Preliminary Study for Development of an Experimental Model of Cancer Chemotherapy Induced Diarrhea

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ABSTRACT

Introduction: Cancer chemotherapy induced diarrhea (CCID) is a frequent problem during anticancer treatment. Patho-physiology of CCID differs between chemotherapeutic agents. Neither there is specific treatment for CCID nor adequate experimental models. The objective of this study was to develop a relevant experimental animal model of CCID, which can help in development of specific therapy for CCID.

Material and methods: In this study thirty six albino rats of either sex were divided into 6 groups of 6 animals each. Group I rats received distilled water and served as control. Group II,III,IV, V, VI received 5-Fluorouracil(5-FU) once intra peritoneal(IP) at doses 10,20,30,40 and 50 mg/kg respectively and served as test groups for experimental model of CID. Standard parameters like stool samples for quality& quantity, incidence of diarrhea, histopathology of intestine and fatal outcome in rats were recorded.

Results: Out of test groups, rats treated with 5-FU 30mg/kg IP single dose developed diarrhea in 50% rats,mean time for onset of diarrhea was 84±16.7 hours and duration was 62±9.16 hours, diarrhea subsided within 7 days with mild changes of intestinal histopathology and 16.7% mortality. Occurrence of diarrhea, change in intestinal histopathology was not remarkable with dosage less than 30mg/kg. In 5 FU 40 mg/kg & 50mg/kg group incidence of diarrhea increased to 66.6% but death rate were further increased to 33.3% in5 FU 40 mg/kg& 83.3% with 50mg/kg.

Conclusion: The results suggest 5-FU 30mg/kg single dose IP to albino rats can be used as a suitable experimental model for CCID for evaluation of novel potential candidate drugs for treatment of CCID.

Keywords: Experimental Model, Cancer Chemotherapy, Diarrhoea, 5-FU

INTRODUCTION

Bone cancer is a common disorder. Various anticancer drugs cisplatin, Cyclophosphamide, 5-Fluorouracil (5-FU) etc are used in bone cancers. Diarrhea is a frequent problem during these anti-cancer treatment.Various anticancer drugs i.e.5-Fluorouracil(5-FU), Cisplatin, Cyclophosphamide, Paclitaxel, Irinotecan, Lapatinib etc. are implicated in Cancer chemotherapy induced diarrhea (CCID).[1,2] In some cases, the diarrhea induced by anti-cancer drugs resolves by withdrawal of therapy, however other cases require treatment. The main modalities of treatment available at present are symptomatic relief with i.v. hydration[2], loperamide[2] or octreotide[3] etc. But no specific therapy available for treatment of CCID. For development of specific therapy of CCID there is

requirement of animal (experimental) models. However there is paucity of studies on development of suitable experimental models of CCID. Hence the present research work aims at developing a clinically relevant animal model of CCID, which can be used for study of potential drugs for treatment of Cancer chemotherapy induced Diarrhea (CCID).

MATERIAL AND METHODS

This study was the initial part of the large study entitled "Effect of Aqueous and alcoholic extract of Curcuma Longa on Cancer Chemotherapy Induced Diarrhea in Animal Model" approved by the Institutional Animal Ethics Committee (IAEC), SCB Medical college, Cuttack (CPCSEA registration no- 431/01/C). The preliminary part of this study was developing an experimental (Animal) Model for Cancer Chemotherapy Induced Diarrhea (CCID), so that effects of extracts of curcuma longa can be tried on this model. This study was conducted in the department of Pharmacology of SCB Medical College and Hospital, Cuttack with support of orthopedic & pathology consultant, between July 2015 to August 2016.

Among conventional anti-cancer drugs 5-FU has been associated with the highest incidences of diarrhea which necessitates an efficacious combination therapy to maximize patient's quality of life whilst on chemotherapy. Hence the present study was planned with the objectives of developing an experimental model of CCIDin albino rats using 5-FU. Literature search revealed 5-FU 20mg/kg as the nonlethal dose for rats[7] & acute toxicity (LD50) was stronger after 5-FU (63mg /kg).[8] Hence to develop a clinically relevant

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animal model of CCID by using 5-FU we have started from 10mg/kg one dose less than 20 mg/kg and proceeded to 20, 30, 40 and 50 mg/kg.

Plan of study: Thirty six albino rats of either sex weighing 120-150 grams were used for the study. All the rats were housed in cages and given standard animal feed and water ad libitum. The rats were divided into 6 groups of 6 animals each and the study was conducted as per the following plan. Group I rats were given Distilled Water and served as Control. For induction of diarrhea for experimental model of CCID, 5-FU injection containing 500mg in 10 ml ampoule (50mg/ml) were procured from the open market. 5-FU was given once intra-peritoneal (IP) to the rats at dose of 10,20,30 40 and 50mg/kg to the groups II,III,IV,V and VI respectively. The rats were observed for a period of 2weeks and the following parameters as mentioned in detail below were studied.

The parameters studied
a) Stool parameters: quality& quantity of stool, incidence, time of onset& duration of diarrhea: After administration of distilled water or 5-FU (in different doses) to individual rats, they were housed in metabolic cage. The stool of each rat was collected into containers at 6 hourly intervals over24 hours for 10 to 14 days. The stool quality (consistency and association of blood and mucus), quantity, the incidence, duration of diarrhea in each group was observed and recorded.

b) Histopathology study of the intestine: Two rats from each group were sacrificed in order to conduct the histopathology study of intestine. Rats were anesthetized by injection ketamine hydrochloride 50mg/kg, a midline incision was given passing through the abdomen, the peritoneal cavity was opened. Then a small segment from the intestine was resected and collected in formalin filled vials which were sent to Department of Pathology for histopathology study.

c) Fatal outcome in rats: The fatal outcome (mortality) in the rats either in the control or 5FU treated groups during the study period were recorded for further analysis.

Treatment provided to the rats for diarrhea : Literature search revealed potential beneficial role of hypotonic ORS around (240 mosm /L) in rat diarrhea.[9] Hence new formula WHO-ORS containing 245 mmol/L[10] was provided sufficiently to the rats during diarrhea. As there is no other definitive treatment recommended forCCID in rats no other treatment was given to the rats.

The data obtained from various groups were fit into tabular formats and expressed as mean±SEM. They were analyzed statistically using one-way ANOVA followed by Student's t test for paired analysis. P-values less than 0.05 imply significance.

RESULTS

The rats were observed for 2 weeks, after administration of the drugs.

The observation of stool parameters: The quality of fecal matter, incidence, time of onset & duration of diarrhea in the rats treated with distilled water or5- FU in different doses are depicted in Table-1.The quantity of fecal matter in the rats treated with distilled water or5- FU in different doses are depicted in Table-2

The fecal matter of the rats in control group were solid, molded, dark brown & rough, weighing 2.14 gm±0.01. The rats treated with 5-FU 10 mg/kg did not have significant difference in stool parameters than the control group. In 5-FU 20mg/kg treated group, only 1 (16.7%) of the rats developed semi liquid diarrheal stool with duration of diarrhea 24 hours which gradually subsided. 5-FU in dose of 30 mg/kg has resulted in diarrhea in 3 (50%) of rats, mean time for onset of diarrhea was 84±16.7 hours (range 72-108 hours) and duration was 62±9.16 hours (range 54 -72 hours) ultimately the diarrhea subsided within 7 days with a molded, dark brown & rough stool. Rats treated with 5-FU 40mg/kg had diarrhea in 66.6% rats. Rats treated with 5-FU 50 mg/kg also had diarrhea in same 66.6% rats with blood & mucous in 1(16.7%) rat, the mean time for onset of diarrhea was 64.5 ±13.30 hours (range 48-78 hours) and duration was 60±16.97 hours (range 48-84 hours) before death or recovery of the rats.

It was difficult to collect all the stool quantity and estimate the exact amount of stool in all the individual rats because of soiling of stool on the body of rat, sometimes mixing of urine with diarrheal stool even in metabolic cage. Hence the quantity of stool mentioned in table -2 is the amount which ever was possible to be collected. It is also evident from table-2, that the stool quantity collected has no linear relationship with the dose of the 5 FU. Again the statistical analysis of comparative stool quantity in rats on day 0 before any drug is given (baseline stool quantity) are highly significantly different (df-35, f-6.814 p<0.001). Hence statistical analysis of stool quantity in different doses of 5FU is not further analyzed. Rather it is evident from table-1 that there is correlation of incidence of diarrhea & change in consistency of stool with different doses of 5 FU.
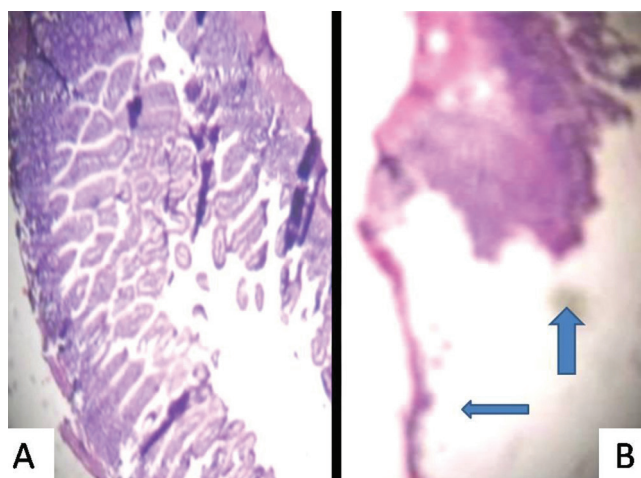

Figure-1: A: Normal intestinal wall of the rat; before 5 FU treatment. **B:** After 5FU treatment: Thin arrow - completely sloughed mucosa; Thick arrow - Focal residual degenerated mucosa.

Group of rats, drug and dose	Number &% of rats developed diarrhea Total n=6	Stool consistency	Time of onset of diarrhea (Mean±SD in hours	Duration of diarrhoea (Mean±SD in hours	Number &% of mortality in rats & time till death (n=6)
Group I control-DW	Nil	Normal	-	-	NIL
Group II 5FU 10mg/kg	Nil	Nil	-	-	Nil
Group III 5FU 20mg/kg	1(16.7%)	Liquid	78	24	Nil
Group IV 5FU 30mg/kg	3(50%)	Liquid- 1 rat Semi-solid- 2 rats	84±16.7	62±9.61	1 (16.7%)
Group V 5FU 40mg/kg	4(66.6%)	Semiliquid -2 Liquid-2 rat	60±9.79	73.5±22.11	3(50%) Time to Death 108-156 hours
Group VI 5FU 50mg/kg	4(66.6%)	semiiquid -1 Liquid-3 rat	64.5±13.30	60±16.97	5(83.33%) Time to Death 96 – 162 hours
Incidence of diarrhea/quality of stool/duration of Diarrhea/ and mortality in different groups of rats.					

Table-1: Analysis of stool parameters

Group of Rats & Dose of 5FU in mg/kg	Mean weight of the Stool (In gram) ± Standard Error							
	Day0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Gr I Distilled Water	2.14±0.01	2.22±0.09	2.98±0.15	2.36±0.27	3.73±0.99	2.99±0.23	3.24±0.317	2.38±0.25
Gr II: 5FU 10mg/kg	2.37±0.06	2.82±0.11	3.55±0.11	3.51±0.21	4.06±0.08	4.70±0.11	3.86±0.07	2.42±0.09
Gr III: 5FU 20 mg/kg	2.28±0.02	5.49±1.10	6.68±1.72	8.41±2.25	8.96±2.14	8.74±2.20	4.84±1.14	3.37±0.61
Gr IV: 5FU 30mg/kg	2.44±0.23	6.37±0.31	9.11±0.77	14.26±0.98	15.88±1.52	14.58±0.96	8.11±1.73	4.09±0.69
Gr V: 5FU 40mg/kg	3.16±0.19	6.71±0.45	12.05±0.66	15.32±0.83	14.05±2.87	9.50±3.10	10.27±2.19	6.69±1.49
Gr VI:5FU 50mg/kg	2.42±0.56	6.91±0.62	11.89±0.59	14.77±1.27	12.60±3.20	11.15±3.62	12.93±2.75	8.20±1.77
Comparative analysis of stool quantity on Day0 among different groups of rats.								

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	6	2.1400	.02828	.01155	2.1103	2.1697	2.10	2.18
2.00	6	2.3667	.15629	.06381	2.2027	2.5307	2.22	2.62
3.00	6	2.2867	.08824	.03602	2.1941	2.3793	2.14	2.38
4.00	6	2.4433	.56422	.23034	1.8512	3.0354	1.63	3.12
5.00	6	3.1150	.48356	.19741	2.6075	3.6225	2.42	3.64
6.00	6	2.4233	.13765	.05619	2.2789	2.5678	2.24	2.62
Total	36	2.4625	.42933	.07155	2.3172	2.6078	1.63	3.64
ANOVA								
CV								

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.431	5	.686	6.814	.000
Within Groups	3.021	30	.101		
Total	6.451	35			

Histopathology study of the Intestine: The intestinal wall structure of the control rat showed, from inside to outside - mucosa, sub mucosa, a muscularis propria and serosa. The mucosa was lined by simple columnar epithelium thrown into villous projections. The predominant cell types were enterocytes with mucous secreting goblet cells interspersed in between. The lamina propria was filled with loose connective tissue, blood vessel and lacteals [Figure1 A]. IN 5FU treated diarrheal rats the intestine was covered by only mucoid covering externally. Histologically the wall structure was found extensively distorted with loss of mucosal integrity, moderate sloughing with focal residual edematous and congested lamina propria evident at some foci [Figure1 B]. In 5FU 50mg/kg had excessive damaged & sloughed out mucosa.

The fatal outcome (mortality trend) of the rats during the study is presented in Table-1. All individual rats in each group were examined daily by veterinary Surgeon of our

institution. It is observed that there was no mortality in the rats during the period of observation in Group-I (Control), Group-II (5 FU 10mg/kg) & group-III (5 F-U 20mg/kg).In 5 -FU 30mg/kg1 out of the 6 rats(16.7% of the rats) has died on the 6th day which had severe diarrhea on 4th day. In 5 FU 40 mg/kg group death rate were further increased to 33.3%. In 5 FU 50 mg/kg group the mortality was >50%i.e. 83.3% (5 out of 6 rats succumbed) all these rats had severe diarrhea with blood & mucus. From table-2 it is evident that there is no direct correlation with the quantity of stool with fatality in the rats.

DISCUSSION

There is paucity of basic research for development of animal model for Cancer Chemotherapy Induced Diarrhea (CCID). Lack of suitable animal models hamper development of effective new drug for management of CCID.

In Our study we have tried to develop a clinically relevant animal model of CCID. It showed that 5-FU 30 mg/kg IP single bolus dose produced diarrhea in 50% of experimental rats with occasional blood & mucus in stool; it started 84±16.7 hours after the injection of 5-FU, reached maximum on 4th day and subsided after 7 days[Table-1]. Mild histological changes in the intestines with loss of mucosal integrity, and congestion of the wall of lamina propria. There was fatality in 16.7% of the experimental rats with this dose.

In contrast, 5-FU10mg/kg did not produce any change in different parameters.5-FU 20mg/kg produced diarrhea in 16.5% rats, no significant histopathological change of the intestine; nor had fatality in experimental rats. 5-FU 50mg/kg produced diarrhoea in 66.6% rats with increase in blood and mucus in stool along with severe epithelial sloughing and ulceration of the intestine(fig 1B). 5-FU 50mg/kg in rats produced a fatality rate of 83.3%. Hence, 5-FU 30mg/kg single bolusdose IP in albino rats can act as a suitable clinically relevant animal model for CCID as in this dose there is diarrhea in 50% of experimental rats.

Different studies have revealed 5-FU 20mg/kg as the nonlethal dose[7] and stronger acute toxicity (LD50) after 5-FU 63mg /kgin rats.[8] But in our study we found some differences i.e. no mortality in 5-FU 10mg/kg & 20mg/kg, 16.7% mortality in 5-FU 30mg /kg; and 66.7% mortality in 5-FU 40 mg/kg. Such differences may be due to the differences in rat species, environmental temperature, quality of drinking water and the level of the animal house and laboratory facilities etc. However we suggest not to increase dose of 5FU in experimental model of albino rats to beyond 30 to 40 mg/kg.

Y.M. Petersonetal[12] had used 5-FU to induce CCID in rats for evaluating a novel potential candidate ZP1816 for treatment of CCID. In that study, though they have concluded that the novel molecule ZP1816 has dose dependently decreased the incidence and severity of 5-FU induced diarrhea but detail regarding the histological changes, and mortality etc. in the dose of 75mg/kg of 5-FU was not mentioned. However in our study we got CCID with 5-FU 30 and 40 mg/kg with changes in intestinal histopathology, lethality of 16.7%with

5FU 30mg/kg & 83.3% mortality in dose of 5-FU 50mg/kg. Studyon Irinotecan in colorectal carcinoma rat model have shown that with 100mg/kg/day there was no diarrhea and no lethality, with 150mg/kg/day 75% of rats developed diarrhea and 50% developed lethality and with 200mg/kg/day there was severe diarrhea in all the rats with 100% lethality.[13] In contrast, our study with normal rats showed that with 5-FU 10mg /kg there was no diarrhea and no lethality;with20 mg/kg diarrhea in16.7% rats and with 30 mg/kg there was diarrhea in 50% rats but 16.7% mortality and further increase in dose to 50 mg/kg led to severe diarrhea with blood and mucus, intestinal ulceration in all the rats and high death rate of 83% of the experimental animals.

This low death rate of rats in our study may possibly be due to use of new formula WHO-ORS containing 245 mmol/L[10] as a treatment for the diarrheal rats. However both studies emphasized that, these cytotoxic drugs can be used only through a narrow range both for therapeutic purpose and for preparation of experimental model.

A rat model developed by J.M.Bowen in 2012 with the targeted anticancer drug, Lapatinib, a receptor tyrosine kinase inhibitor (RTKI) demonstrated that, in normal rats an oral dose ofLapatinib 240 and 500 mg/kg/day for 28 days induced diarrhea with a peak at 3-4 weeks without causing significant histopathological damage in the intestine nor lead to major lethality;[14] but there was no comment on any change in intestinal micro flora. As RTKI is a targeted drug. In contrast in the present study 5-FU was given a single dose IP, diarrhea peaked at 3-4 days and observation period required was 1-2 weeks.

CONCLUSION

On the basis of the results obtained from the present study and comparing the result of other studies; we suggest that, 5-FU given a single 30 mg/kg dose IP to albino rats can act as a clinically relevant suitable animal model for evaluation of novel potential candidate drugs for treatment of CCID. Future studies evaluating intestinal micro flora, blood parameters, effect on weight in the rats with repeated doses of 5FU 30 mg /kg may further establish this experimental model.

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