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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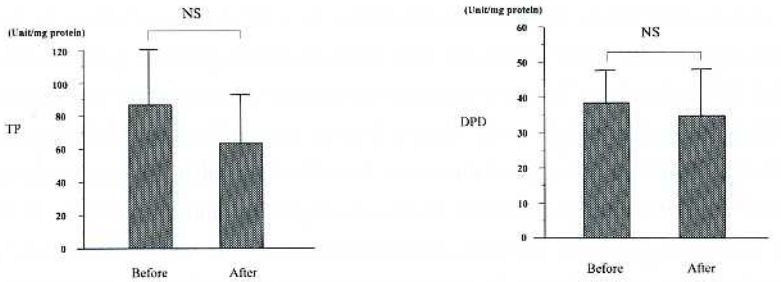

Fig. 4. TP and DPD changes in the Short group.
A, TP expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. There were no significant differences between TP-before and TP-after (n=17; P=0.102).
B, DPD expression levels in the tumors before and after neoadjuvant chemotherapy 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.

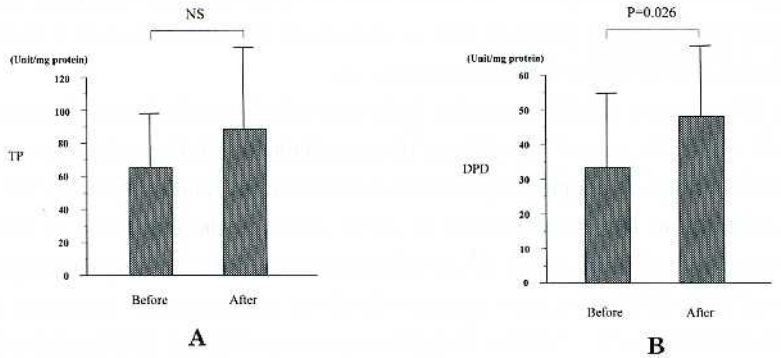

Fig. 5. TP and DPD changes in the Long group.
A, TP expression levels in the tumors before and after neoadjuvant chemotherapy with MMC. There were no significant differences between TP-before and TP-after (n=16; P=0.134).
B, DPD expression levels in the tumors before and after neoadjuvant chemotherapy 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.

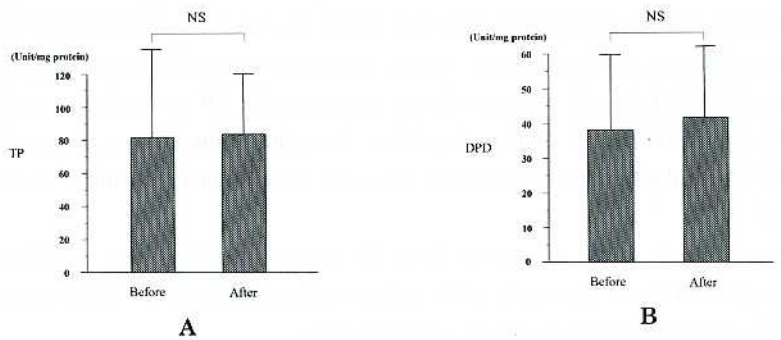

Fig. 6. TP and DPD expression levels in the MMC(-) group.
A, TP expression levels of biopsy and operative specimens. There were no significant differences between TP-before and TP-after (n=29; P=0.63).
B, DPD expression levels of biopsy and operative specimens. There were no significant differences between 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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