Induction of Dihydropyrimidine Dehydrogenase Expression by Mitomycin C in Colorectal Cancer

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ABSTRACT. Since thymidine phosphorylase (TP) is an essential enzyme for the activation of capecitabine to 5-fluorouracil (5-FU) in tumors, TP up-regulators should enhance the efficacy of capecitabine. Dihydropyrimidine dehydrogenase (DPD), on the other hand, is considered to be a key enzyme in the catabolism of 5-FU, and its high expression in a tumor is thought to reduce the efficacy of 5-FU against tumors. The aim of this study was to confirm whether or not mitomycin C (MMC) is a TP and/or DPD regulator. Biopsy specimens were obtained from 62 colorectal cancer patients preoperatively by colonoscopy. After a biopsy, 33 patients received neoadjuvant chemotherapy with MMC and underwent operations after 1-13 days. Using biopsy and operative specimens, TP and DPD levels in the tumors were examined. Patients were divided into three groups; an MMC(-) group (no MMC), a Short group (operation within four days after MMC) and a Long group (operation over six days after MMC). In the MMC(-) and Short groups, no significant differences in DPD levels before and after MMC were observed. In the Long group, on the other hand, DPD levels were elevated (p=0.026). As for TP, MMC did not raise the levels of TP in the MMC(-) and Short groups, but it tended to do so in the Long group (p=0.13). Although MMC appears to be a TP up-regulator, it is also a DPD up-regulator at appropriate intervals.

Key words ① **5-fluorouracil** ② **Dihydropyrimidine dehydrogenase** ③ **Doxifluridine** ④ **mitomycin C** ⑤ **Thymidine phosphorylase**

Capecitabine is an oral fluoropyrimidine that mimics continuous infusion 5-fluorouracil (5-FU). Conversion from capecitabine to 5-FU is dependent on the enzyme thymidine phosphorylase (TP), which is more highly expressed in tumor tissue than in healthy tissue, resulting in preferential generation of 5-FU at the tumor site[1)-3)]. Therefore, under administration of capecitabine, high expression of TP in tumor tissue is believed to indicate high expression of 5-FU in the tumor tissue and is connected to enhanced efficacy of

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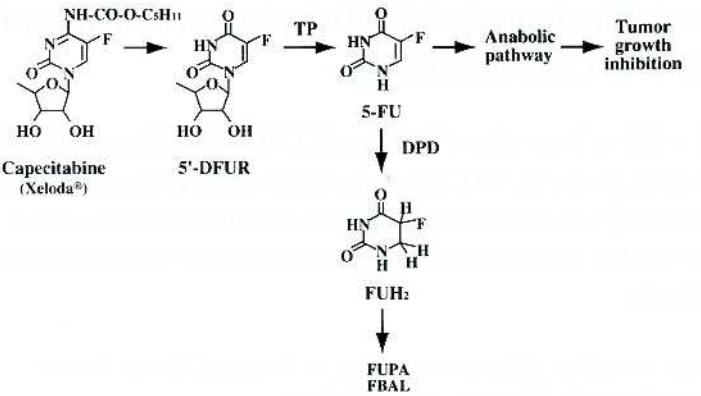

Fig. 1. Catabolism of capecitabine, doxifluridine, and 5-fluorouracil. 5'-DFUR, doxifluridine ; 5-FU, 5-fluorouracil ; TP, thymidine phosphorylase ; DPD, dihydropyrimidine dehydrogenase, FUH2, dihydrofluorouracil ; FUPA, 2-F-3-ureidopropionate ; FBAL, alpha-fluoro-beta-alanine.

capecitabine against the tumor. Therefore, the rationale for combining capecitabine with a TP up-regulator is fairly strong. Although previous studies with a human cancer xenograft model revealed that paclitaxel, docetaxel, mitomycin C (MMC), and X-ray irradiation greatly increased the expression levels of TP, there have been few clinical studies regarding this relationship[4)-6)]. Combination therapy with capecitabine and these TP up-regulators is expected to result in synergistic activities against tumors.

Dihydropyrimidine dehydrogenase (DPD), on the other hand, is considered to be a key enzyme in the catabolism of 5-FU, and its expression in a tumor is thought to reduce the efficacy of 5-FU against the tumor[7),8)] (Fig. 1). In humans, there has been no evidence that the abovementioned expected TP up-regulators, paclitaxel, docetaxel, MMC, and X-ray irradiation, change the levels of TP and DPD in tumors. The objective of this study was to confirm whether or not MMC is a TP and/or DPD regulator and a good partner of capecitabine or other fluoropyrimidines in the treatment of human colorectal cancer (CRC).

MATERIALS AND METHODS

Patients and Study Design

Between December 1998 and July 2001, patients with histologically proven resectable CRC were recruited into the study. Biopsy specimens were obtained preoperatively by colonoscopy from 62 CRC patients. After the biopsy, 33 patients received neoadjuvant chemotherapy with MMC (6mg/m^2) and underwent operations after 1-13 days. Using the biopsy and operative specimens, TP and DPD expression levels in the tumors were examined. This study is not a prospective nor a randomized one. Retrospectively, the patients were divided into two groups; i.e., a no chemotherapy group (MMC (-) group) (n=29) and a chemotherapy group (MMC (+) group) (n=33). The MMC (+) group was also divided into two sub-groups (Short and Long groups) according to the median of the period from administration of MMC to the operation.

Eligibility Criteria

To be eligible for the neoadjuvant chemotherapy, patients had to be <75 years of age and were required to have adequate renal function (creatinine < 1.2 mg /dl, creatinine clearance > 60 ml /min), hepatic function

(bilirubin <1.5 mg/dl, GOT < 40 IU/1, GPT < 40 IU /1), a blood hemoglobin level of > 10 mg/dl, a WBC count of > 4,000/ m^3, and a platelet count of >100,000 /m^3. The Eastern Cooperative Oncology Group performance status (PS) of all the eligible patients was 0. All of the patients who received MMC gave informed consent for the treatment as neoadjuvant chemotherapy. The 29 patents in the MMC (-) group did not receive neoadjuvant chemotherapy because 13 did not fulfill the eligible criteria, 9 did not need neoadjuvant chemotherapy since their cancer was in an early stage, and 7 refused neoadjuvant chemotherapy.

Measurement of TP and DPD expression

Tumor tissues measuring > 0.1 cm^3 were obtained preoperatively by colonoscopy and postoperatively from operative specimens. These were immediately frozen in liquid nitrogen and then stored at -80 ℃ until analysis. The TP and DPD expression in the tumor tissues was quantified with the enzyme-linked immunosorbent assay (ELISA) in collaboration with the Nippon Roche Research Center (Kanagawa, Japan). All the expression measurements obtained from the ELISA were adjusted based on the total protein concentration and were expressed as Unit /mg protein.

Statistical Analysis

In each group, TP and DPD expression levels in the tumors before neoadjuvant chemotherapy with MMC (TP-before and DPD-before) were compared with those after neoadjuvant chemotherapy with MMC (TP-after and DPD-after). The Wilcoxon paired tests were performed for intra-patient analysis of the difference, if any, in TP and DPD expression and the TP/ DPD ratio between the biopsy and operative specimens. The limit of statistical significance was P =0 .05.

RESULTS

Pilot cases

During the early period of this study, two patients gave us informed consent for treatment in the form of neoadjuvant chemotherapy with MMC and for biopsies three times before operation.

Case 1.

A 51-year-old female was admitted to our hospital with a four-month history of blood in her stool. Her height and weight were 152 cm and 46 kg, respectively. A colorectal endoscopic examination revealed an irregular mass in the rectum with a central deep ulceration. The histology of biopsy specimens revealed a well differentiated adenocarcinoma. Chest and abdominal computed tomography disclosed multiple lung and liver metastases. Tumor markers (CEA 27.6 ng/ml, CA19-9 1480 U/ml) markedly increased. The levels of both TP and DPD expression increased rapidly between the seventh and tenth days after administration of MMC (Fig. 2A).

Case 2.

A 72-year-old male was admitted to our hospital with a two-week history of blood in his stool. His height and weight were 164 cm and 45 kg, respectively. A colorectal endoscopic examination revealed an irregular mass in the rectum with a central ulceration, and the histology of biopsy specimens disclosed a moderately

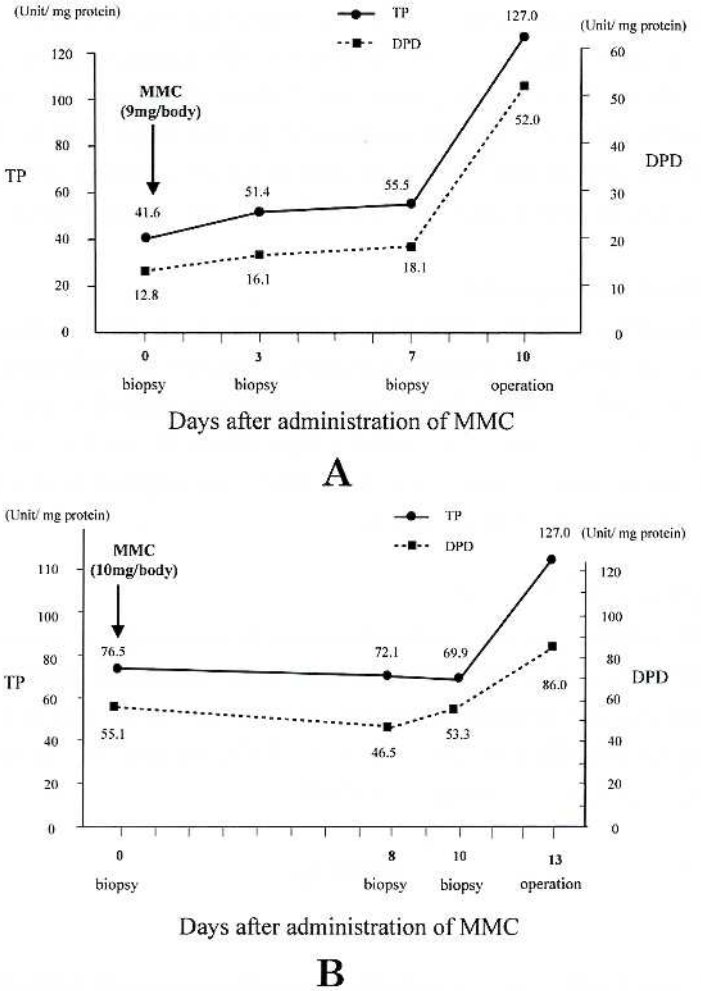

Fig. 2. Causes of TP and DPD changes in pilot cases

A case 1

Not only TP, but also DPD expression levels increased rapidly 7- 10 days after administration of MMC.

B case 2

TP expression levels increased rapidly and DPD levels increased mildly 10- 13 days after administration of MMC.

differentiated adenocarcinoma. The levels of both TP and DPD expression increased between the tenth and thirteenth days after administration of MMC (Fig. 2B).

Patient Characteristics

The characteristics of the patients in each group are shown in Table 1. Elderly patients and early clinical stage patients tended to be grouped in the MMC (-) group. In the MMC (+) group, the periods from MMC administration to operation ranged from one day to 13 days (average; 8.8 days, median; 4 days). The MMC (+) group was divided into two sub-groups according to the median of the periods; i.e., a group which underwent the operation within four days after administration of MMC (Short group) (n=17) and a group which underwent the operation over six days after administration of MMC (Long group) (n=16) (Fig. 3).

Table 1. Patient Characteristics

group	MMC (-)	MMC (+)	
		Short	Long
Number of patients	29	17	16
Males/Females	16/13	12/5	10/6
Mean age (range), years	67.2 (38-86)	59.2 (42-75)	62.7 (21-74)
Tumor site			
colon	14	9	4
rectum	15	8	12
Duke's classification			
A	12	2	7
B	6	10	3
C	7	3	2
D	4	2	4
Mean dose of MMC (range),mg/body	0	9.8 (8-12)	9.2 (8-10)
Mean time between MMC and operation, days	—	2.5 (1-4)	8.8 (6-13)

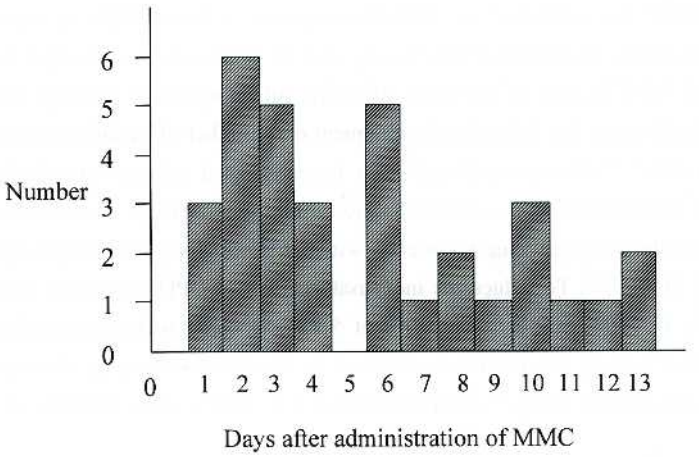

Fig. 3. Distribution of case numbers according to the periods from MMC administration to operation

MMC (+) Group

There were no differences between any of the patients who received MMC (n=33) TP-before and TP-after (76.4 ± 35.2 (SD) vs. 75.6 ± 42.8 Unit /mg protein, P=0.89). There were also no significant differences between those who received DPD-before and DPD-after (36.0 ± 16.4 (SD) vs. 41.0 ± 18.2 Unit /mg protein, P=0.23). As for the TP/DPD ratio, there were no differences between TP/DPD-before and TP/DPD-after (2.0 ± 0.8 (SD) vs. 2.2 ± 0.8, P=0.085).

1) Short Group

There were no differences between either TP-before and TP-after (87.0 ± 34.9 (SD) vs. 63.6 ± 28.8 Unit /mg protein, P=0.102), or DPD-before and DPD-after (38.4 ± 9.9 (SD) vs. 34.5 ± 13.3 Unit /mg

protein, P=0.463) (Fig. 4). Regarding the TP/DPD ratio, there were no differences between TP/DPD-before and TP/DPD-after (2.0 ± 0.7 (SD) vs. 2.3 ± 0.9, P=0.235).

2) Long Group

Although there were no differences between TP-before and TP-after (65.1 ± 33.0 (SD) vs. 88.4 ± 51.8 Unit /mg protein, P=0.134), DPD-after was higher than DPD-before (33.5 ± 21.4 (SD) vs. 47.8 ± 20.5 Unit /mg protein, P=0.026) (Fig. 5). As for the TP/DPD ratio, there were no significant differences between TP/DPD-before and TP/DPD-after (2.0 ± 0.6 (SD) vs. 2.2 ± 0.7, P=0.25).

MMC (-) Group

There were no differences between the TP expression levels in the tumors from biopsy specimens and those from operative specimens (82.0 ± 54.5 (SD) vs. 83.3 ± 36.3 Unit /mg protein, P=0.627) (Fig. 6A). As for DPD, there were also no differences between the DPD expression levels in the tumors from biopsy specimens and those from operative specimens (37.9 ± 22.1(SD) vs. 41.5 ± 20.4 Unit /mg protein, P=0.270) (Fig. 6B). Finally no differences were observed between the TP/DPD ratio in the tumors from biopsy specimens and those from operative specimens (2.4 ± 1.7 (SD) vs. 3.3 ± 5.8, P=0.581).

DISCUSSION

In this study, MMC was identified as a DPD up-regulator in human CRC at appropriate intervals. In combination therapy, MMC might reduce the efficacy of 5-FU against CRC and might not be a good partner for 5-FU. Although 5-FU is one of the most effective single agents in treating solid tumors, its low effectiveness as a single agent has led to the development of a number of modulators intended to enhance its therapeutic effectiveness[9),10)]. Some compounds have been expected to have synergistic anticancer activity with 5-FU[11)]. DPD, on the other hand, is the initial, rate-limiting enzyme in the catabolism of 5-FU, and its expression has been found to significantly correlate with 5-FU sensitivity, with high expression resulting in low sensitivity to 5-FU[7),12)~16)]. To reduce the inexcusable effect of DPD, eniluracil, which is a potent DPD inhibitor that results in 100% oral bioavailability of 5-FU, appeared with considerable expectations[17)~19)]. Although its antitumor activity has been demonstrated, the frequent occurrence of severe toxicity with this regimen has limited its clinical utility[18)]. There have been few reports about DPD up- or down-regulators in clinical studies[20)].

Quantification of TP and DPD expression with ELISA methods in CRC using biopsy specimens is considered to be a technically feasible method for assessing the expression of TP and DPD in tumors. In gastric cancer, immunohistochemical analysis of DPD expression using biopsy specimens has correlated with that of resected specimens[21)]. In this study, in the MMC (-) group, cases receiving no treatment, there were no significant differences in the expressions of TP and DPD between biopsy and operative specimens. Moreover, in this group, both TP and DPD expression in the biopsy specimens correlated with that of the operative specimens; i.e., Pearson's correlation coefficient (r) and the corresponding P values for these correlations were r =0.473 and 0.584, and P= 0.0088 and 0.0006 respectively.

For TP expression, MMC might be a good partner for capecitabine, but for DPD expression, it would not be. Capecitabine is an oral fluoropyrimidine that mimics continuous infusion of 5-FU and seems to be an effective drug against CRC[1),3)]. TP is an essential enzyme for the activation of the oral cytostatic drugs, such

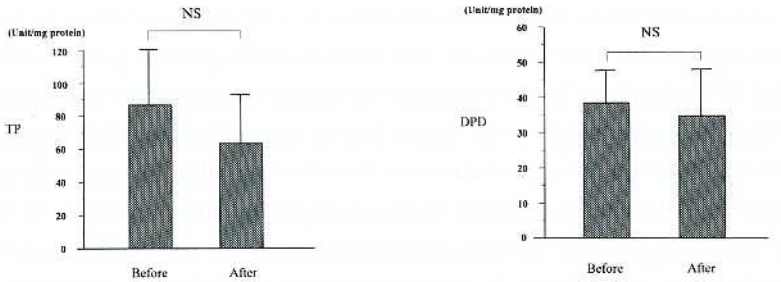

Fig. 4. TP and DPD changes in the Short group.
A, TP expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. There were no significant differences between TP-before and TP-after (n=17; P=0.102).
B, DPD expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. There were no significant differences between DPD-before and DPD-after (n=17; P=0. 463).
NS, not significant. Each bar represents the average value +/-SD.

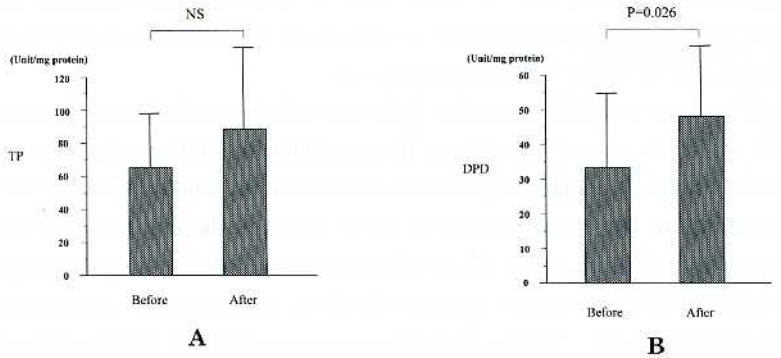

Fig. 5. TP and DPD changes in the Long group.
A, TP expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. There were no significant differences between TP-before and TP-after (n=16; P=0.134).
B, DPD expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. DPD after administration of MMC was higher than it was before (n=16; P=0. 026).
NS, not significant. Each bar represents the average value +/-SD.

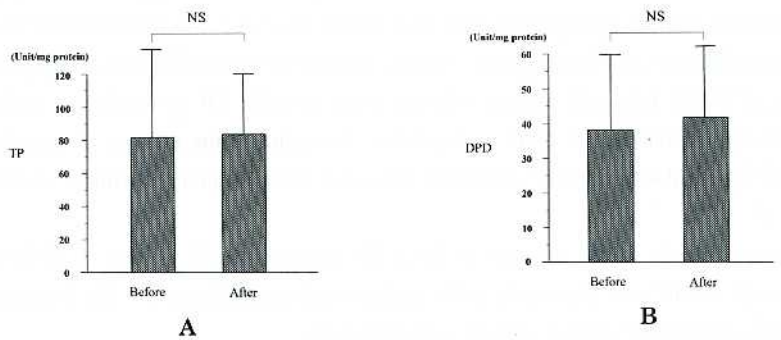

Fig. 6. TP and DPD expression levels in the MMC(-) group.
A, TP expression levels of biopsy and operative specimens. There were no significant differences between TP-before and TP-after (n=29; P=0.63).
B, DPD expression levels of biopsy and operative specimens. There were no significant differences between DPD-before and DPD-after (n=29; P=0. 27).
NS, not significant. Each bar represents the average value +/-SD.

as capecitabine and its intermediate metabolite doxifluridine, to 5-FU in tumors[22] (Fig. 1). In a clinical study with doxifluridine against CRC, there seemed to have been a better response in patients with high TP levels than in those with TP low levels[23]. Thus, the rationale for combining capecitabine or doxifluridine with a TP up-regulator is fairly strong. Several efforts to identify the best partner for capecitabine have been made, and, paclitaxel, docetaxel, MMC and X-ray irradiation were found to up-regulate TP expression in several human cancer xenografts[4)–6)]. Unfortunately, in those studies dealing with animals, changes in DPD expression levels were not given consideration. The abovementioned possible TP up-regulators were expected to be modulators of capecitabine in addition to having their own anti-cancer effect. A few studies were designed using a combination of capecitabine and MMC and tested clinically[24),25)]. In our study dealing with humans, although there were no significant differences, MMC tended to be a TP up-regulator in the Long group. Simultaneously, DPD expression levels were elevated significantly after the administration of MMC in the Long group. TP and DPD were not up-regulated in the Short group. In pilot cases, TP and DPD were up-regulated at more than 10 days after the administration of MMC. These findings may indicate that it takes several days, approximately 10 days, for TP and/or DPD to be up-regulated by MMC. Therefore, MMC appears to be a DPD up-regulator in human CRC at appropriate intervals. However, it is still unclear how long MMC maintains high TP and/or DPD expression levels.

The ratio of TP expression to DPD expression in the tumors before and after the administration of MMC did not change in any of the groups. The efficacy of capecitabine was influenced by not only TP but also DPD. These cytostatic drugs do not play a cytotoxic role until metabolization to 5-FU by TP. Then the 5-FU generated is catabolized to dihydrofluorouracil by DPD. Although the expression of DPD, which is a rate-limiting enzyme in catabolism, might be an independent representation for predicting the efficacy of 5-FU, the ratio of TP to DPD expression in tumors has been emphasized for predicting the efficacy of capecitabine and doxifluridine[23),26),27)]. In this study, the expression of TP and DPD tended to be up-regulated by MMC, but the ratio of TP to DPD expression remained unchanged by MMC in all three groups.

Although the efficacy of MMC itself should not be overlooked, MMC does not seem to optimize the efficacy of capecitabine therapy on the whole. The combination of capecitabine therapy with TP and DPD modulators could be one rational approach, and it would be worthwhile to carry out further studies. As for the expression levels of both TP and DPD, a combination of capecitabine therapy and MMC therapy seems to have additive efficacy, but not synergic efficacy. As a matter of course, a combination of 5-FU therapy and MMC therapy would lead to less than additive efficacy, because elevated DPD does not play a favorable role for the efficacy of 5-FU. It is still unclear whether other possible TP up-regulators, such as paclitaxel, docetaxel, and X-ray irradiation, are DPD up-regulators. To optimize the efficacy of capecitabine therapy, these possible TP up-regulators must be confirmed to be real TP up-regulators without elevation of DPD in humans.

In conclusion, although MMC appears to be a TP up-regulator, it is also a DPD up-regulator at appropriate intervals. MMC may play a role in the activation of capecitabine to 5-FU in tumors, but it might be an antagonistic agent for the efficacy of 5-FU against tumors.

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