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Effect of 3-Aminobenzamide, an Inhibitor of Poly (ADP-Ribose) Polymerase in Experimental Cardiac Hypertrophy

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Abstract: The male Wistar rats were anaesthetized with thiopentone sodium (35 mg kg⁻¹, i.p.) and were subjected to Partial Abdominal Aortic Constriction (PAAC) for 4 wk. 3-aminobenzamide (10, 20 mg kg⁻¹ i.p., b.i.d) treatment was started three days before surgery and it was continued for 4 weeks after surgery. The Left Ventricular (LV) hypertrophy and LV dysfunction were assessed by measuring ratio of LV weight to body weight (LVW/BW), LV wall thickness (LVWT), LV collagen content, protein content, RNA concentration, Left Ventricular Developed Pressure (LVDP), rate of pressure development (dp/dt_{max}) and rate of pressure decay (dp/dt_{min}). Further, Venous Pressure (VP) and Mean Arterial Blood Pressure (MABP) were recorded. The PAAC produced LV hypertrophy by increasing LVW/BW, LVWT, LV protein content and LV RNA concentration. Further, PAAC was noted to produce LV dysfunction by decreasing LVDP, dp/dt_{max}, dp/dt_{min} and increasing LV collagen content. Moreover, PAAC has significantly increased VP and MABP. 3-Aminobenzamide, a PARP inhibitor markedly attenuated PAAC-induced LV hypertrophy, LV dysfunction, increase in VP and MABP. These results suggest that 3-aminobenzamide prevented PAAC-induced cardiac hypertrophy and LV dysfunction which may be due to inhibition of PARP.

Key words: Cardiac hypertrophy, aortic banding, 3-aminobenzamide, PARP

INTRODUCTION

Cardiac hypertrophy is an adaptive response of the heart during which terminally differentiated cardiomyocytes increase in size without undergoing cell division. Initially the hypertrophic response may be adaptive; but chronic increase in wall tension due to prolonged hypertrophy can become detrimental, resulting in cardiac dysfunction and heart failure (Reddy *et al.*, 1996a). However, the signaling mechanisms of hypertrophic cardiac growth are largely unknown. The Poly (ADP-ribose) polymerase (PARP) contributes to DNA repair and maintenance of genomic integrity (Virag and Szabo, 2002). However overactivation of PARP leads to consumption of ATP and cell death (Hong *et al.*, 2004). PARP regulates gene expression of various inflammatory mediators (Le Page *et al.*, 1998), intercellular adhesion molecule-1 (Zingarelli *et al.*, 1998) and transcription of NfκB (Oliver *et al.*, 1999). Recently overexpression of PARP has been implicated in cardiac hypertrophy (Pillai *et al.*, 2005). Moreover, 3-aminobenzamide is documented to inhibit PARP (Liaudet *et al.*, 2001). Therefore the present study has been designed to investigate the effect of 3-aminobenzamide, a PARP inhibitor on pressure overload-induced left ventricular hypertrophy.

MATERIALS AND METHODS

The experimental protocol used in present study has been approved by institutional animal ethical committee. Young male Wistar albino rats weighing 175-225 g were maintained on rat feed (Kisan Feeds Ltd., Chandigarh, India) and tap water *ad libitum*. They were housed in animal house and were exposed to 12 h light and 12 h dark cycle.

Experimental cardiac hypertrophy using Partial Abdominal Aortic Constriction (PAAC): Pressure overloaded cardiac hypertrophy was produced using aortic banding (Shimoyama *et al.*, 1999). Rats anaesthetized with thiopentone sodium (35 mg kg⁻¹ i.p.) were subjected to midline incision of 3-4 cm in abdomen at the end of xiphoid process to expose abdominal aorta between diaphragm and celiac artery. The 4-0 silk suture was placed around the middle of the exposed aorta and it was tightened along with needle of 0.7 mm in diameter. The needle was withdrawn to leave the vessel partially constricted and midline incision was sutured in layers. Neosporin antibiotic powder (GlaxoSmithKline, Mumbai, India) was applied locally on the sutured wound. Rats were allowed to recover and were kept under observation for 4 weeks. Sham operated animals were

subjected to same surgical procedures except partial abdominal aortic constriction. Body weight was monitored weekly.

Morphological and haemodynamic assessments: After 4 week of PAAC, jugular venous pressure (mm H₂O) and carotid mean arterial blood pressure (mm Hg) were recorded using pressure transducer (BIOPAC System, California, USA) in anaesthetized rats. Then the thorax was opened and the heart was excised and placed into ice-cold saline. The heart was immediately mounted on langendorff's apparatus and perfused with Kreb's Henseleit solution (NaCl 118 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; MgSO₄ 7 H₂O 1.2 mM; NaHCO₃ 25 mM; KH₂PO₄ 1.2 mM; C₆H₁₂O₆ 11 mM), gassed with 95% O₂-5% CO₂, pH 7.4, maintained at 37°C. For the measurement of cardiac function, the double distilled water filled latex balloon was inserted through the mitral valve into the left ventricle and left ventricular developed pressure (LVDP) (mm Hg), rate of pressure development (dp/dt_{max}) (mm Hg/sec) and rate of pressure decay (dp/dt_{min}) (mm Hg/sec) were measured using pressure transducer (BIOPAC System, California, USA). Then the auricles and right ventricle of the excised heart were removed. The weight of left ventricle including interventricular septum was recorded and expressed as mg per g of body weight. The left ventricle was divided into three equal slices and wall thickness of each slice was noted at eight different points using ocular micrometer. The mean value of all three slices was calculated.

Biochemical assessments: The left ventricular collagen content was estimated by measuring the hydroxyproline concentration as described earlier (Pelouch *et al.*, 1997; Masutomo *et al.*, 1999; Simko *et al.*, 2002). The dry left ventricular mass was hydrolyzed in 6N hydrochloric acid at 100°C. After resolution in buffer at pH 7.0, p-dimethylaminobenzaldehyde (Ehrlich's reagent) was added to form complex with hydroxyproline. The concentration of hydroxyproline was determined spectrophotometrically at 558 nm. The hydroxyproline concentration (taken as an index of LV collagen) was expressed as mg g⁻¹ weight of left ventricle.

The left ventricle was stored at -80°C in liquid nitrogen for further quantitative estimations. The left ventricle was homogenized and protein content was determined spectrophotometrically at 750 nm by Lowry method (Lowry *et al.*, 1951) and expressed as mg g⁻¹ of left ventricular weight.

The RNA was extracted from homogenized left ventricular tissues using method of Chomczynski and Sacchi (Chomczynski and Sacchi, 1987). RNA

concentration was estimated spectrophotometrically at 260 nm. One absorbancy unit at 260 nm in a 1 cm light path cuvette was assumed to be equal to 40 µg mL⁻¹ of RNA. The purity of RNA was assessed by determining the ratio of absorbance at 260 and 280 nm and the ratio was more than 1.8.

Experimental design: Rats were randomly divided into 6 groups and each group comprised of 12 animals. Group 1, (sham control, n = 12) surgery was performed to expose the abdominal aorta but it was not constricted. Group 2, (PAAC control, n = 12), abdominal aorta was exposed and partially constricted. Group 3, (3-aminobenzamide 10 mg kg⁻¹ treated sham control, n = 12), rats were subjected to surgery without aortic constriction and they were treated with 3-aminobenzamide (10 mg kg⁻¹ i.p., b.i.d) which was started 3 days before surgery and was continued for 4 weeks after surgery. Group 4, (3-aminobenzamide 20 mg kg⁻¹ treated sham control, n = 12), rats were subjected to surgery without aortic constriction and they were treated with 3-aminobenzamide (20 mg kg⁻¹ i.p., b.i.d) as mentioned in group 3. Group 5, (3-aminobenzamide 10 mg kg⁻¹ treated PAAC control, n = 12), rats were subjected to partial abdominal aortic constriction and they were treated with 3-aminobenzamide (10 mg kg⁻¹ i.p., b.i.d) as mentioned in group 3. Group 6, (3-aminobenzamide 20 mg kg⁻¹ treated PAAC control, n = 12), rats were subjected to partial abdominal aortic constriction and they were treated with 3-aminobenzamide (20 mg kg⁻¹ i.p., b.i.d) as mentioned in group 3.

Statistical analysis: Results were expressed as mean±SEM. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range test. The p-value < 0.05 was considered to be statistically significant.

Drugs and chemicals: 3-Aminobenzamide was obtained from Lancaster, Bangalore, India. Agarose and folin ciocalteu reagent were obtained from SRL, Mumbai, India. Proteinase K, sarcosyl, 2-mercaptoethanol and bovine serum albumin were obtained from Sigma-Aldrich, St Louis, USA. All other reagents used in this study were of analytical grade.

RESULTS

There was no significant change in body weight of rats subjected to sham surgery and partial abdominal aortic constriction with or without 3-aminobenzamide treatment.

Table 1: Effect of 3-aminobenzamide on morphological and haemodynamic assessments

Parameters	Sham control	PAAC control	PAAC+3-AB (10 mg kg ⁻¹)	PAAC+3-AB (20 mg kg ⁻¹)
VP (mm H ₂ O)	30±3.2	117.30±4.3 ^a	70.20±3.1 ^b	34.1±2.7 ^b
MABP (mm Hg)	110.2±2.7	168.10±3.65 ^a	149.04±3.1 ^b	119±2.69 ^b
LVW/BW (mg g ⁻¹)	1.87±0.07	2.91±0.06 ^a	2.35±0.06 ^b	1.96±0.04 ^b
LVWT (mm)	2.18±0.07	3.72±0.10 ^a	2.68±0.07 ^b	2.42±0.06 ^b

PAAC indicates partial abdominal aortic constriction, VP indicates venous pressure, MABP indicates mean arterial blood pressure, LVW indicates left ventricular weight, BW indicates body weight and LVWT indicates left ventricular wall thickness. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

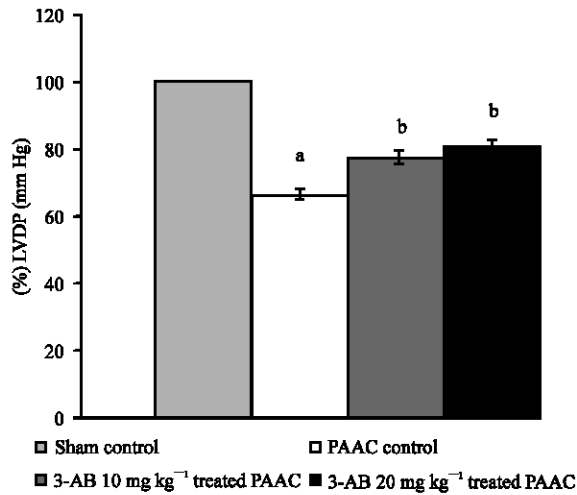


Fig. 1: Effect of 3-aminobenzamide on left ventricular developed pressure, values of Left Ventricular Developed Pressure (LVDP) are expressed as percentage response. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

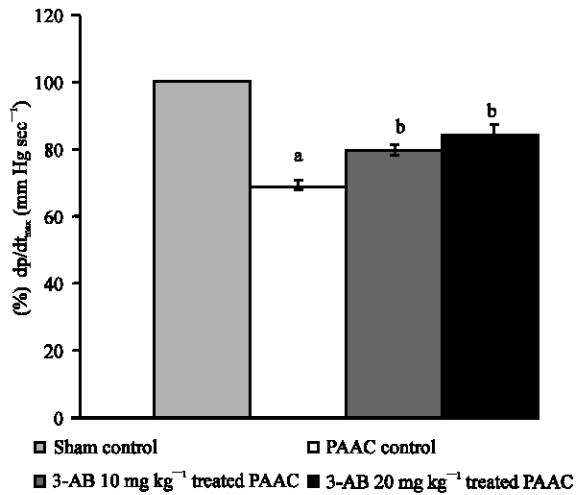


Fig. 2: Effect of 3-aminobenzamide on rate of pressure development, values of rate of pressure development (dp/dt_{max}) are expressed as percentage response. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

Effect of PARP inhibitor on morphological and haemodynamic assessments:

Partial abdominal aortic constriction (PAAC) after 4 weeks of surgery significantly increased venous pressure (VP) and mean arterial blood pressure (MABP) as compared to sham-operated rats. Further, PAAC was noted to decrease left ventricular developed pressure (LVDP), rate of pressure development (dp/dt_{max}) and rate of pressure decay (dp/dt_{min}). The 3-aminobenzamide (10, 20 mg kg⁻¹ i.p., b.i.d) treatment did not modulate VP, MABP, LVDP, dp/dt max and dp/dt min in rats subjected to sham surgery. However it significantly attenuated PAAC-induced increase in VP, MABP and decrease in LVDP, dp/dt_{max} and dp/dt_{min} (Table 1 and Fig. 1-3). The PAAC markedly increased ratio of left ventricular weight to body weight (LVW/BW) (mg g⁻¹) and left ventricular wall thickness (LVWT). The 3-aminobenzamide (10, 20 mg kg⁻¹ i.p., b.i.d) treatment did not affect ratio of LVW/BW and LVWT in rats subjected to sham surgery but the treatment in a dose dependent manner significantly attenuated increase in ratio of LVW/BW and LVWT due to PAAC

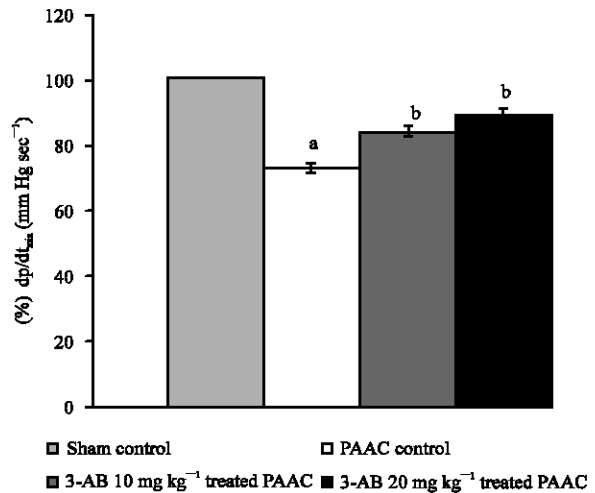


Fig. 3: Effect of 3-aminobenzamide on rate of pressure decay, values of rate of pressure decay (dp/dt_{min}) are expressed as percentage response. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

Table 2: Effect of 3-aminobenzamide on biochemical assessments

Parameters	Sham control	PAAC control	PAAC+3-AB (10 mg kg ⁻¹)	PAAC+3-AB (20 mg kg ⁻¹)
Collagen content	1.67±0.06	4.56±0.09 ^a	3.27±0.08 ^b	2.76±0.07 ^b
Protein content	116.70±5.9	174.80±4.6 ^a	163.70±3.6	127.60±4.1 ^b
RNA conc.	2.61±0.04	3.32±0.06 ^a	2.95±0.05 ^b	2.80±0.04 ^b

Collagen content, protein content and RNA concentration are expressed as mg per gram of left ventricle. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

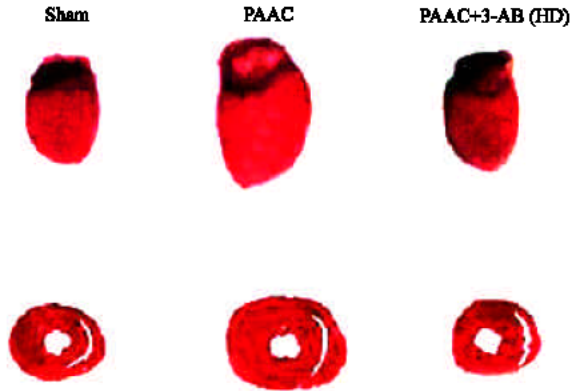


Fig. 4: Effect of 3-aminobenzamide on cardiac morphology

(Table 1 and Fig. 4). Figure 4 shows changes in heart size and changes in Left Ventricular Wall Thickness (LVWT) of rats subjected to PAAC for 4 weeks. PAAC+3-AB (HD) indicates rats subjected to PAAC and treated with high dose of 3-AB (20 mg kg⁻¹ i.p., b.i.d).

Effect of PARP inhibitor on biochemical parameters: The PAAC was noted to increase LV collagen content which was significantly reduced by 3-aminobenzamide (10, 20 mg kg⁻¹ i.p., b.i.d) treatment. Further, PAAC significantly increased protein content (mg g⁻¹) and RNA concentration (mg g⁻¹) in left ventricle. 3-aminobenzamide treatment in low dose (10 mg kg⁻¹ i.p., b.i.d) attenuated PAAC induced increase in protein and RNA concentration but results were statistically significant in case of RNA only. On the other hand 3-aminobenzamide treatment in high dose (20 mg kg⁻¹ i.p., b.i.d) significantly attenuated PAAC-induced increase in protein content and RNA concentration in left ventricle (Table 2).

DISCUSSION

The increase in venous pressure (Philipp *et al.*, 2004), ratio of left ventricular (LV) weight to body weight (Stamm *et al.*, 2001), LV wall thickness (Li *et al.*, 2003), LV protein content (Nagai *et al.*, 1988) and RNA concentration (Reddy *et al.*, 1996a,b) are documented as an index of left ventricular hypertrophy. In the present study Partial Abdominal Aortic Constriction (PAAC) for 4 weeks has produced left ventricular hypertrophy

assessed in terms of above-mentioned parameters as reported earlier (Shimoyama *et al.*, 1999; Reddy *et al.*, 1996b; Philipp *et al.*, 2004). The 3-aminobenzamide has been noted to attenuate PAAC-induced cardiac hypertrophy. Further, 3-aminobenzamide employed in present doses is documented to inhibit PARP (Liaudet *et al.*, 2001) Thus, it may be suggested that PARP may be involved in PAAC-induced cardiac hypertrophy. The aortic banding is reported to upregulate nitric oxide synthase (Dai *et al.*, 2004) and consequently generates peroxynitrite, due to interaction of nitric oxide and superoxide (Virag and Szabo, 2002; Pillai *et al.*, 2005) which may break DNA and activate PARP. PARP enhances expression of growth factors and hypertrophic genes (Pillai *et al.*, 2005; Carrillo *et al.*, 2004; Hassa and Hottiger, 2002). Thus, it may be proposed that PAAC-induced activation of PARP may be responsible to produce cardiac hypertrophy. Therefore, attenuation of PAAC-induced cardiac hypertrophy by 3-aminobenzamide may be due to inhibition of PARP. The noted PAAC-induced increase in venous pressure may be due to reduced left ventricular function. The abdominal aortic constriction may be initially responsible to increase MABP, which has been observed to return to normal value after about one and a half-hour of PAAC. However, MABP has been noted to increase gradually and attain peak level after 3-4 week of PAAC. The marked increase in MABP may be due to PAAC-induced pathological cardiac hypertrophy. The PAAC-induced increase in venous pressure and MABP have been noted to be attenuated by 3-aminobenzamide treatment. It supports the contention that PARP may be implicated in PAAC-induced cardiac hypertrophy.

The left ventricular dysfunction has been associated with decrease in LVDP, dp/dt_{max} and dp/dt_{min} (Osada *et al.*, 1998; Vaughan *et al.*, 1993; Zhang *et al.*, 2005). Further, studies have demonstrated that an increase in left ventricular collagen content may produce cardiac stiffness and fibrosis resulting in cardiac dysfunction (Weber and Brilla, 1991; Weber *et al.*, 1994; Simko *et al.*, 2002). In the present study, the left ventricular dysfunction assessed in terms of decrease in LVDP, dp/dt_{max} and dp/dt_{min} and increase in left ventricular collagen content have been observed in PAAC-induced cardiac hypertrophy. Moreover 3-aminobenzamide

treatment has been noted to attenuate left ventricular dysfunction associated with PAAC-induced left ventricular hypertrophy. It further suggests that PARP may be implicated in PAAC-induced left ventricular dysfunction due to pathological cardiac hypertrophy.

On the basis of above discussion it may be concluded that PAAC-induced cardiac hypertrophy and left ventricular dysfunction may be prevented by 3-aminobenzamide due to inhibition of PARP.

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