Indian J Physiol Pharmacol 2007; 51 (2) : 147–152

EFFECT OF LONG TERM ORAL ADMINISTRATION OF L-ARGININE ON EXPERIMENTALLY PRODUCED MYOCARDIAL ISCHEMIA IN RABBITS

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(Received on July 21, 2006)

Abstract : L-arginine a semi essential amino acid and a precursor of nitric oxide (NO) was orally supplemented in diet (standard rabbit feed) of hypercholesterolemic (n=6) and normal rabbits (n=6) for 16 weeks. Myocardial ischemia was produced in both groups of rabbits by subcutaneous single bolus injection of isoproteronol. Severity of myocardial ischemia was assessed by estimating the serum CPK and AST levels after 6 hour of ischemia-reperfusion. The result suggests that severity of ischemia was lesser in the L-arginine primed hypercholesterolemic group.

Key words : L-arginine, hypercholesterolemia, experimental, myocardial

INTRODUCTION

L-Arginine, normally considered a non-essential amino acid, plays a critical role in cardiovascular protection and immune system support (1). Under conditions of stress, sickness or injury this important amino acid changed in to a conditionally essential one, which means that supplemental L-Arginine must come from the diet. L-Arginine reduces the risk of heart diseases (2) by producing nitric oxide and acts as a powerful anticoagulant that helps to prevent blood platelets from clumping together (3). L-arginine is a precursor of Nitric Oxide (NO), it synthesizes NO in vascular endothelium in presence of NO synthetase 3 and the release of nitric oxide is dependent on cofactors NADP, FAD, FMN, Tetrahydrobiopeterin Thiol, and colmodulin (4).

Hypercholesterolemia may cause dysfunction in the synthesis of NO or release of NO by endothelial cells of the resistance vessels rather than producing abnormalities of the smooth muscle cells (5). Decreased NO generation by the endothelial cells leading to platelet aggregation and the subsequent development of atherosclerosis; this process is generally corrected by L-arginine (6).

It is now established that both oral and

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parenteral administration of L-arginine can increase NO synthesis in various tissues (7). Ignaro et al (8) suggested a dual role of NO in ischemia reperfusion injuries. NO is a potent vasodilator and potential to attenuate ischemia reperfusion injury (9). L-arginine significantly improved angina class, systolic blood pressure at rest, and quality of life (10) Oral L-arginine treatment at a dose of 12.5 g/l in drinking water improves' endothelium-dependent relaxation but fails to improve cardiac function in rats with heart failure (11).

The oral L-arginine supplementations in hypercholesterolemic animals have generally shown beneficial effects, though the data in humans are varied (12). There are only few studies available to show the effect of oral L-arginine administration on experimental myocardial ischemia in rabbits.

Therefore in the present study, the effect of oral administration of L-arginine has been evaluated on experimentally produced myocardial ischemia in normo and hyperlipidemic rabbits.

MATERIALS AND METHODS

Current study approved by local ethical committee was conducted on 24 adult male albino rabbits (Indian breed) between 10–11 months old and weighing between 1.2–1.5 kg body wt. All rabbits employed were acclimatized in ambient conditions of animal house. Due care was taken to avoid noise, stress and infections. Rabbits were put in separate metabolic cages having dimensions of 3 feet × 2 feet for 28 days for stabilization. Each animal was fed with 120 gm standard rabbit feed (Amrut rabbit feed, Chakan oil mills Maharastra) per day and water intake maintained *ad libitum*. Animals were divided in four groups.

Control I Normocholesterolemic (NL) (n=6): Rabbits receiving Standard Rabbit Feed (SRF) 120 g per day and water ad libitum for 16 weeks, were recognized as control-I.

Experimental Normocholesterolemic with L-arginine (NLA) (n=6): Rabbits of this group provided with SRF, water *ad libitum* and 100 mg/kg body wt/day L-arginine dissolved in water for 16 weeks.

Control II Hypercholesterolemic (HL) (n=6): Rabbits of this group kept on hypercholesterolemic diet along with SRF for 16 weeks.

Experimental II hypercholesterolemic with L-arginine (HLA) (n=6): Rabbits of this group provided with Hypercholesterolemic diet, SRF and 100 mg L-arginine dissolved in water for 16 weeks.

Induction of hypercholesterolemia

Rabbits of group HL and HLA made hypercholesterolemic by method employed by Wolfgong Linz et al 1995 (13). Following this method rabbits were kept on diet containing 0.25% cholesterol and 3% coconut oil enriched with 120 gm SRF (hypercholesterolemic diet) daily for sixteen weeks. Serum cholesterol of all the rabbits (NL, NLA, HL & HLA) was estimated (14) using kit from Span Diagnostics India to assess the hypercholesterolemia.

Sample collection

The samples were collected on day one and on 16th week and 6 hour after ischemia reperfusion.

The fasting (12–14 hrs) venous blood samples were taken from the ear vein of rabbits after properly securing them in rabbit holder using scalp vein set 24G. The needle of scalp vein set was inserted in vein priorly dilated by mild heat and local application of xylol, the two ml blood was collected in 10 ml glass syringe connected with other end of scalp vein set. Blood was transferred to test tube and finally the serum was separated out by centrifugation.

Biochemical estimation

Venus blood samples were taken from rabbits of all the four groups. Total serum total cholesterol was estimated by using principle of Wybenga and pilegg, the kit procured from Span Diagnostics India.

Aspartate Amino Transferase (AST) was estimated following the principle of Reitmans and Frankel (15), Creatine Phosphokinase (CPK) was estimated on the principle of Gerhardt W et al (16) using the kit from span diagnostic surat India.

Recording of ECG

Electrocardiogram of all the rabbits was recorded on three channels ECG machine (CHARDIART508, BPL INDIA). Control ECG was initially recorded followed by induction of ischemia and subsequent recording of ischemia.

Experimental myocardial ischemia

After successful induction of hyperlipidemia, global ischemia was produced in rabbits of all the groups by single bolus subcutaneous injection of isoproterenol (0.2 mg/kg body wt) (17) under ECG monitoring. Heart rate was calculated by counting R-R intervals in one minute. Animals were allowed to reperfusion. Serum Creatine Phospho Kinase (CPK) and Aspartate amino transferase (AST) levels were estimated after 6 hrs of reperfusion to assess the severity of ischemia.

Statistical analysis

Mean and standard deviation of all the sets of observations pertaining to the biochemical and electrocardiograph changes were calculated. Significance of the differences was calculated by applying unpaired Student's t test, P value <0.05 is taken as significant.

RESULTS

Effect of hypercholesterolemic diet

The serum cholesterol levels of all the four groups were estimated on day first and on the end of 16th week of experimentation. It was found that cholesterol and coconut oil supplemented diet resulted in significant rise in serum levels of total cholesterol in hypercholesterolemic (HL, HLA) groups (Table I). It was also observed that, in L-arginine taking groups (NLA, HLA) the serum cholesterol levels were raised more in comparisons to their control group.

Effect of IPT injection

Subcutaneous injection of isoproterenol successfully induces the ischemic changes in all the groups of the present study, which were evidenced by ST elevation, and T inversion in lead-II of ECG.

TABLE I: Effect of hypercholesterolemic diet.

Serum cholesterol mg/dl	*Groups*	*NL*	*NLA*	*HL*	*HLA*
	At the start of the study	82.0±6.2	84.0±5.1	84.0±5.1	83.0±6.6
	At 16th week	84.0±5.9*	111.0±4.1*	286.0±6.3**	320.0±6.5**

• P<0.01 (NL vs NLA at 16th week)
• P<0.01 (HL vs HLA at 16th week)

NL: Normal control, NLA: Normal Experimental, HL: Hypercholesterolemic Control HLA: Hypercholesterolemic Experimental.

TABLE II: Serum levels of ischemia markers after 6 hours of ischemica-reperfusion.

Ischemia marker IU/dl	*CPK*		*AST*	
	BI	*AI*	*BI*	*AI*
NL	40.0±5.3	191.0±10.0	20.0±2.6	96.0±5.5
NLA	29.0±5.0	176.0±6.6	22.0±2.2	93.0±4.5
HL	37.0±4.0	205.0±9.5*	26.0±2.8	106.0±54.0**
HLA	45.0±4.7	176.0±14.0*	28.0±2.6	54.0±4.5**

NL: Normal control, NLA: Normal Experimental, HL: Hypercholesterolemic Control HLA: Hypercholesterolemic Experimental, BI: Before Ischemia, AI: After Ischemia.
*P<0.05: HL vs HLA, **P<0.01: HL vs HLA.

Effect of oral L-arginine on severity of ischemia

After 6 hour of ischemia reperfusion, to assess the severity of ischemia, serum AST and serum CPK levels in all the study groups were estimated. It was observed that serum AST and CPK levels were raised in all the study groups as compared with pre ischemic levels of serum AST and CPK. It was worthwhile to note that serum CPK levels in NL and HL groups raise about 5 times (Table II).

DISCUSSION

Various studies with NO donor, NO synthase and NO synthase inhibitor have shown that nitric oxide has potential role in cardio protection (21–23). The L-arginine a nitric oxide precursor has not been tried in oral route in an experimental ischemia model. The current experiment has been planned with an objective of to elucidating the influence of L-arginine on serum cholesterol level and on experimentally produced myocardial ischemia, in normal and hypercholesterolemic rabbits. In present study hypercholesterolemic state in rabbits has been successfully produced, by feeding them 0.25% cholesterol and 3% coconut oil over a period of 16 weeks. HL and HLA groups indicates significant rise in serum cholesterol level. (P<0.05).

A number of studies have shown that acute hypercholesterolemia increases the severity of myocardial ischemia (6, 18–21). In hypercholesterolemic stage, the coronary

vascular reserve is greatly reduced which may carry by reduction in the synthesis or release of NO in coronary bed (4).

Our results suggested that hypercholesterolemia was not checked by oral administration of L-arginine in any groups of study (NLA HLA), while the serum cholesterol levels were raised in L-arginine treated groups. Although the increase in serum cholesterol in HLA and NLA study groups is not clear and need further study.

Experimental ischemia was successfully induced in animals of both study groups by subcutaneous injection of isoproterenol therefore we are in close agreement with Kela et al 1990. However, this method seems to have an advantage over the method employing coronary ligation and reperfusion (21–23) because this method of ischemia induction is non surgical and have very small morbidity or mortality.

The present results indicate that the ischemia was produced in all the groups but the magnitude of myocardial injury produced was lesser in those groups of animals receiving L-arginine as suggested by the levels of ischemia markers CPK and AST (Table II). The change in AST levels was significantly less ($P<0.05$) in hypercholesterolemic rabbits kept on oral L-arginine prior to ischemia induction. The serum AST levels were increase four times in animal of HL group as compared to HLA group ($P<0.01$).

The post ischemic plasma CPK levels in HLA group are also significantly less ($P<0.05$) as compared to HL group.

These results are in close agreement with the finding of Hoshida et al (21) their views are supported by our study. Pretreatment of L-arginine lays an important role in myocardial injury induced by ischemia. In our study the reduction in extent of ischemia is might be due to L-arginine because L-arginine supplementation improved endothelial vasoactive functions in hypercholesterolemics (24). In conclusion our study supports the fact that oral L-arginine prevents the extent of myocardial ischemia.

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