意に予後良好であった(表3)。

【考察】 われわれは、術前のシアル酸が異常値を示す症例、または血清IAP (immunosuppressive acidic protein) 値が異常値を示し、脾臓摘出がなされた症例では、術後PSK併用免疫化学療法が最適な治療法であることを報告した^{14, 15)}。一方、個人を同定出来るHLA抗原を用いて検討を行ったところ、HLA-Bw-40抗原陽性胃癌患者のPSK併用免疫化学療法施行例は予後良好であった³²⁾。しかし、HLA抗原はその特徴として多様性を示すことが知られている。そこで、本研究では、数値化Ⅲ類を用いてHLA抗原の発現頻度からHLA抗原を群として分類し、治療法と予後を検討したところ、HLA抗原のパターンと治療法により予後が異なっていた。このことはHLA抗原のパターン(そのようなHLA抗原を持っている患者)に相対する最適な治療法が存在することを示しているものと考えられる。よって、癌患者のHLA抗原の測定は、個人個人の最適な治療法の予測に有用であると考えられた。

Investigation of Thymidylate Synthase Induction in Colorectal Tissues after Administration of Anticancer Drug, Fluorinated Pyrimidine Fluoride Derivatives

p 101~104

Kitaro Futami et al.

大腸癌に対してUFTならびにTegafurの術前投与を行い、組織内5Fu濃度およびTS阻害率を比較検討するとともに、薬剤非投与例をコントロールとして、抗癌剤投与による組織内TSの変動についても検討を加えた。

【対象と方法】 大腸癌切除症例48例を対象とした。37例は薬剤投与例(UFT投与群20例、Tegafur投与群17例)であり、術前7日間おのおの600mg、分3とし経口連続投与を行った。手術時、腫瘍組織、正常部粘膜、リンパ節(1群領域)を採取し、凍結保存ののち各組織における5Fu濃度、TS量、TS阻害率を測定し、UFT群とTegafur群を比較した。また、11例の薬剤非投与例についても各組織のTS量を測定し、薬剤投与群と比較した。

【結果】 薬剤投与群における5Fu濃度の比較では腫瘍組織においてUFT群がTegafur群に比し、有意に高値であった。また、TS阻害率についてもUFT群で有意に高い阻害率を示した。各組織におけるTS量については、UFT群、Tegafur群とも薬剤非投与群に比較し、有意に高いTS量を示し、UFT群でより顕著であった。腫瘍組織におけるTS量とTS阻害率の関係をみると、TS量に関係なく、TS阻害率は50~60%の値であった。

【考察】 術前1週間連続投与による組織内5Fu濃度およびTS阻害率の検討を行い、腫瘍組織においてUFT群でTegafur群に比し有意に高い値を示し、大腸癌に対してもUFTがより強い抗腫瘍効果を期待しうる因子を備えていると考えられた。抗癌剤による組織内TSの変動については、腫瘍組織において非投与群にくらべUFT群で3.3倍、Tegafur群で2.4倍、正常粘膜でもおのおの3.2倍、2.3倍のTS誘導が生じていた。しかしながら、TS量に関係なくTS阻害率は50~60%に留まっていた。この程度の値が臨床投与量におけるUFT単独投与の限界とも考えられる。

最近の知見にて、5FuとCDDPあるいはLeucovorin併用効果のメカニズムが解明されている。5FuのDNA合成障害に最も重要となるFdUMP-TS-met-hylen hydrofolate (Me-THF) のternary complex形成の上で不足したMe-THFがCDDP代謝過程で補れることにより、ternary complex形成が促進され相乗効果を発揮することが証明されている。今回のTS量が増えても、阻害率に反映しないという結果もその原因をMe-THF枯渇に求めることもできるが、今後TSだけでなく、Me-THFの検索が必要であろう。

大腸癌は胃癌にくらべ、TSにより強く依存し、TS阻害により感受性が高いとも考えられており、薬剤側因子からみて大腸癌に対してもTegafurにくらべTSの誘導が強いUFTの効果が期待されるところである。