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ORIGINAL RESEARCH

Development of an Early-Warning Model for Predicting the Capecitabine-Induced Diarrhea

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Objective: Diarrhea is primary adverse effect of capecitabine (Cap) causing treatment discontinuation. The aim of this study was to construct an early-warning model for predicting the Cap-induced diarrhea.

Methods: A Cap-induced diarrhea model in mice was constructed in 36 mice, and the exposure levels of Cap and its five metabolites were quantified in plasma and colon and correlated their exposure levels to the diarrhea. The concentrations of metabolic enzymes and drug transporters were quantified in the colon of 62 colorectal cancer (CRC) patients, and an early-warning model was constructed using binary logistic regression.

Results: Totally, 15 out of 36 mice were identified as diarrhea mice, and the exposure levels of Cap and metabolites did not show any differences between diarrhea and non-diarrhea mice in plasma, but in colon, the Cap and metabolites, except for dihydrofluorouracil and 5-fluoro-2'-deoxyuridine, presented significantly higher exposure levels in diarrhea mice. Furthermore, the expression levels of metabolic enzymes and drug transporters in the colon differed distinctly between diarrhea and non-diarrhea CRC patients. Finally, a binary logistic model based on cytidine deaminase (CDA) and solute carrier family 22 member 7 (SLC22A7) was constructed for early-warning of diarrhea induced by Cap: Y = 0.028 × CDA (pg/mL) - 0.518 × SLC22A7 (pg/mL) + 1.526, with the area under curve of 0.907 (specificity 100.0%, sensitivity 71.4%) for diarrhea CRC patients.

Conclusion: This study constructed and validated, for the first time, an early-warning model of diarrhea caused by Cap based on metabolic enzymes and drug transporters in normal colon tissue, which may provide a new basis for accurate medication for CRC treatment in clinical practice.

Keywords: capecitabine, exposure level, metabolic enzyme and transporter, diarrhea, predicting model

Introduction

The colorectal cancer (CRC) is one of the most common cancers around the world, with the third incidence and second mortality in all cancer types.[1] Capecitabine (Cap), a first-line oral prodrug utilized to treat CRC, was converted to 5-fluorouracil (5-FU) via three enzymes, including carboxylesterase (CES), cytidine deaminase(CDA) and thymidine phosphorylase (TP),[2–4] and 5'-deoxy-5-fluorocytidine (5'-DFCR), doxifluridine (5'-DFUR), 5-FU, dihydrofluorouracil (FUH_2) and 5-fluoro-2'-deoxyuridine (2'-DFUR) were the major metabolites of Cap.[5,6] Finally, 80% of 5-FU was inactivated by dihydroxypyrimidine dehydrogenase (DPD) and excreted in urine.[7] Although the overall toxicity of Cap was lower than that of 5-FU, there were still more than 57% of CRC patients required dose adjustment and 7% of therapeutic regimens were discontinued due to adverse effects when prescribed with Cap, of which the diarrhea was the

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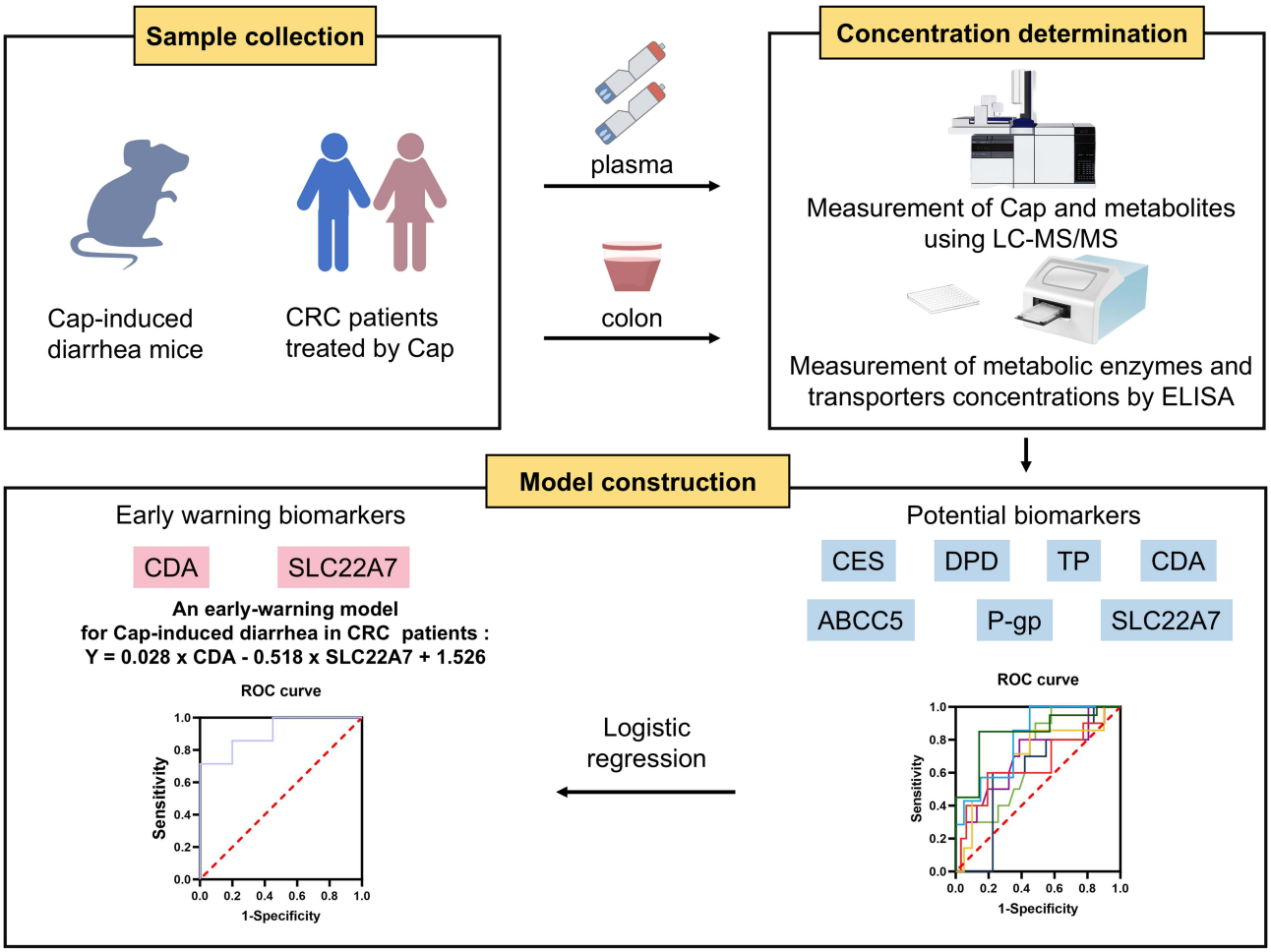
Graphical Abstract



most common.[8–10] Park, et al[11] analyzed the serious adverse effects associated with Cap in Korean patients from 2021 to 2024 and found that the most common adverse effect resulting in treatment discontinuation was ≥grade 3 diarrhea.

At present, studies have shown some factors may be associated with the diarrhea induced by Cap. The factors age >65 and female sex were reported to show higher risk for severe diarrhea during preoperative chemoradiation therapy with Cap.[12] García-González et al[13] speculated that some specific genomic variations may be related to Cap-induced diarrhea, for example, p-glycoprotein*1 (P-gp*1); deficiency or activity decrease of DPD also resulted in reduced clearance of 5-FU, therefore, diarrhea may also be associated with prolonged exposure and accumulation of toxic metabolites.[14] Membrane drug transporters have been recognized as critical determinants of intracellular drug concentrations and had been implicated in both adverse effects development and drug resistance to fluoropyrimidine-based therapies. As the first identified member of the ATP-binding cassette (ABC) transporter superfamily, P-gp (ABCB1) has been well-characterized as a membrane transporter capable of mediating 5-FU efflux from cancer cells, thereby contributing to either drug resistance or intracellular 5-FU accumulation.[15,16] Similarly, ATP-dependent multidrug resistance protein 5 (MRP5, ABCC5), another ABC transporter family member, has been experimentally confirmed to confer 5-FU resistance through transportation of monophosphate metabolites.[17] Solute carrier family 22 member 7(SLC22A7), a member of the SLC superfamily, has been identified as an organic anion transporter responsible for the cellular uptake and efflux of various xenobiotics (eg, drugs) and endogenous metabolites.[18] Some researchers have found significant associations between SLC22A7 SNPs (rs2270860/rs4149178) and capecitabine-induced skin toxicity/severe diarrhea in CRC

patients.[19] Cap-induced diarrhea may involve in intestinal epithelial injury caused by reactive oxygen species.[20–22] A further link has been established between the intestinal toxicity of 7-ethyl-10-hydroxycamptothecin (SN-38), cyclooxygenase-2-mediated inflammation, and prostaglandin release;[23] However, the mechanisms of diarrhea seem to be more than that for Cap.

As the diarrhea may disrupt the therapeutic regimens and even bring severe harm to the patient, early warning of diarrhea is of particularly importance, however, predictive models for diarrhea in CRC patients undergoing chemotherapy based on Cap have not yet been developed. Based on animal model and CRC patients, this study found that Cap-induced diarrhea was related to the concentrations of Cap and its metabolites in the colon, other than that in plasma and this results were supported by liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis, which revealed higher mean accumulations of Cap, 5-FU and FUH2, respectively, in colon tissue than that in plasma, although plasma concentrations of 5'-DFUR and 5'-DFCR in diarrheic mice were higher than those in colon, no statistically significant differences ($p > 0.05$) were observed when compared to plasma concentrations in non-diarrheic mice. This study further analyzed the differences in metabolic enzymes and drug transporters between diarrhea and non-diarrhea CRC patients. Finally, we developed and validated a novel early-warning model based on metabolic enzyme and transporter levels, which was rigorously validated in this study, for predicting diarrhea occurrence in CRC patients receiving Cap treatment.

Materials and Methods

Chemicals and Reagents

Capecitabine (Cap, Xeloda, pharmaceutical grade, ≥99.5% purity, lot. SH2819) was offered by Shanghai Roche Pharmaceuticals (Shanghai, China). Additional reagents and solvents (HPLC grade) used for UHPLC-MS/MS were obtained from Merck (Darmstadt, Germany) unless otherwise specified. Enzyme-linked immunosorbent assay (ELISA) kits (research grade), including DPD, TP, CDA, CES, P-gp, SLC22A7, ABCC5 were purchased from Shanghai Xinyu Biotechnology Co., Ltd. (Shanghai, China, lot. XY202210102, 202305079, XY202309024). All kits were validated for intra-assay coefficients of variation <8% and inter-assay coefficients of variation <11%. 4% paraformaldehyde (ACS grade, lot. GP22123012709), paraffin (histological grade, lot. WGHB319213129), hematoxylin and eosin staining kits (histological grade, lot. GP22122391015) were purchased from Wuhan Servicebio Technology Co., Ltd (Wuhan, China).

Animals and Treatments

Totally 42 ICR mice (SPF grade, 5 weeks old, male) were purchased from Shanghai Sipul-Bicai Laboratory Animal Co., Ltd. (Shanghai, China), and were free-ranging in SPF environment for 7 days before modeling. The protocol of this animal study was approved by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine according to the guidelines (Ethical number: PZSHUTCM210715004) and strictly adhered to the animal welfare guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals. The mice were randomly divided into two groups: control group (n = 6) and experimental group (n = 36). A suspension of Cap in 0.5% CMC-Na solution was prepared at 275 mg/kg and administered intragastrically to the mice in the experimental group for consecutive 14 days (bid), and for mice in the control group, 4 mL/kg of 0.5% CMC-Na was given for 14 days (bid). The occurrence of diarrhea in mice was assessed and the body weight of all mice were weighted every 3 days. After the administration, all the mice were sacrificed on day 15, and the plasma samples, colon samples were collected for the analysis of drug exposure levels of Cap and metabolites and the metabolic enzymes and transporters. The detailed protocol of animal study can refer to our previous study.[24,25]

Hematoxylin and Eosin Staining

Colon sample from mice in each group preserved in 4% paraformaldehyde were embedded in paraffin, sliced into 5 μm-thick sections, and stained with hematoxylin and eosin staining. Morphological changes of the colon samples were observed under the microscope, including the morphology and number of intestinal villi, the number of cup cells, and the depth of crypts, etc.

Patients and Sample Collection

This study enrolled the CRC patient the same as a previously published trial.[26] A total of 62 patients were included, with a mean age of 56.83 years (range 28–81), including 66.13% males. Inclusion criteria were: 1) age ≥18 years old, 2) biopsy-confirmed CRC, and 3) treatment consisting of surgical resection followed by adjuvant Cap-based chemotherapy. The study protocol was approved by the Ethics Committee of Second Affiliated Hospital of Naval Medical Univeristy (Shanghai Changzheng Hospital, 2016SL007, and registered at www.clinicaltrials.gov, NCT03030508), and every patient approved and signed the informed consent. The study was carried out in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The patients who were diagnosed with CRC and adjuvantly treated with Cap-based chemotherapy were enrolled in Shanghai Changzheng Hospital between January 2018 and April 2019. The normal colonic sample were collected in the surgery, washed with normal saline and flash frozen in liquid nitrogen. The samples were finally stored at −80°C until retrieval. Patients were followed up in the treatment period, and an increase in frequency and/or loose or watery bowel movements were classified as diarrhea group according to the Common Terminology Criteria for Adverse Events (CTCAE, Version 5.0), or the patients were designated into non-diarrhea group. While the diagnostic model development did not include diarrhea grading as factor, this study documented diarrhea severity during data collection for baseline characteristic analysis.

Metabolites Extraction and Quantification Analysis

Plasma and colon samples were pretreated with liquid–liquid extraction according to the quantitative method established by our group.[27] Simply speaking, the tissue grinding was first carried out, and about 50 mg of tissue was taken and homogenized after adding 1 mL cold 1 × PBS by tissue tearor (F6/10, Shanghai Fluke Fluid Machinery Manufacturing Co., LTD), then, the extractant (ethyl acetate: isopropanol=19:1, V:V) solution containing internal standard (10 ng/mL for both fludarabine and 5-chloropyrimidine) was added at a ratio of 1:30 (V:V) to extract the Cap and metabolites; the mixture was then processed using the same procedures in the plasma sample pretreatment. Finally, the supernatant was injected directly into Agilent 1290–6460A ultra-high performance liquid chromatography tandem mass spectrometer (UHPLC-MS/MS) system for analysis.

Determination of Metabolic Enzymes and Transporters

Colon samples (~30 mg) from mice were added with cold 1 × PBS in a 1:3 ratio (V:V), and the tissues were thoroughly grinded with tissue tearor. After centrifugation at 4°C, 12000 × g for 20 min, the supernatant was collected for the determination of metabolic enzymes DPD, TP, CDA, CES using the ELISA kits according to the manufacturer's instructions, respectively.

For human colon sample, briefly speaking, the collected colons (~300 mg) from CRC patients were processed using the same way as described above to obtain the supernatant for the detection of metabolic enzymes (DPD, TP, CDA, CES) and transporters (P-gp, SLC22A7, ABCC5) using the ELISA kits.

Statistical Analysis

The data were shown as mean ± SD, and Student's *t*-test or Mann–Whitney *U*-test was performed to compare drug concentrations, metabolic enzymes and membrane transporters contents between diarrhea mice (patients) and non-diarrhea mice (patients), and each data was calculated from at least 5 individual experiments or subjects. $P < 0.05$ was considered statistically significant. A predictive model for Cap-induced diarrhea was developed by incorporating variables (metabolic enzymes and membrane transporters) into a binary logistic regression analysis, identifying those variables that were significantly correlated with diarrhea and calculating the regression coefficients. Predictive capacity, specificity and sensitivity were determined using subject operating characteristic (ROC) curve analysis,[28] and the cutoff value was selected when the Youden's index reached maximum. Data processing and model construction for this experiment were accomplished through GraphPad Prism 10 and SPSS 26.0.

Results

Establishment of Cap-Induced Diarrhea Model in Mice

This study successfully established a Cap-induced diarrhea mouse model to investigate the pathogenesis and risk factors. After two weeks of treatment, 15 (41.6%) mice in the experimental group developed diarrhea, as shown in Figure 1A, which was manifested by sticky perianal hair, yellow and wetter feces (normal feces of mice was black and hard). For the mice in control group, there were no any signs of diarrhea appeared. Throughout the experiment, the weight of mice in the control group continued to increase, in contrast to a significant decrease in the weight of mice in the experimental group (Figure 1B and D). Hematoxylin and eosin-stained sections showed that mice with diarrhea exhibited significantly reduced intestinal villi, atrophied, edematous, and degenerated mucosal structure, with even partial mucosal detachment, infiltration of inflammatory cells, reduction of goblet cells, and shortening of crypts (Figure 1C). In addition, the diarrhea mice in experimental group presented obviously lower body weights than the non-diarrhea mice in experimental group (Figure 1E).

Exposure Level Analysis of Cap and Its Metabolites in Mouse

As the Cap could induce diarrhea only for 41.6% of the mice in the experimental group, all the mice in this group were divided into two subgroups, diarrhea mice and non-diarrhea mice, to compare whether there were any differences in the exposure levels of Cap and its metabolites. As shown in Figure 2A, the 5'-DFCR and 5'-DFUR presented higher exposure levels compared with other metabolites, but there were not any differences of exposure levels of Cap and its metabolites between the two subgroups, while in the colon, all the metabolites except 2'-DFUR displayed relatively high exposure levels, and the exposure levels of Cap and its metabolites in the colon, including 5'-DFUR, 5-FU, and 5'-DFCR, were found to be significantly higher in diarrhea mice than that in non-diarrhea mice (Figure 2B). Exposure pattern of Cap and its metabolites in colon distinctly differed from that in plasma.

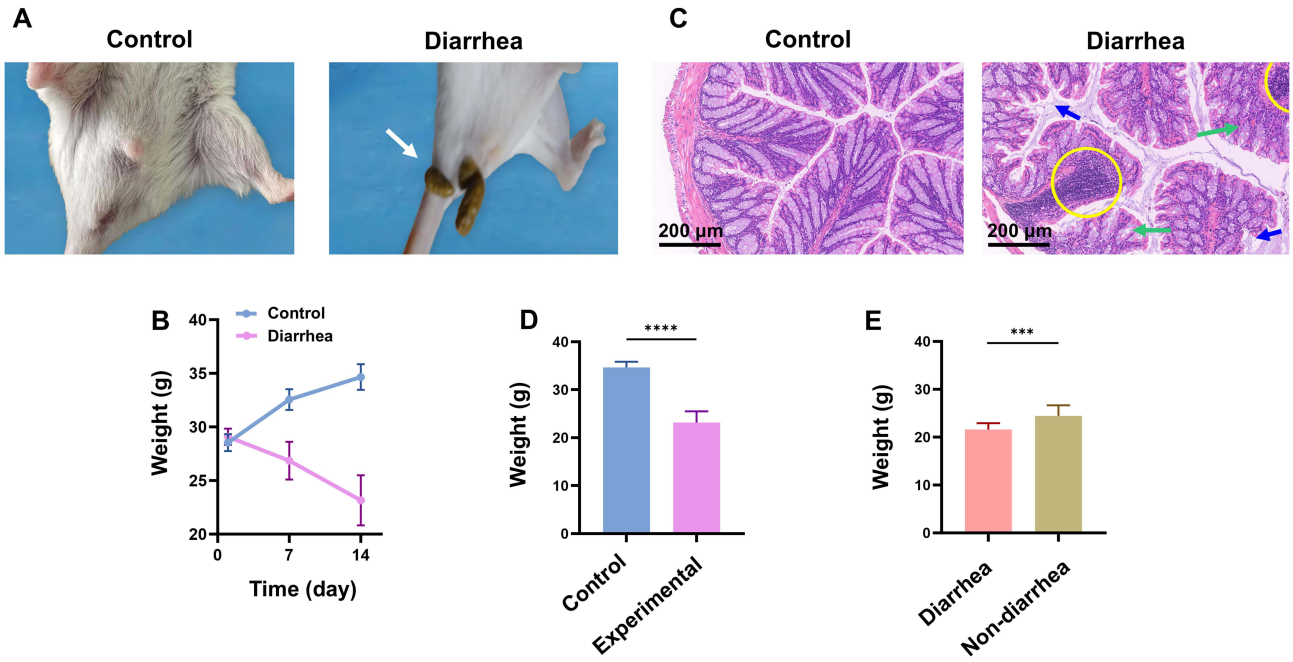


Figure 1 Construction of Cap-induced diarrhea model in mice. (**A**) comparative pictures of perianal conditions in diarrhea and non-diarrhea mice(white arrow for loose stools). (**B**) time-body weight curves of mice in control group and experimental group. (**C**) hematoxylin and eosin staining of colon sample (×20)(blue arrows for villi atrophy, green arrows for crypt damage, and yellow circles for inflammatory infiltrates). (**D**) body weights of mice between control group (n = 6) and experimental group (n = 36) on day 14. (**E**) body weight comparison of diarrhea (n = 15) and non-diarrhea (n = 21) mice in experimental group. ***p < 0.001, ****p < 0.0001.

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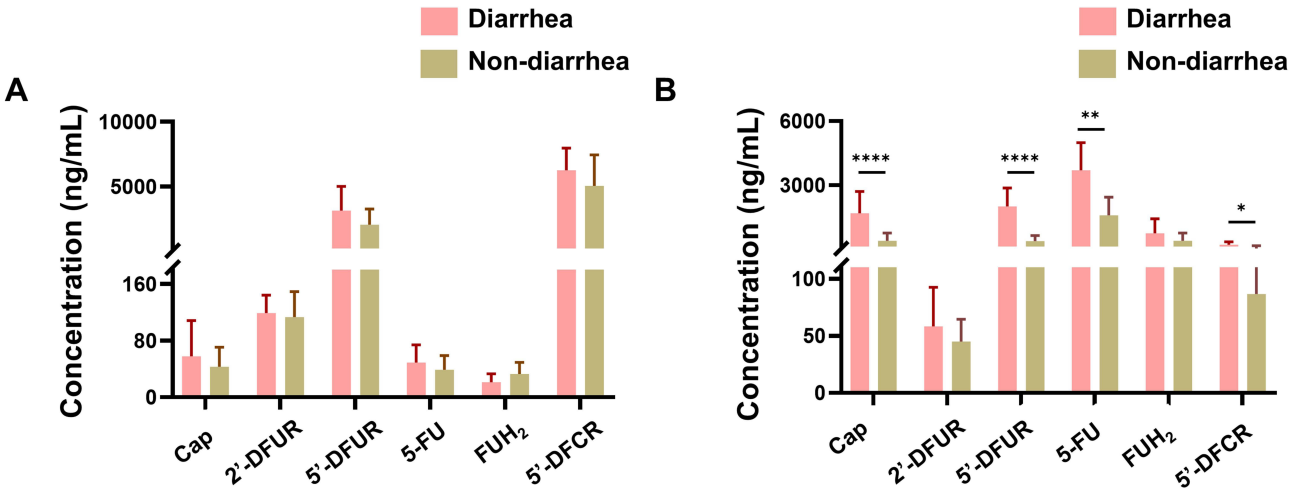

Figure 2 Comparative analysis of exposure levels of Cap and its metabolites in diarrhea (n = 15) and non-diarrhea (n = 21) mice. (**A**) plasma sample. (**B**) colon sample. Each result was obtained from at least five independent experiments or mice. *p < 0.05,**p < 0.01,****p < 0.0001.

Analysis of Metabolic Enzymes and Membrane Transporters in Colon

In animal study, we found higher accumulations of Cap and its metabolites 5'-DFUR, 5-FU, and 5'-DFCR in the colon of diarrhea mice. In consideration of the three steps of catabolism of Cap to generate the 5-FU and the detoxification of 5-FU, this study further analyzed four metabolic enzymes in the colon of mice using ELISA kits, and a clear difference was found only in DPD level between diarrhea mice and non-diarrhea mice (Figure 3A). To improve the reliability of our results and its clinical applicability, we proceeded to analyze the levels of metabolic enzymes and drug transporters of 5-FU (P-gp, SLC22A7, and ABCC5) in the colon of CRC patients receiving adjuvant chemotherapy based on Cap. As in Figure 3B, the metabolic enzyme levels in the colon of CRC patients with diarrhea were obviously higher than those of patients without diarrhea (all $P<0.05$), meanwhile, the levels of drug transporters of 5-FU (P-gp, SLC22A7, and ABCC5) in the colon were much lower in diarrhea CRC patients compared to non-diarrhea CRC patients (Figure 3C).

Construction and Validation of a Diagnostic Model Based on Metabolic Enzymes and Drug Transporters

As differences of levels of metabolic enzymes and drug transporters between the diarrhea CRC patients and non-diarrhea CRC patients were detected (all $P < 0.05$), the possibility of constructing an early-warning model for Cap-induced diarrhea based on metabolic enzymes and drug transporters was further explored. Sixty-two CRC patients were randomly divided into a training

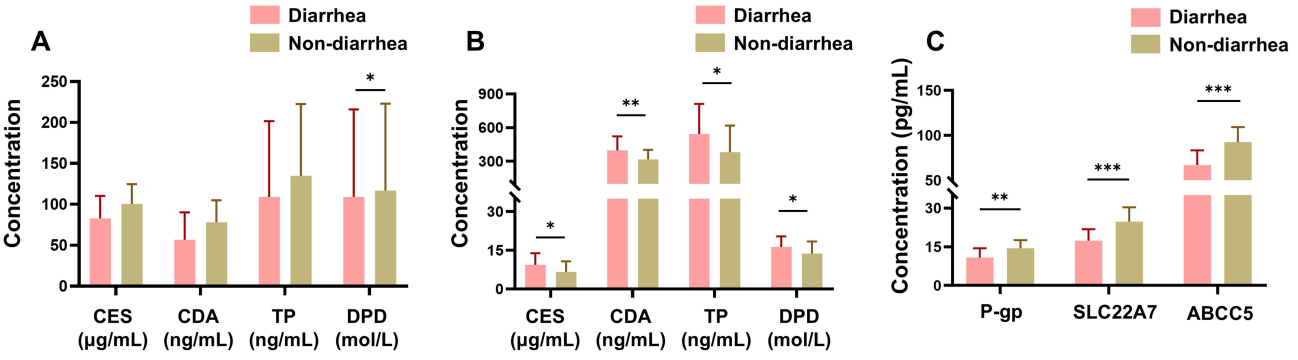

Figure 3 Comparative analysis of metabolic enzymes and drug transporters levels. (**A**) metabolic enzymes levels in colon between diarrhea mice and non-diarrhea mice. (**B**) metabolic enzymes levels in colon between diarrhea CRC patients (n = 18) and non-diarrhea CRC patients (n = 43). (**C**) drug transporters levels in colon between diarrhea CRC patients(n = 9) and non-diarrhea CRC patients (n = 30). Each result was obtained from at least five independent experiments or mice or patients. *p < 0.05,**p < 0.01,***p < 0.001.

set (42 patients) and a validation set (20 patients), the demographic data and clinical factors of the study cohort were summarized in Table 1. In the training set, the individual variable with respect to diagnosing Cap-induced diarrhea was assessed, and the area under the receiver operator characteristic curve (AUC) ranged from 0.603 to 0.843 (Table 2 and Figure 4A). To enhance the diagnostic capacity, this study then constructed a binary logistic model based on all metabolic enzymes and drug transporters; and inclusion and exclusion of variables in the model were tested in various methods, and the best diagnostic model was constructed using backward and conditional methods;[28] finally, CDA and SLC22A7 were selected to establish the diagnostic model. The formula for this diagnostic model was Y = 0.028× CDA − 0.518× SLC22A7 + 1.526 (AUC = 0.907, 95% CI: 0.773–1.000, P = 0.002, sensitivity 71.4%, specificity 100.0%), with a cutoff value of 0.628 when the Youden's index reached its maximum (Figure 4B); subsequently, this diagnostic model was validated in the validation set, resulting in good diagnostic capacity of diarrhea induced by Cap in CRC patients, with 100.0% sensitivity and 66.7% specificity (Figure 4C). These results confirmed that the diagnostic capacity of this early warning model was better than univariate models.

Table 1 Demographic and Clinical Characteristics of Study Cohorts

Items	Training Set	Validation Set
Gender (n, male/female)	42 (31/11)	20 (10/10)
Age (mean ± SD)	57.24 ±10.85	56.45±10.50
Range	31~ 81	37~ 70
CRC stages (n)		
I	1	1
II	19	8
III	19	10
IV	3	1
Therapeutic regimen (n)		
XELOX	33	15
Cap	9	5
Pathological pattern (n)		
Rectal cancer	17	7
Colon cancer	25	13
Diarrhea grades (n)		
I	6	4
II	3	2
III	1	2
NA	32	12
Number of treatments (median, range)	6, 1 ~ 8	6, 1 ~ 8

Table 2 Diagnostic Capacity of Single Metabolic Enzyme or Drug Transporter

Variable	AUC	Standard Error	Asymptotic Significance	Asymptotic 95% Confidence Interval	
				Upper Limit	Lower Limit
DPD	0.603	0.099	0.331	0.410	0.797
TP	0.694	0.085	0.069	0.526	0.861
CDA	0.690	0.100	0.073	0.494	0.886
CES	0.658	0.110	0.147	0.443	0.873
P-gp	0.700	0.123	0.121	0.460	0.940
SLC22A7	0.843	0.084	0.008	0.678	1.000
ABCC5	0.807	0.088	0.017	0.634	0.980

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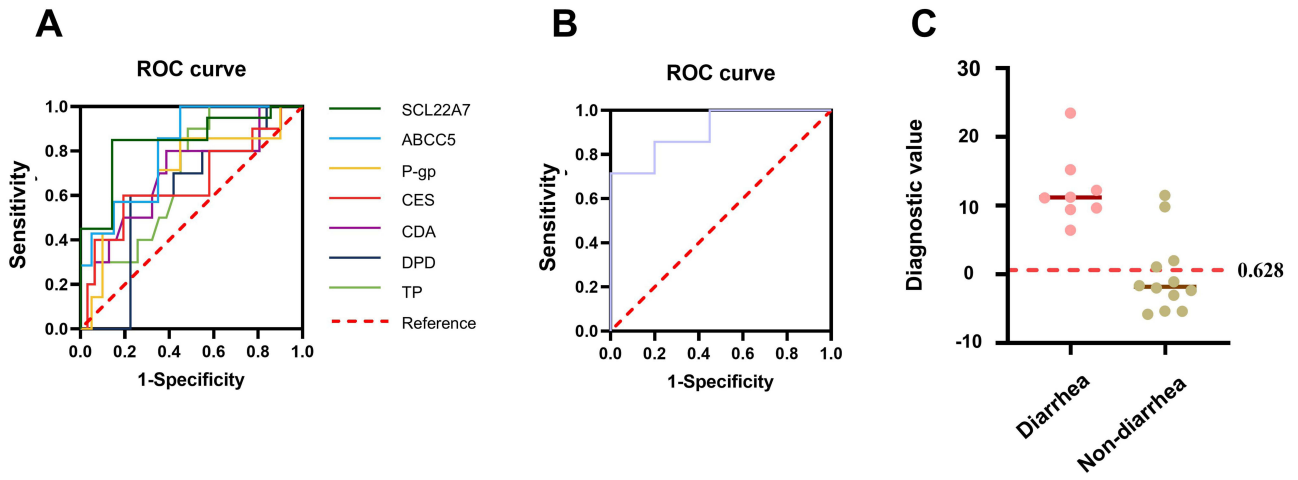

Figure 4 Construction and assessment of diagnostic models. (**A**) ROC curves of single metabolic enzyme and drug transporter for the diagnosis of diarrhea. (**B**) ROC curve of combined metabolic enzyme and drug transporter using binary logistic regression for the diagnosis of diarrhea. (**C**) validation of the diagnostic model constructed using binary logistic regression. Cutoff value = 0.628.

Discussion

The correlation between plasma Cap/5-FU concentration and adverse effects in chemotherapy patients remains controversial. In an assessment of 5-FU plasma levels in CRC patients receiving continuous infusion chemotherapy, there was no statistically significant difference between grades ≥2 and <2 diarrhea in patients.[29] Tong et al found that 5-FU plasma exposure (AUC) were more strongly correlated to hematological adverse effects but less so to gastrointestinal and cardiovascular adverse effects.[30] Zhou et al also stated that the relationships between 5-FU concentration at steady state (C_{ss}) in plasma and pharmacodynamic endpoints (eg, response, mucositis, diarrhea, and hand-foot syndrome) was not confirmed.[31] Much of the current basis for guiding dosing of 5-FU relies on the relationship between 5-FU systemic exposure (AUC) and adverse effects and efficacy,[32] however, this parameter was calculated based on multiple sample collection after administration. A study has proposed a successful method for preventing serious or life-threatening adverse effects in patients with gastrointestinal cancers by analyzing 5-FU and FUH_2 pharmacokinetic parameters,[33] but in this study, the differences of Cap and its metabolite exposure levels between diarrhea and non-diarrhea mice were first found in the colon, but not in the plasma; local exposure levels of Cap and metabolites may better reflect the exposure-adverse effects relationship.

The adverse effects caused by Cap, especially diarrhea, tend to be early onset,[34] so the metabolic enzymes and drug transporters in the colon may correlate to the differential exposure levels of Cap and metabolites and finally the occurrence of diarrhea. The effects of metabolic enzymes and drug transporters on the adverse effects of Cap have been studied in some studies. Lou et al summarized that the pathogenesis of adverse effects may be related to enzymes and drug transporters involved in Cap's disposal and accumulation of Cap metabolites.[35] Many studies have reported an association between DPD activity and 5-FU adverse effects.[36–38] Saif et al speculated that metabolic enzymes may affect Cap-induced adverse effects, namely CES, CDA, TP and DPD,[39] but most of the studies focused on the gene variations, and their influences on the adverse effects of Cap necessitate further confirmation.[40] In our study, levels of metabolic enzymes in the colon were quantified for the first time and compared between the diarrhea and non-diarrhea groups, and the results presented significantly low level of DPD in diarrhea mice, but in diarrhea CRC patients, the level of DPD was obviously lower than that in non-diarrhea CRC patients, while the other three metabolic enzymes CES, CDA and TP showed significantly higher levels in diarrhea CRC patients. The result of DPD in colon from CRC was opposite to that in mice; there may be other factors influencing the DPD catabolicing 5-FU. However, no other study has reported the levels of metabolic enzymes in colon correlating to the diarrhea induced by Cap.

The efflux of nucleoside analogues usually needed the transporters, for example, the ABC transporters; and some solute carrier (SLC) transporters could facilitate the entry of 5-FU to the enterocyte. As the 5-FU was the main cytotoxic product of Cap, this study combined the specific transporters of 5-FU and their expression levels in colon, and the

ABCC5, P-gp, and SLC22A7 were selected and determined in colon. The transport of 5-FU was mediated by SLC transporters, and the SLC22A7, which was lesser expressed in colon after kidney and liver, showed high affinity for this process.[41–43] The SNPs, rs4149178 and rs2270860 of SLC22A7 were found to be obviously associated with > grade 2 diarrhea and > grade 2 skin toxicity, respectively, induced by Cap.[44] In the treatment of CRC, ABC transporters also played important roles. ABCC5 was a transporter involved in drug resistance or cytotoxicity of 5-FU, which has been proved in many studies.[45,46] Hagmann et al examined the high expressed ABCC5 in cells and demonstrated that ABCC5 confers resistance to 5-FU.[47] In Patu-02 cells, knockdown of ABCC5 significantly increased 5-FU cytotoxicity and enhanced 5-FU accumulation.[48] In this study, we found that the ABCC5 showed the highest level among three transporters; and obvious differences of their levels were found between diarrhea and non-diarrhea patients, may contributing to the differential exposure levels of Cap and metabolites. However, the final model did not include the ABCC5 as a variable, and this may be explained by the ways of variable entering the model. The CDA and SLC22A7 were easy to quantify with the commercially available standard kits and the normal colon sample could be collected in the surgery, which pave a way for the routinely determining their levels in clinical practice after a large-scale sample size validation.

In summary, we established a mouse model of diarrhea caused by Cap, and found that exposure levels of Cap and metabolites in colon showed huge interindividual difference, correlating to the diarrhea. In addition, it was found that diarrhea and non-diarrhea CRC patients could be well differentiated by the metabolic enzymes and transporters. A diagnostic model was constructed using CDA and SLC22A7 (AUC 0.907, sensitivity 71.4%, specificity 100.0%), and validated in an independent group (sensitivity100.0%, specificity 66.7%) with excellent sensitivity and specificity. The model may be a better tool for early-warning of Cap-induced diarrhea compared to traditional methods based on plasma exposure levels of 5-FU. However, there are still some limitations in this study. (1) The number of enrolled subjects was small and therefore no grading of diarrhea was applied in this study, and more clinical samples are needed for further validation. (2) Despite the simplicity and efficiency of the study method, in situ multi-omics can also be considered to discover more early-warning biomarkers. 3) more experiments are necessary to explore the toxicity of Cap and metabolites to the colon, which may increase the understanding to the diarrhea induced by the Cap. 4) besides, incorporating the analysis of gut flora may help to elucidate the underlying mechanism of diarrhea induced by chemotherapeutic agents.

Conclusion

In this study, UHPLC-MS/MS was used to quantify Cap and its metabolites in plasma and colon of mice, which confirmed that their exposure levels in colon were not negligible and related to diarrhea. Further investigation demonstrated associations between metabolic enzymes and transporters with Cap-induced diarrhea. A diagnostic model was established: $Y = 0.028 \times CDA - 0.518 \times SLC22A7 + 1.526$, demonstrating strong predictive performance (AUC = 0.907, 95% CI: 0.773–1.000; P = 0.002) with 71.4% sensitivity and 100% specificity. These findings propose novel strategies for optimizing Cap clinical application, although validation based on large-scale cohort remains to be carried out.

Abbreviations

CRC, Colorectal Cancer; Cap, Capecitabine; 5-FU, 5-fluorouracil; SLC22A7, solute carrier family 22 member 7; CES, carboxylesterase; CDA, cytidine deaminase; TP, thymidine phosphorylase; 5'-DFCR, 5'-deoxy-5-fluorocytidine; 5'-DFUR, doxifluridine; FUH_2, dihydrofluorouracil; 2'-DFUR, 5-fluoro-2'-deoxyuridine; DPD, dihydroxypyrimidine dehydrogenase; P-gp*1, p-glycoprotein*1; ABC, ATP-binding cassette; MRP5 (ABCC5), ATP-dependent multidrug resistance protein 5; SN-38, 7-ethyl-10-hydroxycamptothecin; ELISA, enzyme-linked immunosorbent assay; AUC, area under curve; ROC, receiver operator characteristic curve.

Data Sharing Statement

The data generated in this study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Study protocol was approved by the Ethics Committee of Second Affiliated Hospital of Naval Medical Univeristy (Shanghai Changzheng Hospital, 2016SL007, and registered at www.clinicaltrials.gov, NCT03030508), and every patient approved and signed the informed consent.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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