BIOAVAILABILITY STUDY OF ORAL 5-FLUOROURACIL IN COLORECTAL CANCER PATIENTS.

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Summary

The aim of the present study was to determine the bioavailability of 5-Fluorouracil (5-FU) when given with skimmed milk in colorectal cancer patients. Oral 5-FU (500 mg/m^2) was administered with skimmed milk (250 ml) to colorectal cancer patients. Blood was collected in heparinzed tubes at 10, 20, 40 and 60 minutes after drug administration. 5-FU was extracted using ethyl acetate, potassium dihydrogen phosphate and water mixture. The levels of 5-FU in plasma were measured by using HPLC at 260nm. Effect of 5-FU on non-hematological parameters such as mucositis, dermatitis, diarrhea, fatigue, anorexia, pain and alopecia were also studied. In all patients peak plasma concentration (C_{max}) of 19.89±1.02 (Mean±S.D, n=7) was observed at 20 minutes (T_{max}). No dose limiting toxicity of grade 3 was observed in any patient under study. Furthermore, the weights of all the patients remained stabilized during the course of therapy. Therefore, we concluded that oral administration of 5-FU with skimmed milk can safely be given to colorectal cancer patients on long term basis.

Keywords: Skimmed milk, 5-Fluorouracil, Bioavailability, Oral route.

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Introduction

5-FU is widely used as a single drug as well as in combination with other chemotherapeutic agents to treat solid tumors of the gastrointestinal tract (1) However, prolonged drug infusion showed signs of toxicity in two-third of patients (2). To increase the therapeutic efficacy and to decrease its side effects, several modalities of drug administration have been investigated (3). When drug is administered by intravenous route, leucopenia was the principal adverse effect (4).Whereas gastrointestinal ulceration (the dose limiting side effect of constant infusion) and Septicemia was also observed (4). After oral administration, 5-FU has been reported to be poorly absorbed from the gut and is extensively metabolized by the liver microsomal enzymes due to first pass effect (3). Concomitant use of skimmed milk with 5-FU significantly reduced the lethal effects in mice (5). Furthermore, milk also reduces chemotherapy-induced mucositis in hamster (6).

In cancer patients, lactose improves the alimentary status and decreases incidence of leucopenia and thrombocytopenia (7). Retrospective data on our previous experience of adjuvant oral 5-FU with skimmed milk weekly given to 22 patients with colorectal cancer for prolonged period i.e. median duration 1 year (range 6 month to 3 year) showed good tolerability with even free survival . These results strongly suggest that prophylactic use of skimmed milk in patients receiving cancer chemotherapy may be useful in reducing adverse effects associated with commonly used antimetabolities like 5-FU . The purpose of the study was to determine the bioavailability of 5-FU with skimmed milk in colorectal cancer patients. It was also aimed to determine the clinical benefits of 5-FU when given with milk.

Materials and Methods

Materials

Ethyl acetate and potassium dihydrogen Phosphate were purchased from Sigma Chem. Co., USA.

Patient studies

This study was approved by the Ethical committee of Nishtar medical college, Multan, Pakistan and was conducted according to its guidelines. Patients were informed of the procedure and the aim of the study and they were enrolled after giving written consent to participate. Bioavailability study was carried out in fourteen colorectal cancer patients (Table 1).

Eligibility criteria include≥ 18 year; adequate hematopoietic (leukocyte count ≥3,000/µl; absolute neutrophile count ≥1,500/ µl; platelet count ≥100,000/µl; and hemoglobin level ≥10g/dl), hepatic (total bilirubin level ≤2.0 mg/dl; aspartate aminotransferase and Alanin aminotransferase ≤2.5 times normal upper limit), and renal (serum creatinine ≤1.5 mg/dl) functions.

Table.1. Characteristics of colorectal cancer patients

No of patients	14
Sex (Male: Female)	9:5
Age (Years)	
Median	45
Range	35:52
Tumor Primary Sites	
Colon	10
Rectum	4

HPLC assay

The HPLC system Aglient 1100 model attached with Aglient 1100 Diode Array Detector and Chem Station 32 (Aglient) software was used. The system was adjusted to an absorbency of 260 nm. Separation was accomplished via isocratic elution of mobile phase (50 Mm KH_2PO_4) with a flow rate of 1 ml /min. A C_{18} Zorbax Eclipse XBD column (5 micron particle size, 4.6×150 mm) was used. HPLC analysis was conducted at 25 ^{0}C, run time and retention time were 10 minutes and 1.5 minutes respectively.

Standards samples were prepared from stock solution (100 µg/ml) by adding appropriate volume of distill water to make various standard samples (0 -20 µg/ml) to human plasma. A calibration curve was constructed by using 260nm for HPLC analysis. (Fig.1)

500µl of plasma samples were taken in a 15 ml centrifuge tube, 200µl of Phosphate buffer (55Mm KH_2PO_4) and 300 µl of DDW was added to each tube to make volume to 1 ml. 7ml of Ethyl acetate was added in each tube. Tubes were sealed with rubber stopper and Para film. The samples were vortexed for 10 minutes and centrifuged at 4000 rpm. The supernatant (6 ml/sample) was placed in fresh centrifuge tubes and evaporated to dryness under a stream of Nitrogen gas.

Once the samples were evaporated, 500 µl of DDW was added and the mixture was shaken until the residues were completely dissolved. The solubilised sample was filtered through 0.45 micron filter (Millipore) and 30 µl of samples were injected onto HPLC system. The retention time of 5-FU was (1.49 minutes). (Fig. 2)

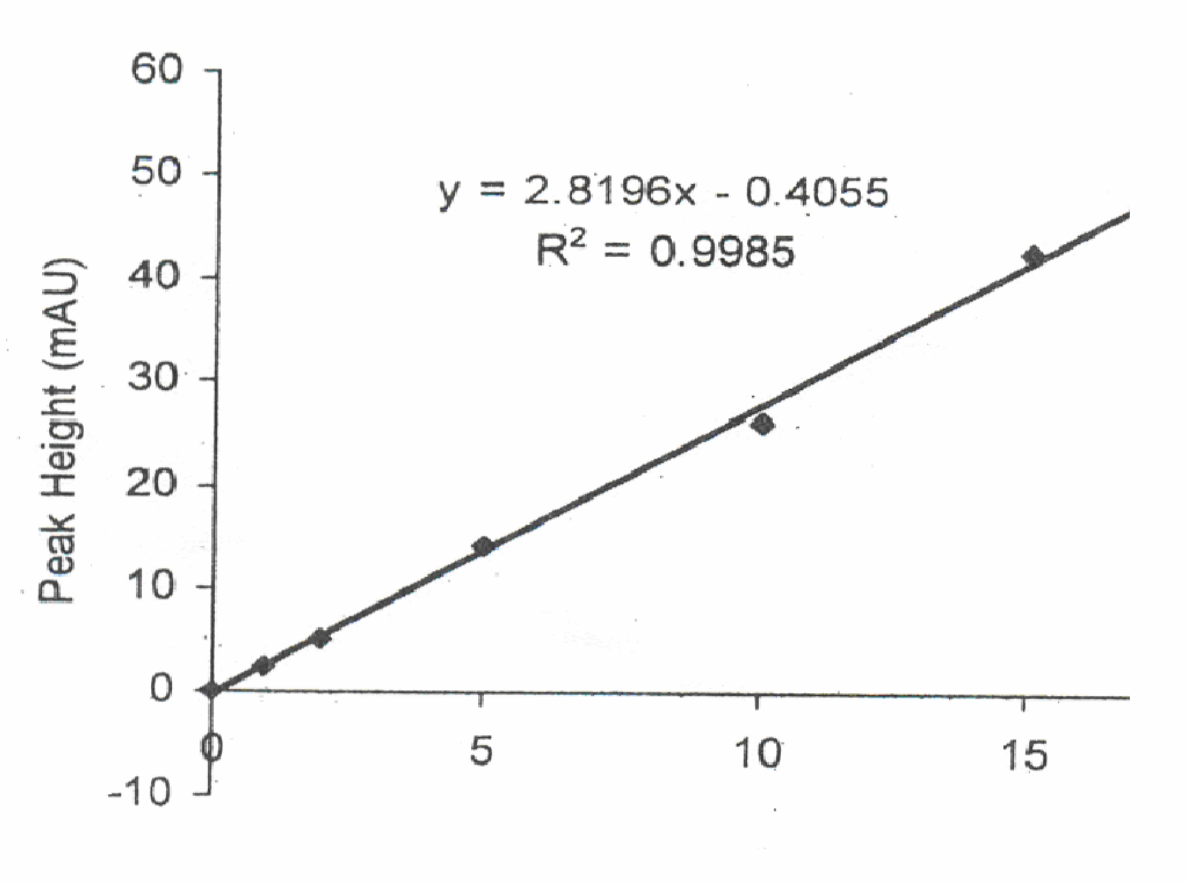

Fig.1. Calibration curve for 5-FU (0, 5, 10, 15, 20 µg/ml) injected 30 µl onto the HPLC system (C18 Zobrax Eclipse XBD column) and eluted by using potassium dihydrogen phosphate, at flow rate of 1ml/min. 5-FU was detected by U.V detector (Aglient 1100 Diode Array Detector) at wavelength of 260nm.

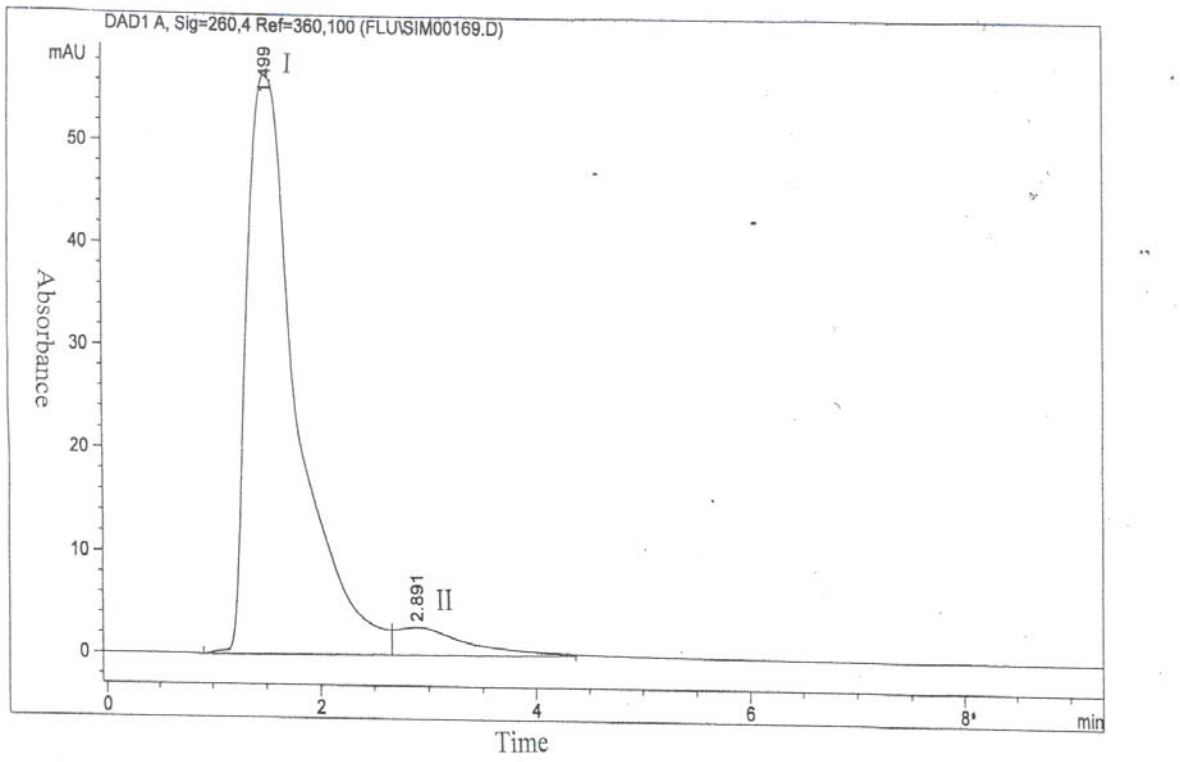

Fig.2. HPLC chromatogram showing the 5-FU (18.88 µg /ml) at a retention time of (1.49 minutes). One metabolite peak of 5-FU was detected at a retention time of (2.89 minutes).

Pharmacokinetic analysis

The concentration time curve of 5-FU was obtained by plotting the mean concentrations (Ci) at each time point (ti) versus time on semi logarithmic scale. The area under the concentration time-curve (AUC) were calculated by the trapezoidal rule

$$AUC=\sum_{i=1}^{n} (Wi-\bar{C}i)$$

With

$$Wi= 0.5(t_{i+1}-t_i)$$

Other parameters such as peak plasma concentration (C_{max}), time for peak concentration (T_{max}), elimination rate constant (Ke) and half-life ($t_{1/2}$) were also calculated.

Results

Pharmacokinetic analysis

In all patients peak plasma concentration (C_{max}) of 19.89±1.02 (Mean±S.D, n=7) was observed at 20 minutes (T_{max}). Results of the pharmacokinetic parameters are given in (Table 2).

Table. 2. Various pharmacokinetic parameters observed after administration of 5-FU with skimmed milk in colorectal cancer patients. (Mean±SEM, n=14).

Parameters	Values
$AUC_{0\text{-}60\ min}$	188.11±162.23
C_{max}	19.89±1.02
T_{max}	20 minutes
K_e	0.058788
$T_{1/2}$	11.7906

Effects of 5-FU on non-hematological parameters

In colorectal cancer patients (n=14) receiving oral 5-FU with skimmed milk, since last 3 months effects on various non-hematological parameters like mucositis, dermatitis, diarrhea, fatigue, anorexia, pain and alopecia were studied . Grading was done to measure severity of toxicity. Toxicity of grade 3 was considered as dose limiting toxicity. Effect of 5-FU on these parameters are summarized in (Table 3).

Effects of 5-FU administration on the weights of Patients

In colorectal cancer patients (n=14) receiving oral 5-FU with skimmed milk, since last 3 months weights of all the patients at the start and at the end of study period (3 months) were measured, and change in weight was determined (Table.4).

Table 3. Effect of 5-FU (500mg/m^2) after concomitant use with skimmed milk 5-FU with skimmed milk (250 ml) on various significant non-hematological parameters in colorectal cancer patients (n=14) at the end of study period (3-months).

Patient no	Dose level	Mucositis	Dermatitis	Diarrhea	Fatigue	Anorexia	Pain	Alopecia
1	500mg	1	0	0	1	1	2	0
2	500mg	0	1	1	0	1	2	0
3	500mg	1	0	0	1	1	1	0
4	500mg	1	2	1		1	1	0
5	500mg	1	2	2	1	1	1	0
6	500mg	0	2	2	1	0	2	0
7	500mg	0	2	2	2	2	1	0
8	500mg	0	1	1	2	1	2	1
9	500mg	1	0	1	0	0	1	0
10	500mg	0	1	1	1	1	0	1
11	500mg	0	1	1	0	2	1	0
12	500mg	0	2	1	0	1		0
13	500mg	1	2	1	0	2	1	0
14	500mg	1	1	0	1	2	0	0

0 was put if no toxicity appears in its respective column

1 was put if mild toxicity appears in its respective column

2 was put if moderate toxicity appears in its respective column

3 was put if severe toxicity appears in its respective column

Table 4. Changes in the weights of colorectal cancer patients received 5-FU chemotherapy with skimmed milk (500mg/m^2 5-FU with 250 ml skimmed milk) at the start and at the end of study period (n=14).

Patient Number	Weight at the start of study	Weight at the end of study period (3 months)	Change in Weight
1	74.50 Kg	74.48 Kg	0.02 Kg
2	78.77 Kg	78.76 Kg	0.01Kg
3	65.23 Kg	65.22 Kg	0.01 Kg
4	66.40 Kg	66.40 Kg	0 Kg
5	63 Kg	63 Kg	0 Kg
6	71.50 Kg	71.49 Kg	0.01 Kg
7	70 Kg	70 Kg	0 Kg
8	75.14 Kg	75.12 Kg	0.02 Kg
9	63.32 Kg	63.29 Kg	0.03 Kg
10	68.26 Kg	68.19 Kg	0.07 Kg
11	59.12 Kg	59.08 Kg	0.04 Kg
12	71.26 Kg	71.21 Kg	0.05 Kg
13	76.85 Kg	76.84 Kg	0.01 Kg
14	58.32 Kg	58.29 Kg	0.03 Kg

Discussion

We are the first to report the bioavailability of 5-FU (500 mg/m^2) with skimmed milk (250 ml) in colorectal cancer patients. We hypothesized that oral administration of 5-FU with skimmed milk may improve absorption of 5-FU from GI tract. We studied various pharmacokinetic parameters of 5-FU when given with milk. Sample were analyzed using HPLC at absorbance of 260 nm and a peak plasma level (C_{max}) of 19.89± 1.02 (mean±S.D, n=14) was observed. These levels are almost double the previously reported levels (8.3µg/ml)

by the same dose i.e 500mg of 5-FU orally in American breast cancer patients (Phillips et al., 1980). The difference in 5-FU levels may possibly be due to the beneficial effect of skimmed milk on the absorption of 5-FU in our patients. Furthermore, difference in the assay procedure /genetic difference may also contribute to the higher levels of 5-FU in our study. Peak plasma concentration was achieved at 20 minutes (T_{max}) that is in agreement with the previously reported results (8). Area under curve of 188.11±162.23was observed in our study. These levels are reasonably high enough to give clinical benefits to our patients as weights of all our patients remained stabilized during the course of study. However in previously reported study 45% of the cancer patients receiving chemotherapy loose 10% of their weight during cancer chemotherapy (9). Our results showed that milk is having some beneficial effects during chemotherapy with 5-FU due to which weights of all the patients receiving oral 5-FU cancer chemotherapy were stabilized.

In previous study colorectal cancer patients gave their preference for oral route as compared to i.v route (10). When we interviewed our patients at the end of 3 months study all the patients also preferred to take 5-FU with skimmed milk rather than i.v route. We further observed that 5-FU with skimmed milk had no side effect on non-hematological parameters. None of the patient showed any toxicity of grade 3 (dose limiting effect).

5-FU when given i.v causes septicemia in colorectal cancer patients that may lead to death (2, 4). Septicemia may possibly be due to a significant decrease in WBCs count in these patients. Therefore, leukocyte count at different time intervals is very important to determine the acute toxicity of 5-FU administration. We observed that 5-FU significantly decreases WBCs count in rabbits after i.v administration. Interestingly, no significant decrease in WBCs count was observed in rabbits receiving 5-FU with skimmed milk. These findings enable us to conclude that skimmed milk protect the colorectal cancer patients from life threatening toxicity such as Septicemia. These findings are in line with the previous data showing that lactose decreases the incidence of leucopenia in cancer patients (7).

5-FU when given with skimmed is reasonably absorbed from the GI tract. This indicates that skimmed milk have some positive interaction with 5-FU at its absorption. 5-FU (500 mg/m^2) have achieved the plasma concentration that reasonably gave clinical benefits to colorectal cancer patients. The weights of all our patients were stabilized during the course of study and their physical conditions were also improved. Oral 5-FU administration with skimmed milk is safe enough and showed no grade 3 toxicity in this study. Our results enable us to conclude that 5-FU can safely be given to colorectal cancer patients with skimmed milk on long term basis.

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