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Discovery of Capecitabine (Xeloda®) and Its Translation to Clinical Studies

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Introduction

This paper reviewed how we developed the anticancer drug capecitabine (Xeloda®) and promoted its clinical studies [1-10]. We started this project in 1986 to design and synthesize orally available cytotoxic compounds that have high tumor selective actions with little myelotoxicity or intestinal toxicity, and therefore could be safely given for daily oral treatment. Cytotoxic drugs generally lack tumor selective activity and act not only on cancer cells but also on rapidly growing normal cells, such as granulocyte progenitor cells in the bone marrow and cryptic cells in the intestinal mucosa, resulting in adverse events in these normal tissues. Consequently, such cytotoxic drugs could not be prescribed frequently for long periods or at a dose level sufficiently high to cure cancer. Cancer patients prefer oral administration at home if it is at least as effective as intravenous administration at hospitals. However, oral administration of cytotoxic drugs gives high drug concentrations at the local sites, intestine and liver, and often results in treatment-associated adverse events.

Our strategy of identifying new oral cytotoxic compounds with high tumor selective action was to design and synthesize an orally available prodrug that generates an active drug by enzymes located in tumors [1-4]. We previously developed a prodrug, 5'-deoxy-5-fluorouridine (5'-DFUR) (Furtulon®), that is metabolized to the active drug

5-FU by thymidine phosphorylase (TP), an enzyme preferentially located in tumors. 5'-DFUR shows better efficacy than does 5-FU in many experimental tumor models and has been clinically used. However, it causes intestinal toxicity and, to a little extent, myelotoxicity when orally given at high doses for long periods. We therefore tried to synthesize in a rational manner a novel fluoropyrimidine with more improved efficacy and safety profiles than those of 5'-DFUR and 5-FU. These studies enabled us to identify capecitabine (Xeloda®, N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) with improved efficacy and increased safety profiles over those of 5-FU, 5'-DFUR and other fluoropyrimidines [1-3].

Drug design and discovery of capecitabine

To minimize the myelotoxicity and increase the tumor selective activity of 5'-DFUR, we at first synthesized 5'-deoxy-5-fluorocytidine (5'-DFCR) [Fig. 1]. It is metabolized to 5'-DFUR by cytidine (Cyd) deaminase, the enzyme highly expressed in the liver, and solid tumors [4] of humans, whereas it is only slightly expressed in immature, growing marrow cells compared to that in mature, normal granulocytes. It was therefore expected that 5'-DFCR would generate 5'-DFUR at high concentrations in the liver and tumors but not in the granulocyte progenitor cells because of this unique localization of the enzyme. Then, to minimize the generation of 5-FU and 5'-DFUR in the intestine, we synthesized hundreds of N^4-substituted-5'-DFCR derivatives that would pass intact through the intestine and then be converted to 5'-DFCR by hepatic enzymes in mice. Among them N^4-trimethoxybenzyl-5'-DFCR (galocitabine) was much less toxic to the bone marrow or intestinal tract than 5'-DFUR and could be given at higher doses showing better antitumor efficacy in mouse tumor models. In the human liver it was, however, not efficiently metabolized to 5'-DFCR in humans because of its very low

susceptibility to the enzyme, acylamidase [1-3].

Then, we tried to identify N^4-substituted 5'-DFCR, which would be converted to 5'-DFCR only by hepatic enzymes from human specimens and monkeys, and also tried, in parallel, to identify hepatic enzymes responsible for such catabolic conversion. Among various N^4-substituted 5'-DFCR synthesized, N^4-alkoxycarbonyl-5'-DFCR derivatives were converted to 5'-DFCR only by carboxylesterase, which activity almost exclusively exists in the liver and hepatoma [4]. Such derivatives given oral would pass intact through the intestinal mucosa and be metabolized to 5'-DFCR in the liver. Capecitabine was selected for further development among a large number of N^4-alkoxycarbonyl-5'-DFCR based on their susceptibility to the human/monkey hepatic carboxylesterase, oral bioavailability in monkeys, chemical stability at acidic pH, etc [2,3].

Tumor selective delivery of the active 5-FU

Capecitabine and its intermediate metabolites, 5'-DFCR and 5'-DFUR, are not cytotoxic by themselves but become cytotoxic after conversion to 5-FU [4]. Capecitabine, administered orally, selectively yielded high intratumor concentrations of 5-FU in human cancer xenograft models as rationally intended [5]. When 5-FU (i.p.) was given at the maximum tolerated dose to mice bearing the HCT116 human colon cancer xenograft, it yielded generally uniform concentrations of 5-FU in the plasma, muscle and tumors (Fig. 2). In contrast, capecitabine, which can be safely given at a much higher dose because of higher safety margin, generated higher levels of 5-FU selectively in tumor tissue. 5'-DFUR (p.o.) also yielded selectively in tumors but at an extent lesser than that by capecitabine. As a result, capecitabine was more effective than 5-FU and 5'-DFUR in the tumor model studied. The tumor selective 5-FU delivery was

also observed in a pharmacodynamic study in patients.

Antitumor Activities

Capecitabine is more potent and has a wider spectrum of antitumor activity than 5-FU, 5'-DFUR, or UFT against 24 human cancer xenograft models of colon, breast, gastric, cervical, bladder, ovarian and prostate cancer [6]. In these experiments, capecitabine administered orally at MTD was effective (defined as >50% growth inhibition) in 18 models and inhibited tumor growth by more than 90% in 7 models (Table 1). In contrast, 5'-DFUR was effective in 15 models and inhibited tumor growth by >90% in only one model. 5-FU and UFT were effective in one and five models, respectively, but they did not inhibit the growth by >90%. Capecitabine thus showed activity against tumors that are resistant to 5-FU and UFT *in vivo*. In similar experiments, capecitabine showed antitumor activity in dose ranges much broader than those of 5-FU, 5'-DFUR, and UFT in mice bearing the human xenografts HCT116 and CXF280, which are respectively the intermediate and the highest level of susceptibility to fluoropyrimidines among various tumor models studied [7]. The therapeutic index (defined as the ratio of the lowest toxic dose to ED_{50}, the minimum dose inhibiting tumor growth by 50%) in the CXF280 model was 94 for capecitabine, versus 2.7 for 5-FU when measured in the only model susceptible to 5-FU.

Preclinical Studies for Additional Clinical Studies

Capecitabine has proved to be clinically effective in breast, colorectal and gastric cancers. To optimize its efficacy, we studied to obtain an insight into its optimal regimen and best partners in combination therapy. In these studies, we observed that tumor levels of TP and DPD (dihydropyrimidine dehydrogenease), the enzymes for respectively generating and catabolizing 5-FU, affected the susceptibility to

capecitabine [6]. Tumors transfected with TP gene became more susceptible to 5'-DFUR in cell cultures, whereas those transfected with DPD gene became less susceptible to capecitabine treatment in human cancer xenografts. In addition, the susceptibility of 24 human cancer xenografts to capecitabine, which was defined as >50% growth inhibition by capecitabine at MTD, correlated well with the TP level ($p = 0.0164$) and TP/DPD ratio ($p = 0.0015$) in tumor tissues (Fig. 3)[6]. Since the inter-patient variability of these enzyme levels is large, these enzyme levels and their ratio would impact on the individual drug response to capecitabine.

We also tried to identify drugs, which up-regulate TP levels in tumor tissues, as rational partners in combination therapy with capecitabine. Previously, we observed that inflammatory cytokines TNFα, IL-1α, and IFNγ induce an increase in both TP mRNA expression and enzyme activity in human cancer cell lines and made tumor cells more susceptible to 5'-DFUR accordingly. We also found that either selected cytotoxic drugs, such as taxanes [8] and cyclophosphamide [9], or X-ray irradiation [10] up-regulate TP activity in tumors, and thereby enhanced the efficacy of capecitabine in human cancer xenografts [Fig. 4]. These cytotoxic drugs and X-ray irradiation simultaneously increased the tumor levels of human TNFα, which in turn up-regulated TP in the tumor cells in the cancer xenografts [8,10]. Interestingly, the TP up-regulation was not observed in the liver or intestine. Capecitabine also showed additive to synergistic efficacy in combination with other standard cytotoxic drugs, such as cyclophosphamide and methotrexate, cyclophosphamide and doxorubicin, gemcitabine, and irinotecan, in selected xenograft models. Furthermore, it was much more effective than 5-FU also in combination therapy.

Conclusion

We rationally designed capecitabine that generates 5-FU preferentially within tumors in humans through three sequential enzymatic reactions. The enzymes include carboxylesterase located in the liver, Cyd deaminase preferentially located in the liver and many solid tumors, and TP, which has a higher concentration in tumor tissues than in healthy tissues. Capecitabine orally given is therefore sequentially converted to 5-FU preferentially in tumors through the non-cytotoxic intermediate metabolites 5'-DFCR and 5'-DFUR. Consequently, it can be safely given at high doses delivering high levels of 5-FU in tumors and showing better efficacy than 5-FU, 5'-DFUR and UFT in tumor models. The minute expressions of Cyd deaminase in the granulocyte progenitor cells may also explain the low myelotoxicity of capecitabine in clinic. Capecitabine is now being proved to be clinically effective for the treatment of breast, colorectal and gastric cancers, and the present approach for drug targeting that utilizes enzymes with unique tissue localization would be useful for identifying tumor-targeting cytotoxic drugs with improved efficacy and safety profiles.

We further provided various preclinical data to promote additional clinical studies with capecitabine. The efficacy of capecitabine in human cancer xenograft models correlated well with TP levels and the ratio of TP to DPD in tumor tissues, the enzymes generating and catabolizing 5-FU, respectively. Therapeutic benefit from capecitabine therapy would therefore increase when it is given to patients with high tumor TP/DPD. Up-regulating the enzyme TP in tumors would also optimize the efficacy. In selected human cancer xenografts, several cytotoxic drugs, such as taxanes, or X-ray irradiation up-regulated the enzyme activity in tumors and consequently enhanced the efficacy of capecitabine. In clinical trials, the combination of capecitabine with taxotere showed survival benefits as compared with taxotere alone in breast cancer patients. Capecitabine in combination with additional TP up-regulators, such as X-ray irradiation, is being clinically assessed with great anticipation.

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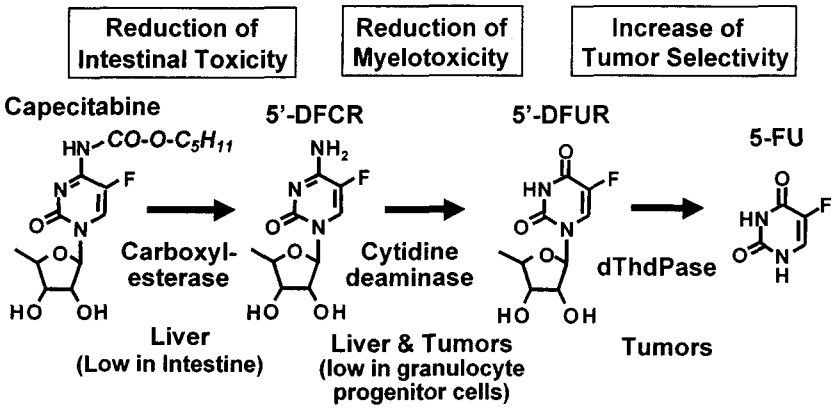

Fig. 1 Drug Design of Capecitabine

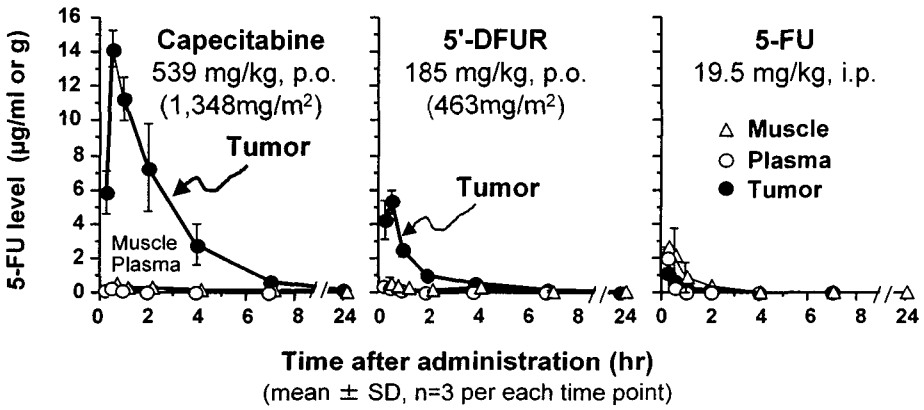

Fig. 2 Tumor selective 5-FU delivery by capecitabine in mice bearing HCT116 human colon cancer xenograft. Three drugs were given at their MTD.

Table 1 Capecitabine efficacy superior to other fluoropyrimidines

Growth inhibition	Response Rate % (Sensitive models / 24 models tested*) Capecitabine	5'-DFUR	5-FU	UFT
>50%	75% 18/24	63% 15/24	4% 1/24	21% 5/24
>90%	29% 7/24	4% 1/24	0% 0/24	0% 0/24

Drugs were orally given at their MTD for 2-4 weeks
24 Models: Colon (7), gastric (3), breast (5), cervix (4), bladder (2), ovary (2) and pancreas (1) cancer xenografts

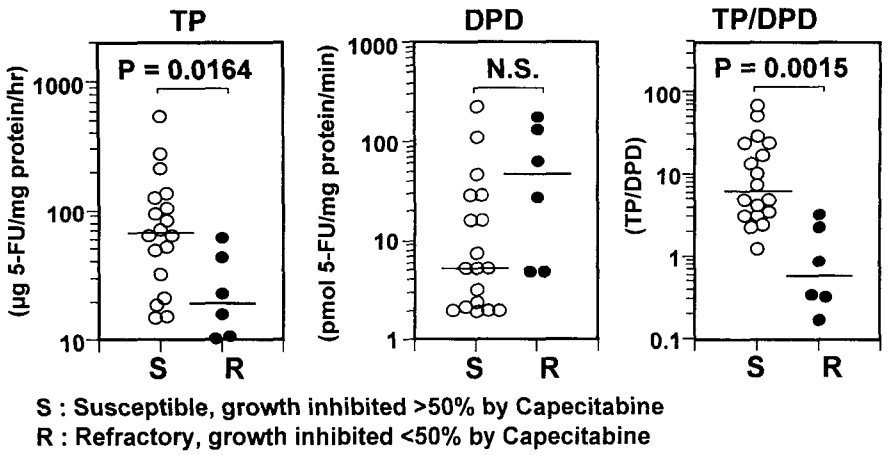

Fig. 3 Positive correlation between the susceptibility of human cancer xenografts (n=24) to capecitabine and tumor TP levels or TP/DPD

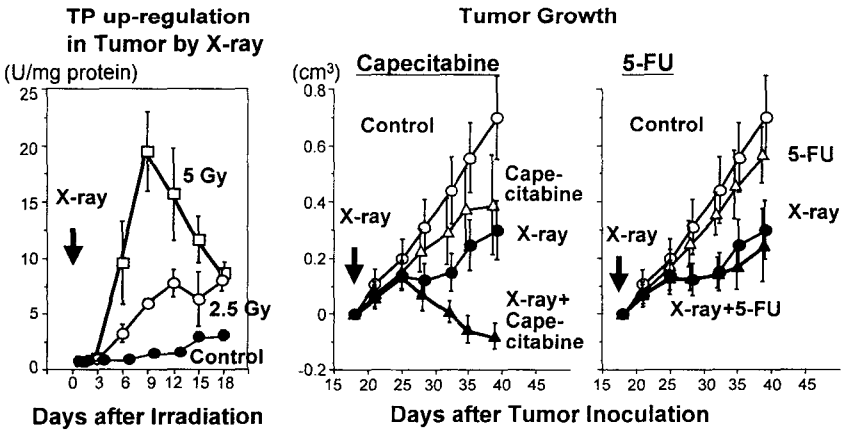

Fig. 4 TP up-regulation in tumors by X-ray irradiation and synergy with X-ray and capecitabine treatment in WiDr human colon cancer xenograft, the line refractory to capecitabine alone