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The Possible Role of TNF- α in Physiological and Pathophysiological Cardiac Hypertrophy in Rats

P BALAKUMAR and MANJEET SINGH

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India.

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ABSTRACT

Pathological cardiac hypertrophy was produced by partial abdominal aortic constriction (PAAC) for 4 wk, while physiological cardiac hypertrophy was produced by chronic swimming training (CST) for 8 wk in orats. Pentoxifylline (30 mg/kg, 300 mg/kg i.p., day-1) treatment was started three days before PAAC and CST and it was continued for 4 wk in PAAC and 8 wk in CST experimental model. The left ventricular 12(LV) hypertrophy was assessed by measuring ratio of LV weight to body weight, LV wall thickness, LV raprotein content and LV RNA concentration. Further venous pressure (VP) and mean arterial blood pres-14 sure (MABP) were recorded. Moreover, DNA gel electrophoresis was employed to assess the myocardial 15 cell death. The PAAC and CST were noted to increase the ratio of LV weight to body weight, LV wall 16 thickness, LV protein content and LV RNA concentration. Further PAAC but not CST significantly in- τ creased VP, MABP and LV necrotic cell death. Pentoxifylline, a TNF- α inhibitor markedly attenuated 18 PAAC induced increase in LV hypertrophy, VP, MABP and LV necrotic cell death; but it did not modulate 10 CST induced LV hypertrophy. These results implicate TNF- α in PAAC induced cell death and pathological 20 cardiac hypertrophy. However, TNF-α may not be involved in CST induced physiological cardiac hyper-21 trophy.

22 Keywords: Aortic banding, Chronic swimming, Cardiac hypertrophy, Pentoxifylline, TNF- α

²⁴duced by exercise [1, 2] and pathological concentric ⁴⁵Ltd., Chandigarh, India) and tap water *ad libitum*. They 25 hypertrophy is associated with altered pattern of mal- 46 were housed in animal house and were exposed to 12-h 26 adaptive cardiac gene expression [3, 4]. Tumor necrosis 47 light and 12-h dark cycle. 27 factor-alpha (TNF- α), a proinflammatory cytokine has 28 been implicated in pathogenesis of myocarditis, 29ischemic heart disease and cardiac dysfunction [5-7]. 30 The prolonged exposures to high concentration of TNF- 50 31α produce cardiac dysfunction [8]. The persistent over 51 ing aortic banding [16, 17]. Rats were anaesthetized $_{32}$ expression of TNF- α has been suggested to be involved $_{52}$ with thiopentone sodium (35 mg/kg i.p.) and midline 33 in cardiac hypertrophy and left ventricular dysfunction 53 incision of 3-4 cm was made in abdomen to expose $_{34}$ [9-11]. Moreover, the role of TNF- α in physiological $_{54}$ aorta between diaphragm and celiac artery. The 4-0 silk 35 cardiac hypertrophy is not yet clear. Pentoxifylline is 55 suture was placed around the middle of aorta and it was 36 reported to inhibit the production of TNF- α [11-15]. 56 tightened along with a 0.7 mm diameter needle. The 37 Hence, the present study has been designed to investi- 57 needle was withdrawn to leave the vessel partially con- $_{38}$ gate the effect of pentoxifylline, an inhibitor of TNF- α $_{58}$ stricted and midline incision was sutured in layers. Neo-³⁹in pathological and physiological cardiac hypertrophy.

MATERIALS AND METHODS

42 has been approved by institutional animal ethical com- 64 tic constriction. Body weight was monitored weekly for 43 mittee. Young male wister albino rats weighing about 654 wk.

Physiological adaptive eccentric hypertrophy is in- 44225-275 g were maintained on rat feed (Kisan Feeds

48 Partial Abdominal Aortic Constriction (PAAC) 49 Induced Pathological Cardiac Hypertrophy

Pathological cardiac hypertrophy was produced us-59 sporin antibiotic powder (GlaxoSmithKline, Mumbai, 60 India) was applied locally on the sutured wound. Rats 61 were allowed to recover and were kept under observa-62 tion for 4 wk. Sham operated animals were subjected to The experimental protocol used in the present study 63 same surgical procedures except partial abdominal aor-

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TNF-a and Pathophysiological Cardiac Hypertrophy

66 Chronic Swimming Training (CST) Induced Physio- 107 67 logical Cardiac Hypertrophy

Physiological cardiac hypertrophy was produced us-69 ing chronic swimming exercise programme [18-20]. ⁷⁰The swimming apparatus was 150 cm in diameter and 7145 cm in height. The water level was maintained at 30 72 cm. Rats were initially subjected to swimming for 30 113 DNA Gel Electrophoresis 73 min twice daily with increments of 10 min daily. The 74 final duration of exercise was adjusted to 90 min; twice 75 daily for 8 wk. Sedentary group animals were allowed 115 of loading dye (40% sucrose, 0.1% bromophenol blue, 76 to take rest without any disturbances. Body weight was 1160.7% sodium dodecyl sulphate) and the mixture was 77 monitored weekly for 8 wk.

78 Morphological and Haemodynamic Assessments

80 (beats/min) using ECG (BPL MK 801, Bangalore, In-122 bromide (0.5µg/mL) was added to the gel for DNA de-⁸¹dia), jugular venous pressure (mmH₂O) and carotid ₁₂₃ tection. 82 mean arterial blood pressure (mmHg) using pressure 83 transducer (BIOPAC System, California, U.S.A) were 124 Experimental Design 84 recorded in anaesthetized rats. The left ventricle includ-85 ing interventricular septum and right ventricle weight 126 each group comprised of six animals. Group 1 (Sham $_{86}$ were noted separately and expressed as mg per g of $_{127}$ control, n=6), surgery was performed to expose the ab-87 body weight. The left ventricle was divided into three 128 dominal aorta but it was not constricted. Group 2 (PAAC ⁸⁸equal slices and wall thickness (mm) of each slice was ₁₂₉ control, n=6), abdominal aorta was exposed and par-89 noted at eight different points using ocular micrometer. 130 tially constricted. Group 3 (Pentoxifylline 30 mg/kg i.p., ⁹⁰ The mean value of all three slices were calculated and 131 day^{-1} treated, n=6), rats were subjected to partial ab-91 noted.

92 Biochemical Assessments

94 trogen for quantitative estimation of biochemical pa-136 i.p., day⁻¹ treated, n=6), rats were subjected to partial 95 rameters. The left ventricle was homogenized and pro-137 abdominal aortic constriction and they were treated with ⁹⁶tein content was determined spectrophotometrically at 138 high dose of pentoxifylline (300 mg/kg i.p., day⁻¹) as 97750 nm by Lowry's method [21] and expressed as mg/g 139 described in group 3. Group 5 (sedentary group, n=6), 98 of left ventricular weight.

100 tricular tissues using method of Chomczynski and Sac-142 chronic swimming exercise. Group 7 (Pentoxifylline 30 101 chi [22]. RNA concentration was estimated spectropho-143 mg/kg i.p., day⁻¹ treated, n=6), rats were subjected to 102 tometrically at 260 nm. One absorbancy unit at 260 nm 144 chronic swimming exercise and they were treated with 103 in a 1 cm light path cuvette was assumed to be equal to 145 low dose of pentoxifylline (30 mg/kg i.p., day⁻¹) 3 days 10440 µg/mL of RNA. The purity of RNA was assessed by 146 before attaining 90 min swimming period and continued 105 determining the ratio of absorbance at 260 and 280 nm 147 for 8 wk after attaining 90 min swimming period. Group 106 and the ratio was more than 1.8.

The DNA was extracted from homogenized left ven-108 tricular tissue using method of Ausubel et al [23]. The 109 concentration of DNA was determined spectrophonotometrically at 260 nm. The protein contamination of DNA was assessed by determining the ratio of absorb-2 ance at 260 nm and 280 nm, which was more than 1.75.

12 µg of extracted DNA was added to equal volume 117 loaded in the well. Electrophoresis was carried out using 1181.8% agarose gel in 1 x TBE buffer (Tris HCl 89 mM, 119 boric acid 89 mM, EDTA 2 mM) for 1.15 hr at 400 mA, 12050V and 3W in submarine electrophoresis apparatus After 4 wk of PAAC and 8wk of CST, heart rate 121 (Pharmacia Biotech, Freibury, Germany). Ethidium

Rats were randomly divided into eight groups and 132 dominal aortic constriction and they were treated with ¹³³low dose of pentoxifylline (30 mg/kg i.p., day⁻¹) which 134 was started 3 days before surgery and was continued for The left ventricle was stored at -80°C in liquid ni-1354 wk after surgery.Group 4 (Pentoxifylline 300 mg/kg 140 rats were allowed to rest without any disturbances. The RNA was extracted from homogenized left ven-141 Group 6 (CST group, n=6), rats were subjected to 1488 (Pentoxifylline 300 mg/kg i.p., day⁻¹ treatment, n=6),

	PAAC induced pathological hypertrophy				CST induced physiological hypertrophy			
	Sham control	PAAC control	PAAC+PTX (LD)	PAAC+PTX (HD)	Sedentary group	CST group	CST+PTX (LD)	CST+PTX (HD)
BW (g)	251.7±3.38	257.2±4.51	252.9±2.58	259.2±3.89	256±3.39	251.4±2.23	251.7±3.27	254.8±3.69
HR (beats/min)	415.5±4.98	409.6±5.68	419±4.43	415.2±4.36	419.2±5.17	368.8±3.34°	389.7±3.38 ^d	397.6±4.61 ^d
VP (mm H ₂ O)	24.2±2.06	$85.4{\pm}2.71^{a}$	66.5±2.74 ^b	38.2±3.26 ^b	24.8±1.70	25.8 ± 2.27	25±1.46	24±2.07
MABP (mmHg)	108.2 ± 2.44	178.6 ± 4.41^{a}	158.2±2.21 ^b	136.8±4.24 ^b	105.5 ± 2.60	103.4 ± 2.42	104.2 ± 3.03	103.8 ± 2.82
LVW/BW (mg/g)	1.97±0.03	$3.25{\pm}0.04^{a}$	2.94±0.02 ^b	2.10±0.03 ^b	1.89 ± 0.03	3.02±0.01 ^c	2.99 ± 0.05	2.95±0.03
RVW/BW (mg/g)	0.51±0.02	0.53 ± 0.01	0.52 ± 0.01	0.49 ± 0.01	0.49 ± 0.02	0.49 ± 0.01	0.51±0.02	0.49 ± 0.02
LVWT (mm)	2.28 ± 0.09	3.98 ± 0.12^{a}	3.28±0.09 ^b	2.44 ± 0.10^{b}	2.08 ± 0.06	3.26±0.13 ^c	3.23 ± 0.08	3.18±0.14
Protein Content	121.5 ± 5.34	175.7±4.69 ^a	156.2±2.78 ^b	135.3±3.92 ^b	127.5±3.03	181.5±4.75 ^c	179.6±3.77	183.5 ± 4.21
RNA Conc.	2.75±0.03	3.42 ± 0.05^{a}	3.14±0.02 ^b	2.84±0.01 ^b	2.55 ± 0.03	3.26±0.11°	3.16±0.04	3.14 ± 0.08

PAAC indicates partial abdominal aortic constriction, CST indicates chronic swimming training. PTX indicates pentoxifylline, LD indicates rats treated with low dose of PTX (30 mg/kg i.p., day⁻¹), HD indicates rats treated with high dose of PTX (300 mg/kg i.p., day⁻¹), BW indicates bodyweight, HR indicates heart rate, VP indicates venous pressure, MABP indicates mean arterial blood pressure, LVW indicates left ventricular weight, RVW indicates right ventricular weight and LVWT indicates left ventricular wall thickness. Protein content and RNA concentration are expressed as mg per gram of left ventricle. Values are mean \pm S.E.M. ^a p<0.05 vs. sham control; ^b p<0.05 vs. PAAC control; p < 0.05 vs. sedentary group; ^d p < 0.05 vs. CST group.

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heart size and (b) changes in left ventricular wall thickness (LVWT) of rats subjected to PAAC and CST. PAAC+ PTX (HD) indicates rats 199 treated with high dose of PTX (300 mg/kg i.p., day⁻¹).

150 they were treated with high dose of pentoxifylline (300 205 did not modulate increase in protein content and RNA 151 mg/kg i.p., day⁻¹) as described in group 7.

152 Statistical Analysis

Results were expressed as mean \pm S.E.M. The data 154 obtained from various groups were statistically analysed 209 155 using one-way ANOVA followed by Tukey's Multiple 210 trophoresis but CST did not produce any such effect. 156 Range test. The p-value < 0.05 was considered to be 211 The DNA smearing is the marker of necrotic cell death. 157 statistically significant.

158 Drugs and Chemicals

Pentoxifylline was obtained from Aventis Pharma 160 Limited, Mumbai, India. Proteinase K, sarcosyl, 2-161 mercaptoethanol and bovine serum albumin were pur- 215 162 chased from Sigma-Aldrich, Louis, St USA. Agarose 216 [16, 17] and chronic swimming training (CST) [18-20] 163 and folin ciocalteu reagent were obtained from SRL, 217 have been employed in the present study to induce car-164 Mumbai, India. All other reagents used in this study 218 diac hypertrophy. Both the experimental models have 165 were of analar grade.

RESULTS

167 Effect of Pentoxifylline on Morphological and 168 Haemodynamic Assessments

There was no significant change in body weight of 226 diac hypertrophy. Pentoxifylline is reported to inhibit 170 rats subjected to sham surgery, 4 wk of partial abdomi- 227 the formation of TNF- α [11-15]. The results of the pre-

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171 nal aortic constriction (PAAC) and 8 wk of chronic 172 swimming training (CST) with or without pentoxifylline 173 treatment (Table 1). PAAC produced no significant 174 change in heart rate but it significantly increased venous 175 pressure (VP) and mean arterial blood pressure 176 (MABP). Pentoxifylline (30 mg/kg, 300 mg/kg i.p., day 177^{1}) treatment in a dose dependent manner significantly 178 attenuated the increase in VP and MABP due to PAAC 179 (Table 1). PAAC increased the ratio of left ventricular 180 weight to body weight (LVW/BW) (mg/g) and left ven-181 tricular wall thickness (LVWT), which were markedly 182 attenuated in dose dependent manner by pentoxifylline 183 (30 mg/kg, 300 mg/kg i.p., day⁻¹) treatment (Table 1 and 184 Fig 1). CST did not produce any marked effect on VP 185 and MABP. Moreover heart rate was markedly reduced 186 as a result of CST and it was attenuated by pentoxifyl-187 line (30 mg/kg, 300 mg/kg i.p., day⁻¹) treatment (Table 1881). The CST markedly increased ratio of left ventricular 189 weight to body weight (LVW/BW) (mg/g) and left ven-190 tricular wall thickness (LVWT). But, pentoxifylline (30 191 mg/kg, 300 mg/kg i.p., day⁻¹) treatment did not modu-192 late increase in ratio of LVW to BW (mg/g) and LVWT 193 due to CST (Table 1 and Fig 1). There was no signifi-194 cant change in ratio of right ventricular weight to body 195 weight (RVW/BW) (mg/g) of rats subjected to sham 196 surgery, PAAC and CST with or without pentoxifylline 197 treatment (Table 1).

Fig 1. Effect of pentoxifylline on cardiac morphology. (a) Changes in 198 Effect of Pentoxifylline on Biochemical Parameters

PAAC and CST significantly increased protein consubjected to PAAC and treated with high dose of PTX (300 mg/kg 200 tent and RNA concentration in left ventricle. Pentoxifyli.p., day⁻¹) and CST+ PTX (HD) indicates rats subjected to CST and 201 line (30 mg/kg, 300 mg/kg i.p., day⁻¹) treatment signifi-202 cantly attenuated PAAC induced increase in protein 203 content and RNA concentration. In contrast to this, pen-149 rats were subjected to chronic swimming exercise and 204 toxifylline (30 mg/kg, 300 mg/kg i.p., day⁻¹) treatment 206 concentration in left ventricle due to CST (Table 1).

207 Effect of Pentoxifylline on Electrophoretic Pattern 208 of DNA

PAAC produced DNA smearing in agarose gel elec-²¹²Pentoxifylline (30 mg/kg, 300 mg/kg i.p., day⁻¹) signifi-213 cantly reduced PAAC induced DNA smearing (Fig 2).

DISCUSSION

The partial abdominal aortic constriction (PAAC) 219 increased ratio of left ventricular (LV) weight to body 220 weight, LV wall thickness, LV protein content and LV 221 RNA concentration which have been observed to in-222 crease in cardiac hypertrophy [24-27]. Pentoxifylline 223 treatment markedly reduced PAAC induced cardiac 224 hypertrophy measured in terms of above-mentioned 225 parameters, but it failed to modulate CST induced car-

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Fig 2. Effect of pentoxifylline on gel electrophoretic pattern of DNA. ²⁷⁹ L-1 represents DNA extracted from left ventricle of sham control ²⁸⁰ heart, L-2 represents DNA extracted from left ventricle of PAAC ²⁸¹⁶. control heart, L-3 represents effect of PTX (30 mg/kg i.p., day⁻¹) on ²⁸² DNA extracted from left ventricle of PAAC control heart, L-5 represents DNA extracted from left ventricle of sedentary group heart, L-6 represents DNA extracted from left ventricle of CST group heart, L-7 represents effect of PTX (30 mg/kg i.p., day⁻¹) on DNA extracted from left ventricle of CST group heart and L-8 represents effect of PTX (300 mg/kg i.p., day⁻¹) on DNA extracted from left ventricle of CST group heart. ²⁸⁵

²²⁸ sent study implicate TNF- α in PAAC induced cardiac ²⁴¹₂₉₂ ²²⁹ hypertrophy. On the other hand, TNF- α may not be in-²⁹³ ²³⁰ volved in CST induced cardiac hypertrophy. ²⁹⁴

²³¹ DNA smearing is an index of necrotic cell death ²⁹⁵10. ²³²[28]. In contrast to the CST experimental model, PAAC ²⁹⁶ ²³³induced cardiac hypertrophy has been noted to produce ²⁹⁷ ²⁹⁸234 DNA smearing which suggest an increase in necrotic ²⁹⁹ ²³⁵ cell death in left ventricle. Moreover, pentoxifylline has ³⁰⁰11. ²³⁶ been noted to attenuate PAAC induced increase in ne- ³⁰¹ ²³⁷ crotic cell death perhaps due to inhibition of formation ³⁰² ²³⁸ of TNF- α .

- 240 PAAC model may be due to reduced left ventricular 306 241 function as suggested by Philipp et al. [29]. The ab- 307 242 dominal aortic constriction may be initially responsible 308 243 to increase MABP, which has been observed to return to 30913. 244 the normal value after about one and a half-hour of ³¹⁰ 245 PAAC. However, MABP has been noted to increase 312 ²⁴⁶ gradually and attain peak level after 3-4 wk of PAAC. 313**14.** 247 The marked increase in MABP in PAAC model may be 314 248 due to pathological cardiac hypertrophy as reported re- 315 249 cently [30]. The PAAC induced increase in venous pres-316 $_{250}$ sure and MABP have been noted to be attenuated by 317 ²⁵¹ pentoxifylline treatment. It suggests that TNF- α induced ³¹⁸ ^{15.} 252 cardiac hypertrophy may be responsible to increase ve-253 nous pressure and MABP. On the other hand, these 321 254 haemodynamic changes have not been noted in CST 32216. 255 induced cardiac hypertrophy.
- In conclusion, pentoxifylline induced inhibition of $^{324}_{325}$ formation of TNF- α may be responsible for the attenua- $^{326}_{325}$ tion of PAAC induced cell death and pathological car- $^{327}_{327}$ to diac hypertrophy. Moreover, TNF- α may not be in- $^{328}_{325}$

²⁶⁰ volved in CST induced physiological cardiac hypertro-²⁶¹ phy.

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Address correspondence to: Prof. Manjeet Singh, Dean of Medicine and Research, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India. Phone: +91 (175) 3046304; Fax: +91 (175) 2283073. E-mail: pbala2002@rediffmail.com