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ORAL ADMINISTRATION OF FENOTEROL MODIFIES INTESTINAL OXIDATIVE STATUS OF MICE

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ABSTRACT: Oxidative stress, the natural consequence of the oxygen metabolism, is normally controlled by antioxidant endogenous defense systems. When these prove to be insufficient, cellular lesions develop that result in ageing but also in some pathological processes. The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defense process against free radicals. Unfortunately, due to the fragility of its molecular structure, non-protected SOD is inactivated in the digestive tract. The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defense process against free radicals. The effect of fenoterol on lipid peroxidation remains unknown, and there were no studies on this subject previously. The present study was undertaken to classify the effect of fenoterol related to lipid peroxidation event and for this aim tissue malondialdehyde (MDA) levels in small intestine was determined. In the present study, we evaluated the lipid peroxidation in mice which were treated with beta agonist fenoterol. The estimation was done in duodenum, jejunum and ileum on days 7,14,21,28. Lipid peroxidation increases from day 7 to 28 after fenoterol administration. The results derived from experimental groups demonstrated that fenoterol induce lipid peroxidation and suppresses oxidative metabolism. Increase of MDA level led us to reach this conclusion. These parameters are basic requirement for oxidative damage and MDA is one of the most important metabolite occurring as a result of oxidative damage.

KEYWORDS: Fenoterol, lipid peroxidation, intestine, free radical, antioxidant

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1.INTRODUCTION

Antioxidative metabolism and lipid peroxidation can be affected by various conditions and substances such as drugs, pesticides, stress and exercise. Lipid peroxidation is a degenerative process which affects the PUFA (polyunsaturated fatty acids) of membrane phospholipids. The general mechanism of this process involve the formation of toxic aldehydes, which react with protein and non- protein substances and result in wide spread changes in cellular membranes. These degenerative processes can be prevented molecularly and enzymatically. Therefore, lipid peroxidation can be estimated directly by determination of reactive oxygen species (ROS) or MDA levels in tissues. Fenoterol has potent anabolic effects on mice skeletal muscle. Fenoterol treatment causes a small increase in fatiguability due to decrease in oxidative metabolism, with some cardiac hypertrophy (Ryall *et al*.,2004). Some free radicles are responsible for the damage of sugars, fatty acids and nucleic acids. Increase in the activity of antioxidant enzymes is required which shows scavenging action. SODs are protein enzymes and their function specifically depends on their quaternary structure. All changes in the environment may, to a greater or lesser extent and more or less irreversibly, modify this structure and therefore the functionality of the SOD. In particular, during gastrointestinal passage, the quaternary structure is modified and the enzyme is inactivated. This is why it is difficult to produce a SOD-rich food supplement that remains active when taken orally. Beta adrenergic agonists have been utilized in many clinical settings, however little attention has paid to their effects on gastrointestinal system. In this study we demonstrate that fenoterol increases MDA in duodenum, jejunum and ileum from doses 1.5mg/kg for 7-28 days. The aim of present study was to evaluate the effects exerted by daily oral administration of fenoterol to mice at doses of 1.5mg/kg for 7-28 days on intestinal physiology. We evaluated the oxidative status in the intestinal mucosa. The effects on the oxidative status could be interesting on the basis of its possible application in some intestinal disease which has been related to the production of free radicals, such as inflammatory bowel disease. Finally, the oxidative status in small intestine was also studied in order to know the possible systemic effects that fenoterol might exert.

2. MATERIALS AND METHODS

Adult Swiss albino male mice of Balb- C strain weighing 25-30g were procured from Central Research Institute (CRI), Kasauli, Himachal Pradesh. They were housed in polypropylene cages under controlled conditions of temperature and light (24 ± 20C; 16 hr day light) and fed upon Hindustan lever pellet diet and water *ad libitum*. All experimental procedures were conducted after the approval of Institutional Animal ethics committee, Himachal Pradesh University (IAEC/BIO/4-2006), Shimla. Mice were randomly assigned into two independent groups: One group containing normal mice served as control and the other group included mice as treated groups. Animals of second group were given daily oral administration of fenoterol (1.5 mg/ kg body wt) for 28 days).

Lipid Peroxidation:

Levels of malondialdehyde index of lipid peroxidation were estimated according to the method of Dhindsa *et al.*,1981 using thiobarbiutric acid. Tissues were homogenized in 2ml of 0.1% TCA in pestle and morter. Homogenate was then centrifuged at 6000 rpm for 15 min. To 1ml of supernatant, 2ml of 0.5% TBA prepared in 10% TCA was added. The test tubes containing the above solution were kept in boiling water bath for 30 min. Tubes were then cooled in ice-cold water bath and then centrifuged. Absorbance of the supernatant was taken at 532nm and 600nm. Difference of the two absorbance was taken as actual value and used for calculating TBA reactive substance malonyldialdehyde formed. The MDA contents were calculated in n moles/ml.

3. RESULTS AND DISCUSSION

Duodenum

The normal mice duodenum shows MDA content 22.6 ± 0.1 n moles/g of fresh tissue wt. at 7 days stage. The MDA production is almost similar at 14,21 and 28 days.MDA production increases to 24.2± 0.04 n moles/g of fresh tissue wt in drug treated mice. Increase in %age from normal to treated is calculated to be 7%. At 14 day stage, a further increase in MDA concentration is noticed and it was found to be 37.8%.Similar trend is observed at 21 days and 28 days stages. The increase in MDA concentration is 56.6% in former and 176% in later.

Jejunum

The jejunum of normal mice exhibits 33.8± 0.1 n moles/g of fresh tissue wt of MDA production at 7 days stage. The MDA production in normal jejunum at 14,21 and 28 days is almost same.Fenoterol treated mice witnesses 36.2± 0.08 n moles/g of fresh tissue wt. The increase in %age is 7.1%. The increase in MDA production is observed from 7-28 days stage in treated mice. At 14 days stage, the MDA concentration is 50.9± 0.4 n moles/g of fresh tissue wt. The increase in %age is 51%. The MDA concentration is 63.3± 0.8 n moles/g of fresh tissue wt at 21 days stage and 77± 1.3 n moles/g of fresh tissue wt at 28 days stage. The % age increase is 88.9% in former and 127% in later.

Ileum

MDA production in ileum of normal mice at 7 day stage is 21.5± 0.06 n moles/g of fresh tissue wt. The MDA production in normal ileum is almost similar at all the stages of investigation. The MDA production in drug treated mice is 51.6± 1.04 n moles/g of fresh tissue wt. The increase in %age is 228%. A further increase in MDA concentration is observed from 14 -28 days of investigation. % age increase during the period of investigation is 242% at 21 days stage and 311% at 28 days stage. Fenoterol results an increase in MDA concentration in treated mice at all the stages of present study.

Table and Fig. III (b)

		Days			
		7	14	21	28
Duodenum	**Normal**	22.6 ± 0.1	22.7 ± 0.06	22.6 ± 0.1	22.6 ± 0.05
	Treated	24.2 ± 0.04*	31.3 ± 0.06*	35.4 ± 0.09*	62.5 ± 0.06*
Jejunum	**Normal**	33.8 ± 0.1	33.7 ± 0.2	33.5 ± 0.09	33.8 ± 0.1
	Treated	36.2 ± 0.08*	50.9 ± 0.4*	63.3 ± 0.8*	77 ± 1.3*
Ileum	**Normal**	21.5 ± 0.06	21.3 ± 0.04	21.6 ± 0.01	21.5 ± 0.1
	Treated	51.6 ± 1.04*	70 ± 1.36*	74 ± 1.36*	88.4 ± 0.12*

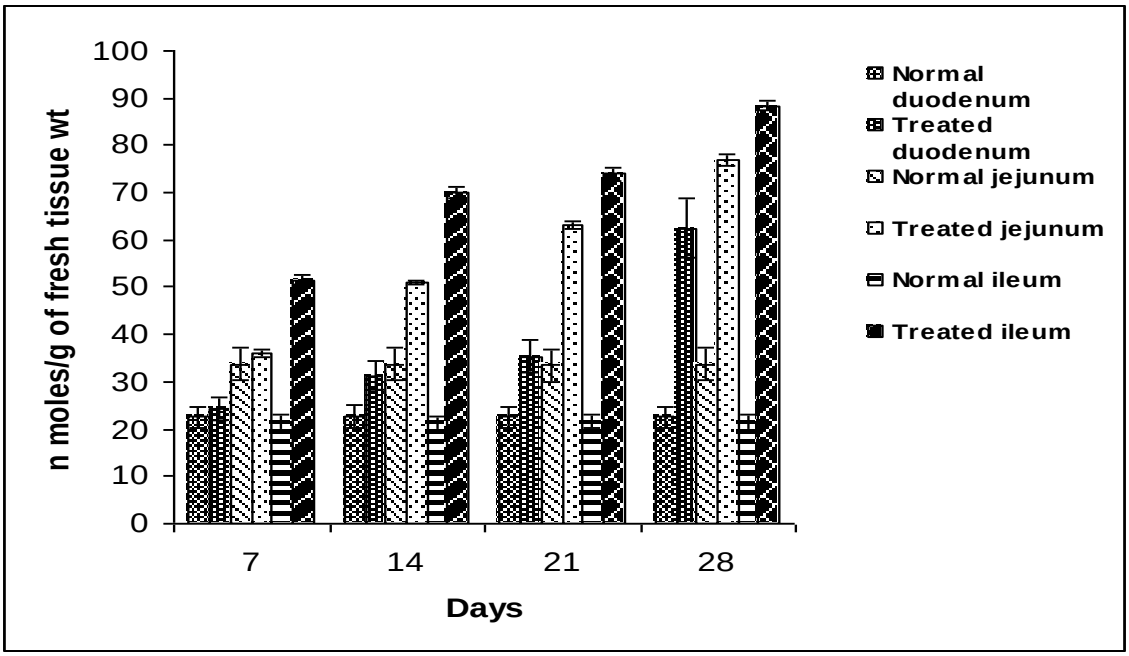

Table and Fig. Lipid peroxide (n moles of TBARS formed/g of fresh tissue weight) in duodenum, jejunum and ileum of normal and drug treated mice during 7-28 days period. Values are mean ± SEM; n = 3 (P*<0.05)

Superoxide dismutase (E.c 1.15.1.1)

Superoxide dismutases are a family of metalloenzymes that catalyse the disproportionation of superoxide (O_2^-) radicals and they play important role in protecting cells against the toxic effects of superoxide radicals produced in different cellular loci. Change in the activities of superoxide dismutase enzyme can be monitored in vitro in the tissues.

SOD activity in Vastus lateralis {Table and Fig. II (a)}

Determination of superoxide dismutase enzyme activity is done in normal and drug treated mice vastus lateralis, duodenum, jejunum and ileum part of small intestine. The control mice vastus lateralis muscle shows 665 ± 2.0 units of enzyme activity. Superoxide dismutase activity increases to 996 ± 1.0 units after drug treatment at 7 days stage. The increase in enzyme activity is calculated to be 49.77%. At 14 day stage, further 68.5% increase of 1124 ± 1.0 units/g of fresh tissue wt./min. is noticed. At 21 day stage, there is a further increase of enzyme activity (1335±1.1units/g). The percentage increase is calculated to be 100%. At 28 days stage, the enzymatic activity is 1995±2.4 units/g of fresh tissue wt./min. The percentage increase is 198.6%. Increase in the enzymatic activity is recorded throughout 7-28 days of investigation. In treated mice the values are statistically significant at all the stages ($P^* < 0.05$).

SOD activity in duodenum {Table and Fig. II (b)}

The enzyme activity is 534 ± 1.3 units/g of fresh tissue weight/min in normal duodenum at 7 day stage. The enzymes activity is almost same at 14, 21, 28 days stage in normal duodenum. Drug treatment shows increase in enzyme activity (665 ± 1.5 units/g of fresh tissue wt/min). The percentage increase is 24.53%. At 14 day stage, further increase of 48.4% enzyme activity is noticed, it is 794 ± 1.7 units/g of fresh tissue weight/min. Administration of fenoterol to the mice influences the activity of enzyme in smooth muscle. Further increase 864 ± 2.1units/g is seen at 21 days stage with percentage increase of 61.79%. At 28 days stage, the enzyme activity further increases to 935 ± .06 units/g of fresh tissue weight/min. The increase in percentage is 74.7%.

SOD activity in jejunum

The jejunum of normal mice at 7 days stage shows enzyme activity 464±1.4 units/g of fresh tissue weight/min. the activity is almost steady in normal jejunum throughout the experiment. Oral administration of drug to the mice demonstrates increase in the enzyme activity. At 7 day stage after drug treatment the enzyme activity is 594±2.1units/g. The enzymetic activity is further increases to 636 ± 1.0 units/g of fresh tissue weight/min after 14 days of treatment. The increase in percentage is calculated to be 37%. The SOD activity is 766 ± 1.3 units/g of fresh tissue weight/min at 21 days stage and the percentage increase is 64.73%. The percentage increase is calculated to be 86% at 28 days stage (865 ± 0.6 units/g). A continuous increase in the enzyme activity is seen during 7-28 days of investigation.

SOD activity in ileum

The enzyme activity in normal mice is 663 ± 1.5 units/g of fresh tissue weight/min at 7 days stage. The enzyme activity is almost same at 14, 21, 28 days stage in normal ileum. After drug treatment a regular increase in enzyme activity is observed. The enzyme activity in ileum is 735 ± 0.6 units/g of fresh tissue weight/min after 7 days of drug treatment. The increase in enzyme activity is 10.8%.

The SOD activity is 865 ± 0.6 units/g of fresh tissue weight/min at 14 days stage with percentage increase of 30.2%. The enzyme activity increases to be 926±0.9 units/g of fresh tissue weight at 21 days stage. The increase is 39.45%. Similarly, at 28 days stage, the enzyme activity enhances to 50.2%. A regular trend of increase in enzyme activity in noticed after drug treatment in skeletal and smooth muscle of mice. In treated mice, the values are statistically significant at all the stages (P* < 0.05).

Table and Fig. II (b)

		Days			
		7	14	21	28
Duodenum	**Normal**	534 ± 1.3	535 ± 2.2	534 ± 1.1	535 ± 1.1
	Treated	665 ± 1.5*	794 ± 1.7*	864 ± 2.1*	935 ± 0.6*
Jejunum	**Normal**	464 ± 1.4	464 ± 2.4	465 ± 2.5	465 ± 0.6
	Treated	594 ± 2.1*	636 ± 1.0*	766 ± 1.3*	865 ± 0.6
Ileum	**Normal**	663 ± 1.5	664 ± 0.4	664 ± 0.6	663 ± 0.6
	Treated	735 ± 0.6*	865 ± 0.6*	926 ± 0.9*	996 ± 1.3*

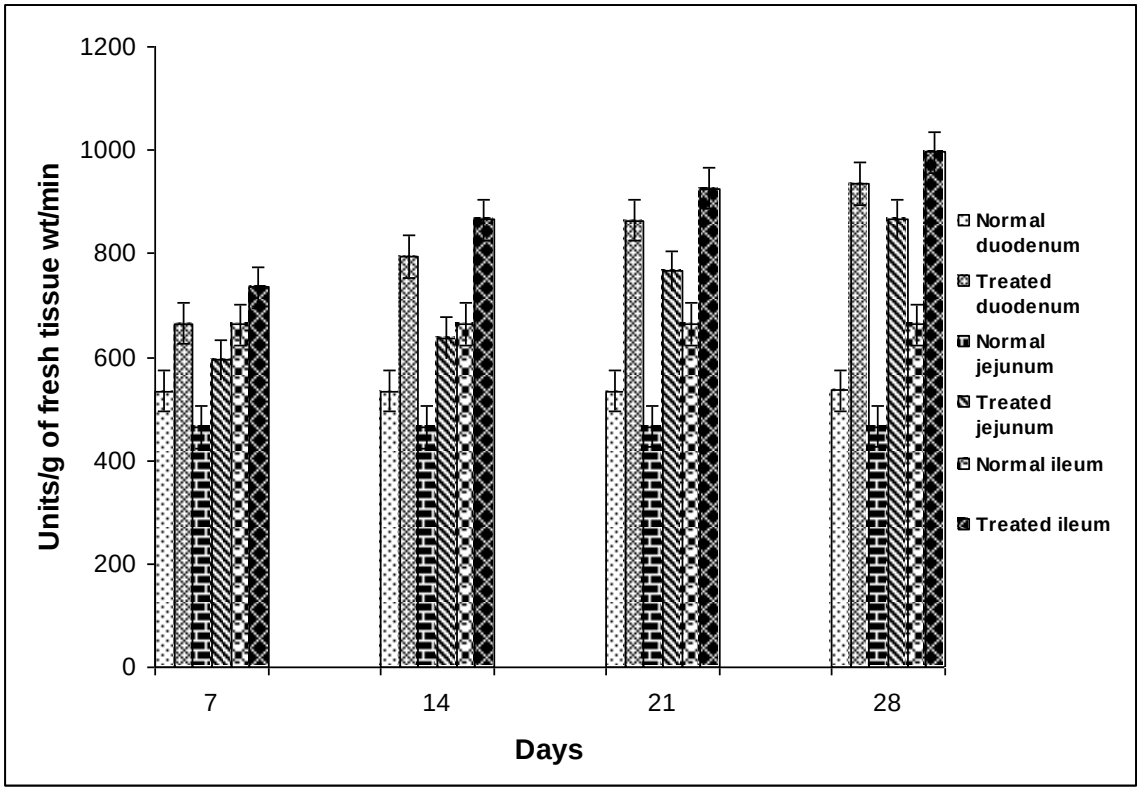

Table & Fig II(b):	Superoxide dismutase specific activity (units/g of fresh tissue wt/min) in duodenum, jejunum and ileum of normal and drug treated mice during 7-28 days period. Values are mean ± SEM; n=3 (P*<0.05)

DISCUSSION

Under normal circumstances, the levels of superoxide anion (O_2^-) produced by the one electron reduction of molecular oxygen) are kept under tight control by endogenous superoxide dismutase (SOD) enzymes, the enzymatic activity of which was discovered in 1969 by McCord and Fridovich. Superoxide anion is formed via a large number of pathways, including normal cellular respiration, inflammatory cells, endothelial cells and in the metabolism of arachidonic acid. In acute and chronic inflammation, the production of superoxide anion is increased at a rate that overwhelms the capacity of the endogenous SOD enzyme defence system to remove it. The consequence of this imbalance results in superoxide anion mediated damage. In the present study we demonstrate that Fenoterol causes a marked increase in reactive oxygen species production and MDA levels, used as marker of lipid peroxidation, in the mice small intestine. At the same time, fenoterol is a significant scavenger of reactive oxygen species, and enhances SOD activity. The increase in SOD activity indicates that the small intestine antioxidant machinery is activated in response to excessive generation of reactive oxygen species. SOD catalyses the conversion of superoxide anions to molecular oxygen and hydrogen peroxide, which requires to be scavenged further by tissue thiols, such as GSH, and by catalase (Bannister *et al.*, 1987; Fridovich, 1995). Furthermore, apart from its own toxicity, hydrogen peroxide in the presence of iron leads to the generation of toxic hydroxyl radicals (Blake *et al.*,1987).

Collectively, the results reported here confirm the presence of a significant level of oxidative stress in our experimental model. Mitochondria are the major source of reactive oxygen species, which are mainly generated at complexes I and III of the respiratory chain (Kudin *et al*., 2005). There is now evidence indicating that rosiglitazone and pioglitazone exert direct and rapid effects on mitochondrial respiration, inhibiting complex I (Brunmair *et al*., 2004) and complex III (Dello Russo *et al*.,2003) activity. As fenoterol partially disrupt the mitochondrial respiratory chain, both electron transport and superoxide anion generation are affected. Moreover, a novel mitochondrial target protein for PPAR-γ agonists ("mito- NEET") has recently been identified (Colca *et al*., 2004). MitoNEET was found associated with components of complex III, suggesting how PPAR-γ agonists binding to mitoNEET could selectively block different mitochondrial targets. Fenoterol's ability to influence mitochondrial function might contribute to their inhibitory effects on reactive oxygen species.The list of patho-physiological conditions associated with the production of superoxide anion expands every day. The most exciting realization is that there appears to be a commonality to the tissue injury observed in various disease states; namely, superoxide anion, produces tissue injury (and associated inflammation) in all tissues in similar ways. Tissue injury and inflammation form the basis of many disease pathologies: ischemia and reperfusion injuries, radiation injury, hyperoxic lung damage, atherosclerosis and so forth. This provides a unique opportunity to manipulate numerous disease states with an agent that selectively removes superoxide anion. The challenge in the future will be to understand the signal transduction mechanisms used by superoxide anion so as to modify key components of the inflammatory response, as this will undoubtedly elucidate important molecular targets for future pharmacological intervention. The important role of oxidative stress is well known in a great many diseases: neurodegenerative diseases (Alzheimer's disease, Parkinson's disease), atherosclerosis, rheumatoid arthritis, Crohn's disease and even certain cancers. Free radicals are also known to contribute to the aging process. For this reason, we are currently witnessing the development of a great many antioxidant products (functional food and drugs). However, their bioactivity with oral administration is often low, thereby limiting their efficacy. In addition, the products available on the market are made to correct a possible deficiency and do not specifically stimulate the antioxidant endogenous defenses. Reactive oxygen species have been indicated as one of the earliest and most important components of tissue injury after reperfusion of ischemic organ and the extent of brain injury appears to depend on the experimental pattern of ischemia/ reperfusion: free radical production is continuous during ischemia, while during reperfusion it is primarily confined to the early stage when fresh oxygen is supplied to the ischemic region (Nita *et al*., 2001). The brain is very susceptible to the damage caused by oxidative stress, due to the high rate of oxidative metabolic activity, high polyunsaturated fatty acid contents, relatively low antioxidant capacity and inadequate neuronal cell repair activity (Traystman *et al*., 1991).Overproduction of

reactive oxygen species results in oxidative damage, including lipid peroxidation, protein oxidation and DNA damage, which can lead to cell death (Floyd, 1999; Love, 1999; Phillis, 1994).

4. CONCLUSION

In conclusion, we show here for the first time that fenoterol exert harmful effects on small intestine of mice by enhancing oxidative stress and inflammatory response. However, further direct evidence and mechanism of action of these drugs shows that their use should be banned.

CONFLICT OF INTEREST

The authors declare that no competing financial interests exist.

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