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ScienceDirect
European Journal of Pharmacology 556 (2007) 136–143
www.elsevier.com/locate/ejphar

# Aspirin restores normal baroreflex function in hypercholesterolemic rats by its antioxidative action

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Received 12 September 2006; received in revised form 31 October 2006; accepted 6 November 2006
Available online 18 November 2006

**Abstract**

Besides its well-known effects on platelet aggregation, aspirin has been suggested to be an antioxidant and is also known to improve the lipid profile. In the present study we tested the hypothesis that aspirin by its antioxidant effect, improves haemodynamic profile and baroreflex sensitivity in rat model of hypercholesterolemia. Hypercholesterolemia was induced in Wistar rats by feeding 1% cholesterol rich diet for 10 weeks. Lipid profile, lipid peroxidation and reduced glutathione were estimated in serum. Haemodynamic changes and baroreflex were measured in anaesthetized rats. Hypercholesterolemic rats showed significant increase in total cholesterol, low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and atherogenic index and significant decrease in high-density lipoprotein-cholesterol (HDL-C). Significant rise in blood pressure, heart rate and attenuation of baroreflex sensitivity were also found in hypercholesterolemic rat. Aspirin in the dose of 100 mg/kg showed significant decrease in total cholesterol, LDL-C, VLDL-C and atherogenic index and significant increase in HDL-C. Aspirin treatment prevented the rise in blood pressure, heart rate and significantly improved baroreflex sensitivity in hypercholesterolemic rats. Hypercholesterolemic rats showed free radical generation, evident by a significant increase in serum lipid peroxidation and significant reduction in serum reduced glutathione content. Aspirin treatment significantly decreased lipid peroxidation and significantly increased reduced glutathione content. We have demonstrated that aspirin improves baroreflex response and prevents the rise in blood pressure and heart rate possibly by reducing sympathetic activity due to its antioxidant effect in experimentally induced hypercholesterolemic rats.


*Keywords:* Aspirin; Hypercholesterolemia; Baroreflex; Lipid peroxidation; Reduced glutathione; Blood pressure; Free radical; Antioxidant

## 1. Introduction

Hypercholesterolemia is a condition characterized by high levels of lipoprotein containing cholesterol in blood. If left untreated, it leads to the development of atherosclerosis (Saini et al., 2004). There is strong evidence that hypercholesterolemia increases production of oxygen free radicals. (Gokkusu and Mostafazadeh, 2003; Prasad and Kalra, 1993; Ross, 1986) which may play an important role in the pathogenesis and/or progression of cardiovascular diseases, especially atherosclerosis and hypertension (Wu et al., 2002; Dhalla et al., 2000).

Arterial baroreceptors are mechanosensitive nerve endings located in carotid sinuses and aortic arch are activated with increase in arterial pressure (Chapleau et al., 1991). This leads to reflex inhibition of sympathetic efferent nerve activity and excitation of parasympathetic efferent nerve activity resulting in a fall in the heart rate and vasodilatation buffers the rise in pressure. Conversely, during hypotension, there is an increase in sympathetic efferent nerve activity and inhibition of vagal efferent nerve activity causing reflex tachycardia response and vasoconstriction that restores normal blood pressure (Fahim, 2003). Thus, the baroreceptor reflex function in a negative feedback manner regulates the level of arterial pressure (Chapleau et al., 1989).

Conclusive evidence shows that baroreceptor modulation of heart rate is impaired in animals and patients with atherosclerosis (Li et al., 1996; Angell-James, 1974; Vlachakis et al., 1976). The decreased baroreceptor sensitivity in atherosclerosis has generally been ascribed to increase in vascular stiffness, degeneration of baroreceptor endings and increased arterial

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doi:10.1016/j.ejphar.2006.11.029

pressure (Hosomi et al., 1986; Cox et al., 1980; Angell-James, 1974). However, it has been suggested that oxygen free radicals produced in atherosclerosis may contribute to baroreceptor dysfunction. Li et al. (1996) demonstrated that exposure of isolated carotid sinus to superoxide dismutase and catalase increases the baroreceptor sensitivity in atherosclerotic rabbit with no effect on normal rabbits. Furthermore, exposure of carotid sinus of normal rabbits, to exogenous free radicals generated by xanthine–xanthine oxidase attenuates the baroreceptor activity, suggesting oxygen free radicals mediated baroreceptor dysfunction (Li et al., 1996).

The cardiovascular beneficial effects of aspirin are generally attributed by its platelet inhibitory activity (Wu et al., 2002). However, aspirin may also prevent atherosclerosis by inhibition of vascular smooth muscle cell proliferation (Kodama et al., 2000) and reduction in pro-inflammatory mediators (Cyrus et al., 2002; Hussain et al., 1998). Other direct effects of aspirin on the integrity of the vascular wall have been reported. Firstly, free radical scavenging properties of aspirin and its capacity to protect endothelial cells from the deleterious effects of hydrogen peroxide (Aruoma and Halliwel, 1988; Wollard et al., 1990; Podhaisky et al., 1997). Secondly, aspirin prevented development of hypertension and reduced insulin resistance in chronically glucose fed rats (El-Midaoui et al., 2002) and reduced vascular superoxide anion production through lowering the NAD(P)H oxidase activity in normotensive and spontaneously hypertensive rats (Wu et al., 2002). It has been shown that salicylate, a metabolite of aspirin or other non-steroidal anti-inflammatory drugs such as ibuprofen or indomethacin, did not modify the generation of free radicals, suggesting that the inhibition of cyclooxygenase perse does not account for the antioxidant effects of aspirin. (Wu et al., 2002; Grosser and Schröder, 2003).

Taken together, the above data support the hypothesis that baroreflex dysfunction in hypercholesterolemia may be due to generation of free radicals and aspirin may posses antioxidant activity. Therefore, the present study was designed to investigate the preventive and therapeutic effect of aspirin on lipid profile, its antioxidant potency and its effect on haemodynamic changes and baroreceptor mediated blood pressure regulatory mechanism tested by bradycardia response to rise in arterial pressure by phenylephrine and tachycardia response to fall in arterial pressure by sodium nitroprusside in experimentally induced hypercholesterolemic rats.

The dose-response curves of aspirin on inhibition of superoxide anion production in earlier studies indicated that a maximum effect was reached at about the dose of 100 mg/kg/d, which was chosen for the present study (Wu et al., 2002; El-Midaoui et al., 2002).

## 2. Materials and methods

### 2.1. Animals

Healthy male Wistar albino rats, weighing between 250–300 g, were obtained from the animal house of Vallabhbhai Patel Chest Institute (VPCI), University of Delhi, New Delhi, India. They were housed in polyethylene cages in groups of four rats per cage and were kept in room temperature maintained at 25±2 °C with a 12-h light/dark cycle. Experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The Institutional Animal Ethical Committee, VPCI, approved this study.

### 2.2. Chemicals

Aspirin, phenylephrine hydrochloride, sodium nitroprusside, urethane, 1-2-dithio-bis-nitrobenzoic acid (DTNB), and thiobarbituric acid were obtained from Sigma USA. Cholesterol powder (CDH, India) and enzyme kits for serum lipid profile (Monozymes India).

*1% Cholesterol supplemented feed:* In crushed pellet diet, cholesterol (1%w/w) powder was mixed; the pellets were reconstituted with water and dried properly to avoid any fungal contamination.

### 2.3. Experimental design

A systematic study was performed on the adult male rats divided in six groups. Each group comprised of 8 animals.

Group 1: Control rats fed with normal pellet diet: Rats of this group were maintained on normal balanced diet for 10 weeks.

Group 2: Rats fed with normal pellet diet along with aspirin: In order to study the effect of aspirin on normal rats, aspirin was given in the dose of 100 mg/kg/d, p.o., with normal pellet diet for 10 weeks.

Group 3: Rats fed with cholesterol mixed pellet diet: For experimentally induced hypercholesterolemia, rats were maintained on pellet supplemented with 1% cholesterol for 10 weeks.

Group 4: Rats fed with cholesterol mixed pellet diet plus aspirin: In order to examine the protective/preventive potential of aspirin in hypercholesterolemia, rats were fed with cholesterol mixed pellet diet along with aspirin in the dose of 100 mg/kg/d, p.o., for 10 weeks.

Group 5: Hypercholesterolemic rats reverted to normal diet for next 10 weeks: In order to evaluate the effect of normal diet on pre-existing hypercholesterolemic condition, rats first maintained on cholesterol mixed pellet diet for 10 weeks were followed by normal pellet diet for next 10 weeks.

Group 6: After treatment with aspirin along with normal diet for 10 weeks in hypercholesterolemic rats: In order to find out the therapeutic potential of aspirin in pre-existing hypercholesterolemic condition, in this group, rats were initially given cholesterol mixed pellet diet for 10 weeks followed by aspirin in the dose of 100 mg/kg/d, p.o., with normal pellet diet for next 10 weeks.