Short Report

Protective Effect of Digoxin on Impaired Chronotropic Responsiveness to Adrenergic Stimulation in Cholestatic Rats

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ABSTRACT

Decreased cardiac responsiveness to adrenergic stimulation has been observed in cholestatic liver disease, but the cause remains unclear. Previous reports have suggested that nitric oxide overproduction might have a role in cholestasis-induced bradycardia via inhibition of L-type calcium channels. In the present study, the digoxin has been used to increase cardiac Ca^{2+} transient in male Sprague-Dawley rats with obstructive cholestasis and the chronotropic responsiveness to adrenergic stimulation was evaluated. Cholestasis was induced by surgical ligation of the bile duct under general anesthesia and sham-operated animals were considered as control. The animals were divided into two groups, which received either digoxin (10, 20 ~g/kg/day) or saline. One week after the operation, spontaneously beating atria were isolated and chronotropic responses to epinephrine were evaluated in a standard oxygenated organ bath. The basal spontaneous beating rate of the atria in the cholestatic animals was not significantly different from that of sham-operated rats *in vitro*. Meanwhile, cholestasis induced a significant decrease in chronotropic effect of epinephrine. This effect was corrected by daily administration of digoxin (20 ~g/kg/day). The results also showed that plasma alkaline phosphatase activity was increased by bile-duct ligation, and digoxin treatment had no effect in the elevation of this marker of liver damage. The protective effect of digoxin on impaired chronotropic responsiveness to adrenergic stimulation in cholestatic rats might be related to increase of Ca^{2+} transient. However, further studies are necessary to confirm the molecular basis of this effect. *Iran. Biomed. J. 7 (2): 85-88, 2003*

INTRODUCTION

Cholestatic liver disease is associated with bradycardia and attenuation of β-adrenergic chronotropic responses [1-4]. Recently, we have shown that a nitric oxide (NO) dependent mechanism might be involved in the bradycardia of cholestasis [4]. Systemic inhibition of NO synthase (NOS) corrected the decreased chronotropic and inotropic response to -adrenergic stimulation in cholestatic rats, which supports the hypothesis of NO overproduction in cholestasis [4, 5]. The reason for cholestasis-induced NO overproduction is not known yet, however, different hypotheses including chronic endotoxemia and accumulation of endogenous opioids have been postulated [6, 7].

The mRNA of endothelial type NOS has been shown to be expressed abundantly in atria as well as ventricular cardiomyocytes [8], supporting the role of NO in the regulation of cardiac function. Different investigators have showed the inhibition of plasma membrane L-type calcium channels by NO [9, 10]. This NO/cGMP-dependent mechanism has an important role in physiological regulation of heart rate, as well as pathophysiological aspects of cardiovascular disorders [9]. Based on evidences reported about NO overproduction in cholestasis [4, 7, 11, 12], it has been postulated that the Ca^{2+} transients may be abnormal in cholestatic liver disease [4]. This hypothesis has been recently confirmed by Ward *et al.* [13] that plasma membrane L-type calcium channels are functionally depressed in cardiomyocyte of bile duct-ligated (BDL) rats.

Cardioglycoside drugs like digoxin are well known in cardiology and medicine. They can

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Table. 1. Comparison of alkaline phosphatase activity (ALP), arterial oxygen pressure (Pa O_2), arterial blood oxygen saturation (O_2 saturation) and plasma digoxine level in bile duct-ligated (BDL) and sham-operated (SHAM) rats given digoxin (10, 20 μg/kg.day) or saline. Data are shown as Mean ± SEM. Eight rats were used in each group.

Group	ALP (IU/L)	PaO$_2$ (mmHg)	O$_2$ saturation (%)	Plasma digoxin level (~g/ml)
SHAM (saline)	636 ± 85	72.0 ± 3.1	93 ± 1	0.00 ± 0.00
SHAM (dig. 10)	590 ± 76	77.3 ± 3.0	94 ± 2	0.36 ± 0.04
SHAM (dig. 20)	612 ± 74	76.2 ± 3.3	95 ± 2	0.63 ± 0.06
BDL (saline)	1153 ± 243	74.1 ± 3.7	94 ± 2	0.00 ± 0.00
BDL (dig. 10)	1264 ± 326	75.2 ± 2.6	90 ± 3	0.39 ± 0.05
BDL (dig. 20)	1223 ± 299	72.3 ± 3.1	92 ± 2	0.59 ± 0.06

indirectly increase Ca^{2+} transient in cardiomyocyes via modulation of Na+/K+ pump [14].

According to Ca^{2+} transient defects in cholestatic heart, pharmacological interventions for enhancement of Ca^{2+} transient in cardiomyocytes might have a role in treatment of cardiac complications in cholestatic liver disease. The aim of present study is to evaluate the effect of digoxin administration on impaired chronotropic responsiveness to adrenergic stimulation in cholestatic rats.

MATERIALS AND METHODS

Animal manipulations. The present investigation conforms to the Guide for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH publication no. 85–23; revised 1985). Male Sprague-Dawley rats (n = 48) weighing 200–250 g were used in the experiments. All animals were given free access to food and water. A laparotomy was performed under general anaesthesia induced by an intraperitoneal (i.p.) injection of ketamine HCl (50 mg/kg; Gedoon Richter, Hungary) and xylazine HCl (Bayer AG, Germany; 10 mg/kg). The bile duct was isolated and double ligated, as described previously [11]. Sham-operated (SHAM) age-matched rats served as controls. The sham operation consisted of laparotomy and bile duct identification and manipulation without ligation. One day after the operation, animals received daily i.p. injection of digoxin (10 and 20 μg/kg/day; Sigma, USA) or saline for 6 days. One week after the surgery, rats were anaesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg; Merck, Germany). Carotid artery was cannulated for arterial blood collection and the heart of each animal was removed for study. Arterial blood was collected for arterial blood gas analysis (PO$_2$ and O$_2$ saturation). Plasma alkaline phosphatase (ALP) activity and plasma level of digoxin were also measured with colorimetric and radioimmunoassay methods, respectively. The biochemical assays were done according to the methods described by kit manufacturer in Nour Pathobiology laboratories (Tehran, Iran).

Isolated whole atrium study. The atria were dissected out from isolated hearts in oxygenated modified Krebs' solution and suspended vertically under isometric conditions under 500 mg tension in a 50 ml glass chamber. The temperature of the bathing solution was 37°C and the pH was 7.4. The composition of the modified Krebs' solution was (in mM): NaCl, 118; KCl, 4.7; CaCl$_2$, 2.6; MgCl$_2$, 1.2; NaH$_2$PO$_4$, 1; NaHCO$_3$, 25; glucose, 11.1; EDTA, 0.004 and ascorbic acid, 0.11 [4]. The solution was oxygenated with a mixture of 95% O$_2$ and 5% CO$_2$. Isometric spontaneous contractions were recorded with an isometric transducer and displayed on DMR-4B Physiograph (Narco Biosystem, USA). To avoid artifacts evoked by dissection, an equilibration period of 30 min was allowed before evaluation of the function of the isolated atria. After the equilibration period, the basal spontaneous rate of each atrium was recorded and then epinephrine (1 to 1000 nM; Fluka, Switzerland) was added to the organ bath to study the chronotropic response to adrenergic stimulation.

Statistical analysis. All data were presented as the Mean ± SEM. Statistical evaluation of the data was performed by analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple comparisons. *P*<0.05 was considered statistically significant.

RESULTS

One day after laparotomy, BDL rats showed manifestations of cholestasis (jaundice, dark urine and steatorrhoea). The cholestatic animals showed weight loss from 240 ± 6 to 222 ± 8 g (*P*<0.05), while SHAM rats revealed a slight increase in

bodyweight (234 ± 6 vs 242 ± 7 g). After animals were killed, plasma ALP activity was found to be significantly higher in BDL rats than in SHAM animals *P*<0.01 (Table 1).

The digoxin administrated during one week had no effect on the elevation of ALP in BDL and SHAM animals, however induced a dose-dependent increase in plasma level of digoxin. As shown in Table 1, plasma levels of digoxin was not significantly different among BDL animals compared with SHAM rats at any given doses. Arterial blood gas analysis also represented similar pattern in BDL and SHAM animals and digoxin administration did not alter the PO_2 and O_2 saturation values (Table 1).
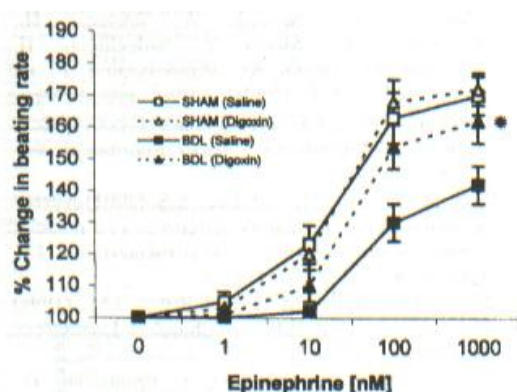
Fig. 1. Chronotropic effect of epinephrine in isolated atrium of bile duct-ligated (BDL) and sham-operated (SHAM) rats given digoxin (20 µg/kg.day) or saline. Data are shown as MEAN ± SEM. Eight rats were used in each group.

Figure 1 shows the positive chronotropic effect of epinephrine in isolated atria of SHAM and BDL rats given digoxin (20 µg/kg/day) or saline. The BDL rats showed a hypo-responsiveness to the positive chronotropic effect of epinephrine (*P*<0.05, Rmax). There was no significant difference in the basal beating rate of isolated atria between BDL and SHAM rats. Digoxin, administered during one week, had a significant (*P*<0.05, Rmax) improvement in chronotropic effect of epinephrine in BDL rats. This pattern was not observed in SHAM animals (Fig. 1).

DISCUSSION

The present study showes that chronotropic responsiveness to epinephrine is impaired in BDL animals. Decreased cardiac responsiveness to adrenergic stimulation has been observed in liver disease, but the cause remains unclear. Because cardiomyocyte contraction depends on Ca^{2+} influx entering via L-type calcium channels to activate Ca^{2+} release from the sarcoplasmic reticulum, Ward *et al.* [13] postulated that the Ca^{2+} transients might be abnormal in cirrhotic cardiomyocytes. They have actually shown that in BDL rats, plasma membrane L-type calcium channels quantitatively decreased and functionally depressed, whereas intracellular systems (which includes the ryanodine receptor, sarcoplasmic reticulum Ca^{2+}-pump adenosine triphosphatase), are intact [13]. Based on this report, we used cardioglicoside, digoxin to increase Ca^{2+} transient and the results represented a significant improvement in responsiveness of isolated atrium to the adrenergic stimulation (Fig. 1). Digoxin treatment did not decrease plasma ALP activity in BDL rats; therefore amelioration of hepatic injury by digoxin is less likely to have a role in the protective effect of digoxin. Morover, there was no significant difference in arterial blood PO_2 and O_2 saturation between BDL and SHAM rats indicating that cholestasis does not induce severe heart failure in the experimental animals.

This finding is in agreement with the clinical observations. Patients with obstructive jaundice have normal cardiac contractility at rest but they show blunted responsiveness to physical and pharmacological stimulations [15]. Such myocardial refractoriness to β-stimulation may contribute to the susceptibility of jaundiced patients to postoperative shock and acute renal failure [15].

So far, the mechanism of cholestasis-induced impaired cardiac Ca^{2+} transient has not been clarified directly. However, we have suggested a NO-dependent mechanism for explanation of cardiac complications in cholestasis [4]. Cholestatic subjects exhibit increased formation of NO related molecules such as S-nitrosothiols and 3-nitrotyrosine [16]. The reason for NO overproduction in liver disease is not completely understood and the question of which isoform of NOS is primarily responsible for the overproduction of NO in liver disease constitutes an area of major controversy. Vallance and Moncada [6] originally proposed that upregulation of inducible NOS (iNOS) secondary to endotoxaemia was the primary source of elevated NO levels. Since then, conflicting data have emerged suggesting that constitutive NOS (cNOS) alone, iNOS alone, or both enzymes are upregulated in patients or animal models of liver diseases [17]. Indirect evidence for cNOS, as the main source of elevated NO, derives from the studies that endothelial denudation

normalizes vascular levels of NO [18]. Recently, upregulation of tetrahydrobiopterin (BH4) has been proposed to have a causative role in NO overproduction of liver disease, which confirms role of a cNOS-dependent mechanism in the pathophysiology of liver disease [19]. Regardless to the mechanism of NO overproduction, inhibition of L-type calcium channels might have a pathophysiologic role in the cardiac complications of cholestatic subjects. Further studies are necessary for understanding the molecular basis of these effects.

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