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FECAL EXCRETION PATTERN OF BILE ACIDS IN RATS FED HIGH FAT DIETS AND NEOMYCIN IN INDUCED COLON TUMORIGENESIS

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Abstract : Neomycin augments colon tumorigenesis in 1,2 - dimethylhydrazine treated rats fed polyunsaturated fat diet and decreases fecal cholic acid excretion, while it inhibits tumorigenesis with increased cholic acid and decreased deoxycholic acid excretions in rats fed high cholesterol diet. Participation of other fecal bile acids seems to be insignificant in relation to colon carcinogenesis.

High fat diets were held responsible in the incidence of colon cancer in epidemiological studies as well as experiments involving animals[1-4]. Populations in the high risk group reportedly have higher concentrations of intestinal anaerobic bacteria and their enhanced metabolism in gut resulting in increased fecal excretions of acid sterols[5,6]. Studies from this laboratory focussed on the observations that dietary lipids influence the concentration of gut flora with subsequent production of promoters or co-carcinogenic agents[7-9]. Neomycin, a well known hypocholesterolemic drug is known to have a significant effect upon sterol metabolism in gut [10,11]. Present study reports the effect of neomycin on the fecal excretion pattern of bile acids in rats with colon tumours induced by 1,2 - dimethylhydrazine (DMH) fed safflower oil and cholesterol diets.

Eighty Sprague Dawley CD male weanling rats weighing 30-40g purchased from Charles River Breeding Laboratories, MA, USA, were randomised, divided into two groups, each group consisting of forty rats and fed the basal polyunsaturated fat diet (safflower oil) listed in Table 1 supplemented with the following :

Group 1: 20% safflower oil (basal diet)

Group 2: 20% safflower oil, cholesterol 1% and cholic acid 0.3% (cholesterol diet).

These dietary groups were subdivided again, each consisting of twenty rats, into control (A) which was without neomycin and neomycin treated group (B). Neomycin sulfate (35 mg/kg body weight) has been administered orally (210 mg/l in drinking water). These four dietary groups were designated group 1A, 1B, 2A and 2B. The sucrose level was adjusted accordingly with addition or omission of nutrients from basal diet. 4.53 Kcal was supplied by 1g of the basal diet with 36% of calories as fat. Cholic acid was used in cholesterol diet in order to maintain a prolonged hypercholesterolemia in rats. After 2 weeks on diet, the control and neomycin treated rats of both the dietary groups received intramuscular injections of DMH(10mg/kg body weight) in normal saline once each week for 15 weeks. The animals were kept in metabolic cages (21°C ±1 animal farm) and maintained on their respective diets for another 20 weeks during which they were weighed twice weekly, physically checked for

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moribund animals and food consumption duly recorded. Animals which died during the experimental period were autopsied immediately. Twenty four hour stool output was collected from rats of all the four dietary groups every 90 days. After 37 weeks, all the animals were killed and their organs were removed and examined in gross. Small and large bowels were washed in ice-cold saline and formaldelyde and examined for the presence of lesions following routine histological procedures.

Table 1. Composition of 20% polyunsaturated fat diet (Basal diet)[a]

Constituents	% Composition
Safflower oil	20.0
Casein (Vitamin free)	18.0
Sucrose	51.6
Choline Chloride	0.3
Salt mixture	4.0
Vitamin mixture	1.1
Alphacel	5.0

[a]Salt mixture (Hegsted) obtained from ICN Pharmaceuticals Inc, Cleveland, OH, USA. Vitamin composition of diets as reported in Broitman et al, 1977 (Ref.1).

The feces collected were homogenised with methanol, solvent evaporated in a roto-vap under reduced pressure and subsequently lyophilised. Bile acids were quantitated following the methods of Grundy et al[12], Deschner et al[13] and Raicht et al[14] with some modifications made in our laboratory. The powdered fecal material was subjected to Soxhlet extraction using radioactive glycocholic acid (~50,000 cpm) as recovery standard, saponified with 10(N) NaOH and extracted with hexane and water. The acid sterols from the aqueous layer were extracted with chloroform : methanol (2:1, v/v), evaporated with an azeotropic mixture of benzene : methanol (86:14, v/v) and converted into their methyl esters. Thin layer chromatography was performed on silica gel plates, first run in benzene and then in isooctane : isopropanol : acetic acid (120:40:1). The fraction containing the different bile acids were converted into their trimethylsilyl derivatives and quantitated using a Hewlett Packard Model 873 gas chromatograph on a 6 ft column packed with 3% OV-101 on 100-120 mesh Supelcoport with column temperature of 260° C and inlet and detector temperature of 280° C. All the analyses were performed using duplicate aliquots for reliable results. Statistical analysis was done by student 't' test and analysis of variance (ANOV) and differences were considered to be significant at $p<0.05$ for the appropriate degrees of freedom.

Table 2. Influence of neomycin on colon tumorigenesis in rats fed polyunsaturated fat and cholesterol diets

		Incidence of tumorigenesis		Average no. of tumours per rat	
	Diets	Small bowel	Large bowel	Small bowel	Large bowel
Group 1:	A. Control	22%	70%	0.35 ± 0.40	0.90 ± 0.30
	B. Neomycin treated	22%	98%[d]	0.50 ± 0.20	3.00 ± 0.20[c]
Group 2:	A. Control	20%	100%	0.35 ± 0.10	3.00 ± 0.70[a]
	B. Neomycin treated	25%	80%[d]	0.50 ± 0.20	1.13 ± 0.30[b,c]

[a]as compared to dietary group without neomycin and cholesterol, $p<0.01$ (ANOV); [b]as compared to dietary group with neomycin and without cholesterol, $p<0.01$ (ANOV); [c]as compared to dietary group without neomycin, $p<0.01$ (ANOV); [d]as compared with corresponding dietary group without neomycin, $p<0.01$ (Chi square test).

It is evident from Table 2 that neomycin has enhanced large bowel tumorigenesis in rats on safflower oil

diet (group 1). Lowering of serum cholesterol and shunting it out through the gut with the help of a hypocholesterolemic agent has been reported to increase colon carcinogenesis[11,15], which has been corroborated well with our finding of augmentation of large bowel tumours with administration of neomycin in rats fed safflower oil diet (group 1A vs 1B, $p<0.01$, ANOV). But neomycin has decreased the incidence of tumorigenesis when the diet has been supplemented with cholesterol and cholic acid (group 2A vs 2B, $p<0.01$, ANOV) in our study indicating a complex role of neomycin in conjunction with dietary cholesterol and cholic acid in inhibiting large bowel tumorigenesis. The exact mechanism could not be explained at this point. However, there seems to be no significant variation in the number or incidence of small bowel tumours in both group 1 and group 2 diets on addition of neomycin (Table 2). The enhanced large bowel tumorigenesis in rats fed group 2A diet compared to those fed group 1A diet (group 1A vs 2A, $p<0.01$, ANOV) has presumably been due to the inclusion of cholic acid, a known promoter of colon cancer, in the diet, and not cholesterol, since dietary cholesterol is known to inhibit colon tumorigenesis in rats[16,15].

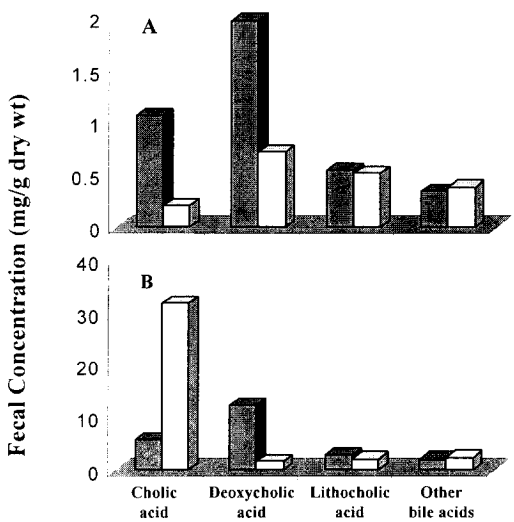

Figure 1. Fecal bile acid profile in rats fed high fat diets and neomycin. A. Dietary group 1 (20% safflower oil). Control; Neomycin treated. B. Dietary group 2 (cholesterol diet). :Control; : Neomycin treated. Values are expressed as mean ±S.E, taking 20 rats per dietary group. Other bile acids collectively represent the profiles of α and β - muricholic acid, chenodeoxycholic and ursodeoxycholic acids.

Figs. 1A and B represent the fecal excretion pattern of the various acid sterols viz. cholic, deoxycholic, lithocholic, α-and β - muricholic, chenodeoxycholic and ursodeoxycholic acids, when rats with induced colon cancer are fed safflower oil and cholesterol diets, respectively, with or without addition of neomycin. The scale of concentration in Fig. 1A is twenty times magnified with respect to that in Fig 1B. Concentrations of α- and β -muricholic, chenodeoxycholic and ursodeoxycholic acids have been presented as other bile acids in Fig. 1A and B. Concentrations of all the bile acids have been increased in general when rats are fed cholesterol diet compared to those fed safflower oil in our study, with cholic acid exhibiting the highest fecal concentration in rats fed cholesterol diet with neomycin (group 2B). In both the dietary groups, cholic acid has shown increased fecal excretion with decrease in tumorigenesis (groups 1A vs 1B, 2A vs 2B). The reason could not be ascertained satisfactorily, since cholic acid has been found to be promoter of colon carcinogenesis in rats[16]. Fecal concentration of deoxycholic acid has been found to be highest in cholesterol containing diet (group 2A) compared to other dietary groups

($p<0.01$, t-test). Although deoxycholic acid excretion has not shown any significant variation in rats fed safflower oil diet (group 1), it has been decreased remarkably in rats fed cholesterol diet when neomycin has inhibited large bowel tumorigenesis (Table 2, group 2A vs 2B, $p<0.01$, ANOV). Fecal bile acids in general have been shown to play an enhancing role in colon carcinogenesis and from earlier animal model studies, lithocholic and deoxycholic acids have been found to act as colon tumour promoters in the large bowel [18,5,16,17]. In this context, it is worthwhile to mention the recent importance of fecal water in relation to colon carcinogenesis[19]. However, in the present study, we have investigated only the fecal excretion pattern of different bile acids. Lithocholic acid seems to have no role to play in tumour formation, in our experiment. Other acid sterols like α and β - muricholic, chenodeoxycholic and ursodeoxycholic acids have remained almost the same without any significant variation among the control and neomycin administered dietary groups. The fecal bile acid excretion pattern which has emerged from this study when rats were fed neomycin and different high fat diets may provide a better understanding of the relationships among nutrition, microflora, bile acids and colonic cancer.

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