Effects of Dietary Advanced Lipid Oxidation End-products on Colitis Healing in Albino Rats

***Adeleye, G. S.**
Nwozor, C. M.
Okafor, C. B.
Department of Physiology, Anambra State University, Uli, Nigeria
****E-mail:*** *adeguyton@yahoo.com*

ABSTRACT

This Experiment is undertaken to investigate the effects of dietary advanced lipid oxidation endproducts on colitis healing in albino rats. 45 albino rats (divided into 3 groups - control, low dose and high dose ALE groups) of average weight of 200g were used for this study. Colitis was induced in all groups using 6% acetic acid. The low and high doses were fed with 7.5g and 15g ALE respectively for 20 days.The control animals ate normal rat chow. The stools of all animals were scored according to the scale of Masonobu et al (2002) for 20 days. On days 7, 14 and 20 three animals were sacrificed from each group and 8cm of the colon was cut out for weight measurement and gross morphological scoring.The results show that on day 6 colitis scores were 0.63 ± 0.03 (control), 0.80 ± 0.03(low dose),0.73 +_ 0.03(high dose). On day 20, 0.33 ± 0.03 (control), 0.50 ± 0.03 (low dose), 0.50 ± 0.02 (high dose). ALE reduced colitis healing rate compared to the control. This study concludes that ALE aggravated acetic-acid induced colitis in albino rats.

Keywords: *Lipid oxidation, Colitis, Albino rats, Healing*

INTRODUCTION

The oxidation of unsaturated lipid results in significant generation of dietary advanced lipid oxidation endproducts (ALEs) which are in part cytotoxic and genotoxic compounds. Colitis is an inflammation of the colon and are of many types: ulcerative, ischemic, crohn's disease, collagenous, chemical colitis among others (Romano *et al* 2008). Lipid oxidation in foods is one of the major degradative processes responsible for changes in flavour, color, and texture. The oxidation of unsaturated lipids results in significant generation of cytotoxic and genotoxic compounds (Addis, 1986 and Kubow, 1992). The free radicals generated by the process of lipid oxidation not only generate cytotoxic compounds but also co-oxidize vitamins such as vitamin A and carotenoids, vitamin E and vitamin C, and thereby impair the nutritional quality of the foods (Kanner 1994, Gorelik, Lipidot , Shaham and Granit 2005). The process of lipid oxidation is initiated when a hydrogen atom is removed from a methylene group in the hydrocarbon chain of a lipid molecule and especially from a dietary polyunsaturated fatty acid (PUFA) such as linoleate, linolenate and arachidonic acid, but also from eicosapetaenoic acid and docosahexaenoic acid. High - fat and high-cholesterol foods not only affect endogenous lipoprotein production and catabolism, but probably also lead to transient exposure of arteries to cytotoxic chylomicron remnants and advanced lipid oxidation endproducts (Cohn 2003). The western diet contains large

quantities of oxidized fatty acids, oxidized cholesterol, cytotoxic aldehydes and phospholipids because a large proportion of these in the diet are often consumed in fried, heated, processed and long stored form. Hundreds of volatile and non volatile decomposition products have been identified in cooking oils subjected to commercial frying conditions most of these are aldehydes (Frankel, 1998; Chang, Peterson and Ho, 1978). Colitis is an inflammation of the colon. There are many types of colitis viz: autoimmune, iatrogenic, idiopathic vascular and infectious (Beutin, 2006). Signs seen on colonoscopy include: colonic mucosal erythema, ulcers, bleeding. How a given colitis is treated is dependent on its etiology, e.g infectious colitis is usually treated with antimicrobial agents (e.g antibiotics), autoimmune mediated colitis is treated with immune modulators or immune suppressants. This study is therefore undertaken to investigate the effects of dietary advanced lipid oxidation endproducts on acetic acid induced colitis in albino rats.

MATERIALS AND METHOD

This study was carried out in the department of physiology, faculty of Basic Medical Sciences, Anambra State University, Uli, Anambra State. Forty- five healthy adult albino rats of wistar strain weighing between 180-220g were used in the study. The animals were housed under standard conditions of temperature (23 ± 20^{o}C) and humidity and 12 hours light (7.00am - 7.00pm). They were kept in wire meshed cages and fed with commercial rat pellets (Greg feeds, Uli centre, Anambra State) and allowed water *ad libitum*. The animals were divided into three groups of 15 rats each. Colitis was induced in all the groups. Group 1 served as control and received normal rat chow and water. Group 2 and 3 received low and high doses of ALEs respectively.

Preparation of Dietary Advanced Lipid Oxidation End-Product: ALE used was red palm oil bleached at a high temperature obtained from the local market.

Induction of Experimental Colitis: Albino rats weighing between 180-220g each were used for the experiment. Colitis was induced according to the previously described method (Jagtap, Shirke and Phadke, 2004). Animals were deprived of food for 24hrs before the induction of colitis but allowed free access to water - rats were anaesthetized with thiopental and a flexible catheter (diameter 2mm) was inserted into the anus and the tip was advanced 8cm proximal to the anus.1ml of acetic acid 6% was instilled into the colon through a cannula for 30seconds after which fluid was withdrawn. To prevent spillage of solution from rectum, animals were allowed to hang in air by holding their tails for 45-60 seconds.

Administration of Dietary Advanced Lipid Oxidation Endproduct: The rats in the experimental groups were given ALE by oral dosing method using the oral cannula. Group 1 (control) did not receive ALE. Group 2 (low dose) received 7.5g of ALE mixed with 92.5g of feed daily for 20 days. Group 3 (high dose) was given 15g of ALE mixed with 85g of feed daily for 20 days.

Stool Scoring: Every morning, all rats from the three groups were scored. Each of the rats from each group was brought out kept on a white paper the tail of the rat was held upwards and then the rat passed stool from its anus. The stool was examined physically and then scored. The following scoring patterns of Masonobu et al (2002) were used.

0 - Normal
1 - Soft stool but still formed
2 - Soft/wet stool/unformed stool
3 - Soft/wet stool + blood traces
4 - Bloody diarrhea

After scoring the mean, standard deviation, and standard error of mean were calculated.

Gross Morphological Damage Scoring: Every one week, 3 animals from each group were sacrificed. The rats were dissected using a scapel. The colon was traced to the anus and was cut. 10cm of the colon was cut open and the inside washed with clean water from a running tap. A hand lens of x3 magnification was used to examine and score the internal structure of the colon. The following scoring pattern of gross morphological damage was used.

0 - No damage
1 - Localized hyperemia with no ulcers.
2 - Linear ulcer with no significant inflammation.
3 - Linear ulcer with inflammation at one side.
4 - More sites of ulcers and inflammation, the size of ulcer < 1cm.
5 - Multiple inflammation and ulcers, the size of ulcer > 1cm.

After scoring, the mean, standard deviation and standard error of mean were calculated. The colons from each group were weighed on an electronic weighing balance and values taken. The data obtained were expressed as mean ± SEM (standard error of mean). The student's t-test was applied and P-values were determined. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Stool Scoring*:* On day 6 the scores were 0.63 ± 0.03 (control) 0.8 ± 0.03 (low dose), 0.73 ± 0.03 (high dose). On day 20, 0.33 ± 0.03 (control) 0.50 ± 0.03 (low dose), 0.50 ± 0.02 (high dose).

Gross Morphological Damage: On day 7 the scores were 2.00± 0.00 (control), 2.5± 0.05 (low dose), 3.00 ± 0.00 (high dose). On day 20, 1.00 ± 0.00 (control) 3.00 ± 0.00 (low dose), 3.67 ± 0.07 (high dose).

Colon weight: On day 7 the scores were 1.24 ± 0.00 (control), 1.44 ± 0.00 (low dose) 1.69 ± 0.07 (high dose). On day 20, 1.35 ± 0.09 (control), 1.60 ± 0.03 (low dose), 1.67 ± 0.02 (high dose).

The oxidation of unsaturated fatty acids results in significant generation of dietary advanced lipid oxidation endproducts (ALEs) which are in part cytotoxic and genotoxic compounds. The gastrointestinal tract is constantly exposed to dietary oxidized food compounds. After digestion a part of them are absorbed into the lymph or directly into the blood stream. Some of the dietary ALEs, which are absorbed from the gut to the circulatory system, seem to act as injurious chemicals that activate an inflammatory response which affects not only circulatory system but also organs such as liver, kidney, lung, and the gut itself (Kanner 2007). Acetic acid induced colitis is one of the commonly used experimental

models while screening natural products and drugs active against inflammatory bowel disease. Consistent with previous reports, in thus study, intrarectal administration of acetic acid caused diffused inflammatory leucocyte infiltration, ulcerated mucosa and necrosis (Hager, Medany, Eter, Arafa and Eur, 2007). The present study showed that dietary advanced lipid oxidation endproducts increased tissue damage in rat model of colitis induced by acetic acid based on stool scoring, colon weight and gross morphological damage.

CONCLUSION

This study is an experiment conducted to examine the effects of dietary advanced lipid oxidation end-products on colitis healing in albino rats. From the study the weight of damaged colon tissue is considered an indicator of the severity and extent of inflammatory response. ALE administered groups showed an increase in colon weight and macroscopic scores for the inflammation. Based on this study it can be concluded that ALEs aggravated acetic acid induced colitis in albino rats. ALEs have been shown to produce oxycholesterols and especially, 7-oxygenated species which are highly cytotoxic toward endothelial cells (Lamaire-Ewing, Prunet, Montange and Vejux, 2005).

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