

Effect of luteolin on glycoproteins metabolism in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis

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Abstract

We have investigated the effect of luteolin, a flavonoid on colon cancer induced in rats by a colon specific carcinogen 1, 2 dimethylhydrazine (DMH). Rats were randomized into 5 groups of 10 animals each. Rats in group 1 received 1.0 ml of 0.5% carboxymethyl cellulose (CMC) everyday via intragastric intubation and served as the untreated control. Group 2 rats received luteolin via intragastric intubation (p.o.) at a daily dose of 0.2 mg/kg body weight. The rats in groups 3 to 5 received DMH [20 mg/kg body weight] injection once a week subcutaneously for the first 15 weeks. The group 4 rats received luteolin as in group 2 starting one week before the DMH injections and continued till one week after the final exposure [DMH + luteolin (Initiation)]. Groups 5 rats received luteolin as in group 2 starting one week after the cessation of DMH injections and continued till the end of the experiment [DMH + luteolin (Post-initiation)]. 20mg/kg body weight of DMH was administered subcutaneously once a week for the first 15 weeks and then discontinued. Luteolin 0.2mg/kg body weight/everyday p.o was administered at the initiation and also at the post-initiation stages of carcinogenesis to DMH treated rats. The animals were sacrificed at the end of 30 weeks. The incidence and number of tumors in the colon were significantly higher when the rats were administered DMH, as compared to DMH + luteolin (initiation and postinitiation) groups. Decrease in sialic acid was observed in the colon, intestine and increase in sialic acid was observed in the liver of DMH treated rats as compared to control animals. Increases in glycoconjugates (Total hexoses and fucose) were observed in the DMH treated rats as compared to control animals. Oral administration of luteolin restored the levels of glycoconjugats during DMH induced colon carcinogenesis. Thus, the present study indicates that luteolin has protected the cell surface and maintained the structural integrity of

the cell membranes during DMH induced colon carcinogenesis.

Keywords: Colon cancer, 1, 2-dimethylhydrazine, luteolin, glycoproteins

Introduction

Colon cancer mortality in developed countries has been steadily rising throughout most part of this century (Bandaru 1999). Mucosal cells of the gastrointestinal tract of rats are rich in glycoproteins. Glycoproteins are a group of complex proteins containing covalently bound oligosaccharides attached to their polypeptide backbone. Hexoses, fucose and sialic acid form the monosaccharide units of the oligosaccharides attached to proteins. Glycoproteins are also important components of intracellular matrix, cell membrane and membranes of the subcellular organelles (Emmetot 1973). They play a vital role in the maintenance of structural integrity of the membrane lipid bilayer. Cellular glycoproteins can be broadly classified into 2 types, secretory and structural components of both the plasma membrane and membranes of the various subcellular organelles. They play a role in cell-to-cell contact, growth regulation and as binding sites for hormones and lectins. Membrane glycoproteins play important roles in cellular phenomenon that undergoes alterations during cancerous transformation (Singhal and Hakomori 1990; Zhao et al. 2006). Cell surface glycoproteins also have important roles in the transport of vitamins and lipids in signal transduction, as hormone receptors and in immunological specificity (Paulson 1989).

Alterations in cell surface glycoproteins like the transplantation antigen of the colon cancer cells and the appearance of tumor specific antigens, have been extensively studied. Some of the changes that occur on the malignant cell surface are due to modified glycoproteins and glycolipids, aberrant fucosylation being one of the most frequent phenomena associated with oncogenic transformation (Alhadeff 1989; Hakomori 1982). It has been pointed out that the tumor cell may modulate its surface by increasing its fucose levels to escape recognition by the immune system (MacDougall et al. 1987). These alterations may contribute to some of the abnormal characteristics of tumor cells, which include decreased adhesion to the substratum and uncontrolled growth (Youakim and Herscovics 1985).

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Chemoprevention has the potential to be a major component of colorectal cancer control. Several investigators have over many years conducted research on agents with potential chemopreventive properties and have elucidated their modes of action. Although full explanation of the intricacies of the causes, development, and control of colon cancer is awaiting further research, the growing knowledge about mechanisms by which chemopreventive agents act defines opportunities to use specific agents at critical stages of cancer initiation, promotion and progression. Flavonoids occur naturally in plant kingdom. They are intensively studied for their role in human health, including cancer prevention. Luteolin is a 3', 4', 5, 7-tetrahydroxy flavone, which usually occurs as glycosylated forms in celery, green pepper, perilla leaf and camomile tea. It has been found to possess antitumorogenic (Yasukawa 1989), antioxidant (Shimoi et al 1984) and anti-inflammatory/antiallergic (Veda et al. 2002) properties. It has also been recognized as a hydroxyl radical scavenger and an inhibitor of protein kinase C.

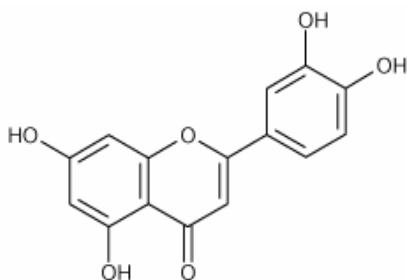


Figure 1: Diagrammatic representation of Luteolin

The effect of luteolin on the metabolism of glycoproteins in 1, 2-dimethylhydrazine induced colon carcinogenesis, is yet to be unraveled. So we studied the effect of luteolin on the tissue bound carbohydrates during the initiation and postinitiation stages of colon carcinogenesis.

Materials and methods

Chemicals

DMH was obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

Preparation of luteolin

Luteolin powder was suspended in 0.5% carboxymethyl cellulose (CMC) and each animal received 1ml of luteolin suspension at a dose of 0.2mg/kg body weight everyday (Anastasia et al. 2002).

Tumor Induction

DMH was dissolved in 1mM EDTA just prior to use and the pH was adjusted to 6.5 with 1mM sodium carbonate to ensure the stability of the chemical. Animals were given a weekly subcutaneous injection of DMH in the groin at a dose of 20mg/kg body weight for 15 weeks (Nalini et al. 2004).

Experimental Animals

Male Wistar rats 100-120g bodyweight were obtained from the Central Animal House, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India and maintained at 27 ± 2°C with 12h-light/12h-dark cycles. Commercial pellet diet

containing 4.2% fat (Hindustan Lever Ltd., Mumbai, India) was powdered and mixed with 15.8% peanut oil making a total of 20% fat was fed to rats throughout the experimental period of 32 weeks (including 2 weeks of acclimatization) to all the rats. Water was given ad libitum. Rats were randomized into 5 groups of ten animals each.

Table 1: Composition of the diet

	Commercial diet	Peanut oil	Total
Protein	17.7	-	17.7
Fat	4.2	15.8	20.0
Carbohydrate	50.5	-	50.5
Fiber	3.4	-	3.4
Mineral	6.7	-	6.7
Vitamin	1.7	-	1.7

Treatment Schedule

Rats in group 1 received no treatment and served as the untreated control. Group 2 animals received luteolin by intragastric intubation daily at a dose of 0.2mg/kg body weight everyday. Rats in groups 3 to 5 received DMH [20mg/kg body weight] injection once a week subcutaneously for the first 15 weeks. Group 4 rats received luteolin as in group 2 starting one week before DMH injections and continued till one week after the final exposure [DMH + luteolin (initiation)]. Group 5 rats received luteolin as in group 2 starting one week after the cessation of DMH injections and continued till the end [DMH + luteolin (postinitiation)]. The experiment was terminated at the end of 30 weeks and all the animals were killed by cervical dislocation after an overnight fast. The colon was split open longitudinally and gross tumors were counted. Colonic and intestinal tissues were then processed and used for various biochemical estimations. The experimental protocol is clearly represented below in Figure 2.

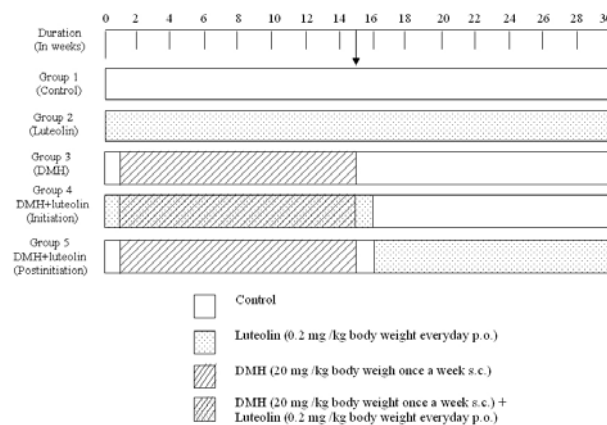


Figure 2: Experimental Protocol

The experiment was terminated at the end of 30 weeks and all the animals were killed by cervical dislocation after an overnight fast. The colon was split open longitudinally and gross tumors were counted. Colonic and intestinal tissues were then processed and used for various biochemical estimations.

Extraction of tissue glycoproteins

The tissues were rinsed in ice cold 0.15 M NaCl to remove blood-borne contaminants and the water was blotted off. It was chopped after weighing in an electronic balance and each sample was extracted for lipids by the method of Folch et al (Folch et al. 1957).

The defatted tissues were used for estimation of carbohydrate moieties of glycoproteins.

Biochemical estimations

The protein bound Sialic acid, hexose and fucose were estimated by the methods Lindberg et al. (1991), Neibes et al. (1972) and Dische and Shettles (1948) and of respectively. Tissue protein was determined by the method of Lowry et al. (1948).

Statistical Analysis

The data presented here are means ± SD of 10 rats in each group. The results were analysed using one-way analysis of variance [ANOVA] and the group means were compared by Duncan's Multiple Range Test [DMRT] using SPSS version 12 for windows. The findings were considered statistically significant if p<0.05.

Results

The average growth rate of the animals in the control and experimental groups are shown in Figure 1. It was observed that the weight gained by rats in the control group > luteolin group > DMH + luteolin (initiation) group > DMH + luteolin (postinitiation) group > DMH group. The effect of luteolin on incidence, percentage, multiplicity, and number of tumors per tumor bearing rat and size of colonic tumors in DMH-treated rats are summarized in Table 2. There were no tumors in the control rats (group 1) and luteolin-treated control rats (group 2). In rats treated with DMH injections (group 3) the tumor incidence in the colon was 100% and the average size of the tumor was approximately 2cm. Luteolin supplementation to DMH treated rats during the initiation stage of carcinogenesis (group 4) reduced tumor size and incidence (10%). Luteolin supplementation during the post-initiation stage (group 5) also resulted in significantly reduced tumor size (0.5cm) and incidence (20%) as compared to the unsupplemented DMH treated rats.

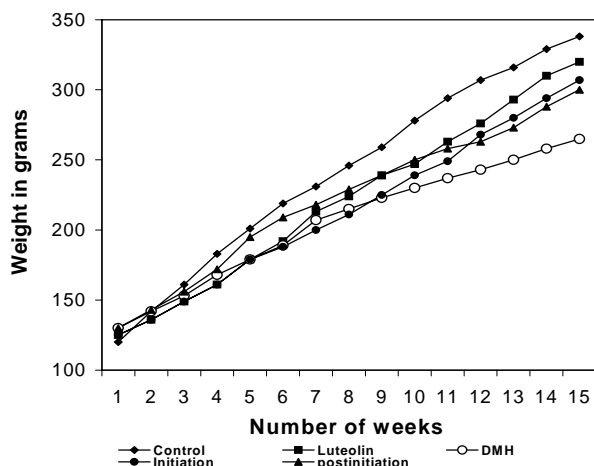


Figure 3: Effect of luteolin and DMH on the average growth rate on control and experimental animals.

Table 3 gives the levels of sialic acid, hexose and fucose in the colon, intestine and liver of control and experimental animals. The levels of sialic acid were significantly decreased in the colon and intestines and increased in liver, whereas the hexose and fucose levels in the colon, intestines and liver were significantly elevated in DMH-treated rats (group 3) as compared to control animals (group1). On luteolin supplementation both during the initiation as

well as the postinitiation phases (groups 4 and 5) the levels of sialic acid were significantly increased in the colon and intestines and decreased in the liver, whereas the hexose and fucose levels in the colon, intestines and liver were significantly decreased as compared to unsupplemented DMH – treated (group 3) animals.

Table 2: Incidence of colonic neoplasms

Group	Number of rats examined	Number of rats with tumor	Incidence of tumor (%)	Tumor size
Control	10	0	0	-
Luteolin	10	0	0	-
DMH	10	10	100	2cm
DMH+luteolin (initiation)	10	1	10	-
DMH+luteolin (postinitiation)	10	2	20	0.5cm

Discussion

The data presented here clearly indicate that the administration of the procarcinogens DMH to rats, in the presence of luteolin, brings about profound alterations in the metabolism of glycoproteins.

Sialic acid is widely distributed in mammals and occurs as a terminal component at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids and it has been implicated in a number of phenomena including metastatic spread, contact phenomenon, tumor antigenicity, transport process and viral receptors (Filipe 1969). In the present study, we observed that the tissue sialic acid levels were significantly decreased in the colon and intestines and increased in the liver of DMH treated rats (group 3) as compared to control rats (group 1). Our study also revealed that tissue sialic acid has significant positive correlation with colon cancer, which is in accordance with previous reports. The observed alterations may be either due to decreased synthesis or increased degradation of glycoprotein antigens. These alterations in glycoproteins of the cancerous tissue may be qualitative, quantitative or both (Hakomori et al. 1984). Colon is an area of the intestinal tract with a high luminal population of bacteria, many of which secrete neuraminidases (Filipe 1969). Since 4-O-acetyl sialic acids are resistant to such neuraminidases, the glycoproteins in this area would be expected to be relatively resistant to loss of sialic acid, whereas this may not be the case with other sugars. Sialic acid probably protects the glycoproteins from proteolysis (Faillard and Schauer 1972; Reid et al. 1975) thus guarding the underlying epithelial cells. Such a protective function may account for the presence of O-acetylated sialic acids being confined to the colon and furthermore, may explain the difference in the sialic acid concentrations of the upper and lower colon. The elevated sialic acid levels in the liver could be due to selective increase in existing specific sialylated sequence or a tumor associated de novo synthesis of specific sialylated sequence. The current results support the notion that the alterations seen in cell surface glycoproteins during oncogenic transformation can be the result of altered expression of glycosyl transferases. The presence of sialyltransferases in malignant cells could lead to altered or even unique glycoconjugates.

Earlier we have revealed an increase in the activity of enzymes of the intestinal microbes in DMH treated rats as compared to control rats (Nalini et al.1998). This may lead to increased hydrolysis of the other types of neuraminic acids present there in, resulting in an overall decline in the sialic acid content of the colon in DMH treated rats. Moreover it is known that the activity of a set of glycosyl transferases such as the N-acetyl galactosaminyl transferases and galactosyl transferases are decreased significantly, whereas sialyl transferase activity is increased significantly in cancer tissues resulting in the accumulation of incomplete oligosaccharides, and

Table 3: Effect of luteolin on tissue sialic acid, fucose and hexose in control and experimental rats

Groups	Sialic acid (mg/g protein)				Fucose (mg/g protein)			
	Proximal colon	Distal colon	Intestine	Liver	Proximal colon	Distal colon	Intestine	Liver
Control	51.49 ± 4.05 ^a	45.96 ± 3.61 ^a	47.82 ± 3.76 ^a	24.36 ± 1.91 ^a	27.23 ± 2.14 ^a	28.46 ± 2.24 ^a	31.91 ± 2.51 ^a	22.72 ± 1.78 ^a
Luteolin	47.13 ± 3.71 ^b	53.27 ± 4.19 ^b	48.82 ± 3.84 ^a	22.36 ± 1.76 ^a	24.56 ± 1.96 ^b	23.92 ± 1.88 ^b	29.45 ± 2.31 ^b	20.15 ± 1.58 ^b
DMH	30.49 ± 2.40 ^c	31.58 ± 2.48 ^c	32.62 ± 2.56 ^c	39.26 ± 3.09 ^b	39.56 ± 3.11 ^c	37.19 ± 2.92 ^b	39.56 ± 3.11 ^c	48.59 ± 3.82 ^b
DMH+luteolin (initiation)	51.53 ± 4.05 ^a	45.76 ± 3.60 ^a	47.49 ± 3.73 ^{ab}	27.28 ± 2.14 ^d	28.43 ± 2.23 ^{ad}	24.62 ± 1.93 ^b	26.34 ± 2.07 ^c	24.12 ± 1.89 ^a
DMH+luteolin (postinitiation)	49.86 ± 3.92 ^{ab}	42.49 ± 3.34 ^d	44.62 ± 3.51 ^b	25.68 ± 2.02 ^{ad}	30.52 ± 2.40 ^d	27.58 ± 2.17 ^a	28.96 ± 2.28 ^b	26.73 ± 2.10 ^d

Groups	Hexose (mg/g protein)			
	Proximal colon	Distal colon	Intestine	Liver
Control	181.26 ± 14.27 ^a	173.65 ± 13.67 ^a	228.63 ± 18.01 ^a	162.35 ± 12.78 ^a
Luteolin	174.01 ± 13.70 ^a	162.31 ± 12.78 ^a	229.68 ± 12.68 ^a	196.57 ± 15.47 ^b
DMH	294.86 ± 23.21 ^c	281.36 ± 22.15 ^c	298.67 ± 23.51 ^b	275.69 ± 21.70 ^c
DMH+luteolin (initiation)	183.21 ± 14.42 ^a	175.23 ± 13.79 ^a	249.86 ± 19.67 ^b	174.86 ± 13.76 ^a
DMH+luteolin (postinitiation)	198.54 ± 15.63 ^b	193.58 ± 15.24 ^d	273.15 ± 21.50 ^d	191.53 ± 15.08 ^b

Values are mean ± S.D of ten rats in each group. Values not sharing a common superscript (a-d) differ significantly at $p < 0.05$ (DMRT)

thus substantial alteration in the carbohydrate composition. The finding also supports our results that the sugar commonly present on the non-reducing termini of the carbohydrate moieties of glycoproteins, sialic acid is much reduced in tumor tissue (Filipe 1969). In addition the synthesis of one type of oligosaccharide chain is known to be unaffected by the development of tumor while the synthesis of another family of oligosaccharides is generally affected (Filipe 1969; Lamont et al. 1974).

Luteolin administration during both the initiation (group 4) and postinitiation (group 5) phases of carcinogenesis significantly decreased the levels of sialic acid in the colon and intestines as compared to unsupplemented DMH treated rats. The presence of luteolin can prevent the degradation of the carbohydrate portions of glycoprotein by the bacterial microflora in these tissues to some extent. Moreover luteolin is known to have both antioxidant and prooxidant effects (Veda et al. 2002). The prooxidant effect of luteolin generates reactive oxygen species in the cancer tissues which in turn results in lipid peroxidation, damage to membranes and apoptosis of the cancer cells. Membrane damage can result in alterations in the membrane glycoproteins and thus the protein bound sugars.

Elevations in fucose content in the tissues of cancer patients have been reported by some investigators and are of considerable interest because of their potential applications as diagnostic and prognostic markers (Patel et al 1994; Wang et al. 1995). Recently many biochemical studies have shown that changes in fucose content appears to be associated with tumor progression rather than with malignant transformation (Patel et al 1994; Wang et al. 1995). In our study we have observed increased levels of fucose and total hexoses in DMH treated rats as compared to control rats. Similar observations were reported by other researchers in different tumor tissues (Wang et al. 1995; Hakomori et al. 1984; Nuck et al. 1992). Already, Kemmner (Kemmner et al. 1992) described increased fucose content in cells from metastasizing colon tumors. Though the exact cause of increase in fucose levels in malignancy is not known, it may be due to the enhanced concentration of glycoproteins and glycolipids, and also the enhanced degree of fucosylation of these compounds. The turn-over of fucose residues in glycoconjugates is achieved by the implication of fucosyltransferases and fucosidases. Hence, the elevated fucose levels observed in tissues of DMH-treated rats could be explained atleast in part by the decreased α -L-fucosidase activity. On luteolin administration during both initiation

(group 4) and postinitiation stages (group 5) of carcinogenesis the levels of total hexoses and fucose were decreased significantly as compared to DMH treated rats (group 3). This may be due to the protective role of luteolin in reducing the tumor incidence.

Thus luteolin supplementation had a significant modulatory role on tissue protein bound carbohydrates in DMH induced colon carcinogenesis. The present study indicates that luteolin has protected the cell surface and maintained the structural integrity of the cell membranes during DMH induced colon carcinogenesis. Moreover luteolin has a marked protective effect against colon cancer, the exact mechanism for which has to be elucidated.

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