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Denopamine, a β1-Adrenergic Agonist, Prolongs Survival in a Murine Model of Congestive Heart Failure Induced by Viral Myocarditis: Suppression of Tumor Necrosis Factor-α Production in the Heart

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Objectives. **This study was designed to examine the effects of denopamine, a selective β1-adrenergic agonist, in a murine model of congestive heart failure (CHF) due to viral myocarditis.**

Background. **Positive inotropic agents are used to treat severe heart failure due to myocarditis. However, sympathomimetic agents have not been found beneficial in animal models of myocarditis.**

Methods. **In vitro: The effects of denopamine on lipopolysaccharide-induced tumor necrosis factor-α (TNF-α) production was studied in murine spleen cells. In vivo: Four-week-old DBA/2 mice were inoculated with the encephalomyocarditis virus (day 0). Denopamine (14 μmol/kg), denopamine (14 μmol/kg) with a selective β1-blocker metoprolol (42 μmol/kg), or denopamine (14 μmol/kg) with metoprolol (84 μmol/kg) was given daily, and control mice received the vehicle only. Survival and myocardial histology on day 14 and TNF-α levels in the heart on day 6 were examined.**

Results. **In the in vitro study, TNF-α levels in treated cells were significantly lower than in controls ($p < 0.05$). In the in vivo study treatment with denopamine significantly improved the survival of the animals (14 of 25 (56%) treated, vs 5 of 25 (20%) control mice), attenuated myocardial lesions, and suppressed TNF-α production (66.5 ± 7.5 pg/mg of heart in treated mice vs 113.5 ± 15.1 pg/mg of heart in control mice, mean ± SE). There was a strong linear relationship between mortality and TNF-α levels ($r = 0.98$, $n = 4$, $p < 0.05$). These in vitro and in vivo effects of denopamine were significantly inhibited by metoprolol.**

Conclusions. **These results suggest that denopamine may exert its beneficial effects, in part, by suppressing the production of TNF-α via β1-adrenoceptors.**

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Congestive heart failure (CHF) is the most serious clinical consequence of viral myocarditis. Furthermore, because viral myocarditis may lead to dilated cardiomyopathy (1–3), appropriate treatment should be administered during the acute phase of the disease. Positive inotropic agents are regularly used in the treatment of severe cardiac decompensation due to myocarditis despite reserved opinions regarding their use in the treatment of CHF (4,5). Several inotropic agents have been tested in animals (6), although catecholamines and other sympathomimetic agents have not been found beneficial in animal models of myocarditis.

Denopamine, [(−)-α-(3,4-dimethoxyphenethylaminomethyl)-4-hydroxybenzylalcohol], is an orally active, selective β1-adrenergic agonist that has no catecholamine moiety in its chemical structure (7,8). The purpose of this study was to examine the effects of denopamine on short-term survival of mice with acute viral myocarditis due to encephalomyocarditis virus (EMCV) infection (9,10). In addition, because tumor necrosis factor-α (TNF-α) plays an important role in the pathophysiology of myocarditis (11–13) and CHF (14–20) the effects of denopamine on TNF-α production were also measured.

Methods

Drug preparation. Denopamine was synthesized by Tanabe Pharmaceutical Co., Ltd. (Osaka, Japan), and metoprolol by Sigma Chemical (St Louis, MO, USA). They were mixed in phosphate buffered saline (PBS) for the purpose of these experiments.

In vitro studies. *Preparation of spleen cells and lipopolysaccharide stimulation.* Spleen cells obtained from 4-week-old DBA/2 mice were dissociated mechanically by squeezing the spleen through a mesh screen and cultured in RPMI-FBS on round-bottomed microplates. Each well contained 3×10^6 spleen cells/mL and the plate was incubated at 37°C in 5% CO_2

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Abbreviations and Acronyms

CHF = congestive heart failure
EMCV = encephalomyocarditis virus
TNF-α = tumor necrosis factor-α
LPS = lipopolysaccharide
ELISA = enzyme-linked immunosorbent assay
BW = body weight
HW = heart weight

with 1 μg/mL lipopolysaccharide (LPS) (Difco Laboratories Inc., Detroit, MI, USA).

Time course of LPS-induced TNF-α production. After 2, 5, 6, or 22 h of incubation, 0.1 mL samples of supernatant (n = 3) were obtained from each well for assay of murine TNF-α. Control samples were not exposed to LPS.

Effect of denopamine on LPS-induced TNF-α production. Nine quantities each of 0.1, 1, 10, or 100 μmol/L of denopamine were added to each well. The control cultures received the vehicle only (n = 9). After 5 h of incubation, 0.1 mL of supernatant from each well was collected for assay of murine TNF-α.

Effect of denopamine and metoprolol on LPS-induced TNF-α production. Denopamine (1 μmol/L), denopamine (1 μmol/L) with metoprolol (1, 5, or 10 μmol/L), or metoprolol (10 μmol/L) was added to each well (n = 4). The control cultures received the vehicle only (n = 4). After 5 h of incubation, 0.1 mL of supernatant from each well was collected for assay of murine TNF-α with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bio-source International, Camarillo, CA, USA), performed in accordance with the manufacturer's instructions. The sensitivity of the kit is 3 pg/mL.

In vivo studies. *Pharmacokinetic study.* The pharmacokinetics of denopamine after its daily oral administration in a dose of 14 μmol/kg per day were studied in seven 4-week-old inbred male DBA/2 mice. After 3 days of treatment, blood was sampled from the animals' tails immediately before and 1, 2, 3, and 5 h after administration of denopamine. Blood was mixed with 500 mg of ethylenediamine-N,N,N′,N′-tetraacetic acid, disodium salt, dihydrate (EDTA × 2 Na) and 500 KIE of aprotinin solution, and centrifuged to separate the plasma, which was stored at −80°C. The plasma concentration of denopamine was measured by liquid chromatography (21). The sensitivity of the method is 0.6 nmol/L.

Experimental infection. Four-week-old inbred male DBA/2 mice were inoculated intraperitoneally with 0.1 mL of the M variant of EMCV diluted in Eagle's minimal essential medium to a concentration of 100 plaque-forming units/mL. The day of virus inoculation was defined as day 0 for the subsequent studies.

Treatment protocols. Protocol 1: The effects of denopamine were examined against an untreated control group. Denopamine was administered in a dose of 14 μmol/kg per day, whereas control mice received the vehicle only. Protocol 2: To test whether the effects of the drug are mediated by β1-adrenoceptors, the effects of selective β1-blockade on denopamine were examined in the same experimental model. Denopamine alone, 14 μmol/kg/day, was administered to 25 mice; 10 mice received denopamine, 14 μmol/kg/day, and metoprolol, 42 μmol/kg/day, in combination; 20 mice received denopamine, 14 μmol/kg/day, and metoprolol, 84 μmol/kg/day, in combination; 25 control mice received the vehicle only.

Survival experiments. Because in this model most mice die of CHF within 14 days after virus inoculation (9,10) survival was measured over a 14-day period in this study.

Assay of TNF-α levels in the heart. Because the expression of TNF-α mRNA in the heart peaked 5 to 7 days after inoculation (22), the effect of denopamine on TNF-α production in the heart was measured on day 7 for protocol 1 and on day 6 for protocol 2, at which time the surviving animals were sacrificed by cervical dislocation and their hearts were removed under sterile conditions. After measurements of the body and heart weights, the heart was divided in two sections along its short axis, at the mid left ventricular level. One half was stored at −80°C, and used for ELISA of TNF-α levels in tissue homogenates using a modification of the methods described by Pizarro et al (23), Sekido et al (24), and Torre-Amione et al (25). Briefly, frozen sections of tissue, suspended in microtubes with 1.5 mL of phosphate buffered saline solution containing 0.05% NaN_3 at 4°C, were ultrasonically homogenized on ice at 50 W (ASTRASONTM Model XL2020, Misnox Inc., Farmingdale, NY, USA), and sonicated for 10 to 20 s (26), while temperature in the suspension was maintained at 4°C during homogenization. Heart tissue homogenates were centrifuged at 14,000 rpm at 4°C for 20 min, and the resultant supernatant was collected to measure TNF-α levels. Murine TNF-α was assayed with commercially available ELISA kits (Genzyme Co., Cambridge, MA, USA) according to the manufacturer's instructions. The sensitivity of each kit is 15 pg/mL, respectively. TNF-α levels were expressed as pg/mg of heart.

Histologic examination. The other half of the heart was used for histologic examination. The specimens were fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The extent of cellular infiltration and myocardial necrosis was graded by two observers blinded to the treatment assignments, and scored as follows: 0 = no lesions; 1+ = lesions involving < 25% of the myocardium; 2+ = lesions involving 25% to 50%; 3+ = lesions involving 50% to 75%; and 4+ = lesions involving 75% to 100%. The scores assigned by the two observers were averaged.

Statistical analysis. Each value is expressed as mean ± SE. Survival was measured by the Kaplan-Meier method, and the log-rank test was used to determine the significance level. Statistical comparisons of TNF-α levels, heart weight-to-body weight (HW/BW) ratios and histologic scores were performed by Mann-Whitney *U*-test or Kruskal-Wallis test for in vivo and in vitro experiments. A simple regression was performed to verify the linearity of the relationship between the survival rate and TNF-α levels. A p value <0.05 was considered statistically significant.

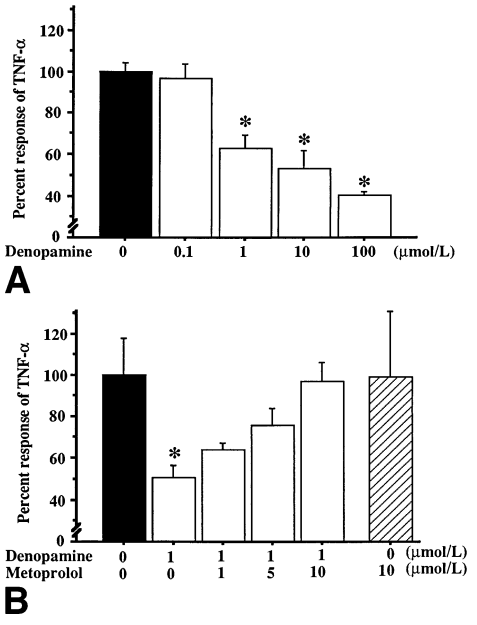


Figure 1. **(A)** Effect of increasing concentrations of denopamine on 1 μg/mL LPS-induced TNF-α production in murine spleen cells. LPS-induced TNF-α production was suppressed by denopamine in a concentration-dependent manner. Values are means ± SE of 9 cultures. *$p < 0.05$ versus the control culture stimulated by LPS. **(B)** Effect of denopamine and metoprolol on 1 μg/mL LPS-induced TNF-α production in murine spleen cells. LPS-induced TNF-α production was significantly suppressed by denopamine, 1 μmol/L. Metoprolol blocked the suppression of TNF-α production in a concentration-dependent manner. Values are means ± SE of 4 cultures. *$p < 0.05$ versus the control stimulated by LPS, denopamine with metoprolol concentration of 10 μmol/L, or metoprolol alone (10 μmol/L).

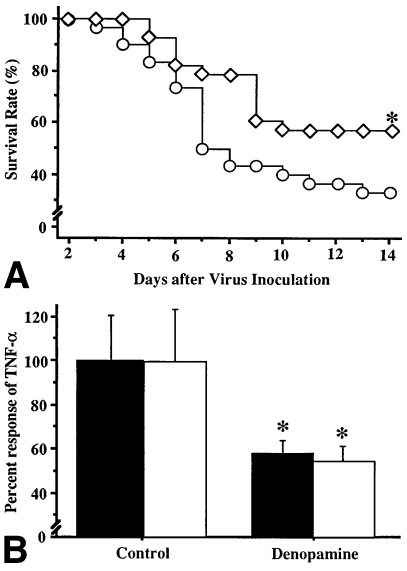


Figure 2. **(A)** Significant improvement by denopamine in survival of mice to day 14 after EMCV inoculation. ○ = control group (n = 30); ◆ = 14 μmol/kg denopamine treatment group (n = 28). *$p < 0.05$ **(B)** Effect of denopamine on TNF-α levels in the heart on day 7 and day 14. TNF-α in the hearts of mice treated with denopamine (n = 17 on day 7; n = 7 on day 14) was significantly lower than in those of control animals (n = 11 on day 7; n = 6 on day 14). Solid bars = day 7; clear bars = day 14. Values are means ± SE. *$p < 0.05$ versus control group.

Results

In vitro studies. *Time course of LPS-induced TNF-α production.* Spleen cell TNF-α levels were 69.4 ± 5.6 pg/mL at 2 h, 380.6 ± 19.4 pg/mL at 5 h, 350.0 ± 47.2 pg/mL at 6 h, and 52.8 ± 0.2 pg/mL at 22 h of incubation following LPS stimulation. TNF-α production by spleen cells after LPS stimulation peaked at 5 h of incubation, whereas in controls it averaged 11.1 ± 0.2 pg/mL over the 22 h period.

Effect of denopamine on LPS-induced TNF-α production. Denopamine suppressed LPS-induced TNF-α production in a concentration-dependent manner (Fig. 1A). Compared with a stimulated control of 510.2 ± 87.2 pg/mL, concentrations of the drug of 0.1, 1, 10 and 100 μmol/L decreased TNF-α levels by 96.9 ± 6.7%, 62.7 ± 6.5%, 53.2 ± 8.8%, and 40.3 ± 1.5%, respectively (n = 9 each). At the 3 highest drug concentrations (1, 10, and 100 μmol/L), TNF-α levels were significantly lower than in the control culture stimulated by LPS ($p < 0.05$).

Effect of denopamine and metoprolol on LPS-induced TNF-α production. Denopamine (1 μmol/L) suppressed LPS-induced TNF-α production, whereas metoprolol blocked that suppression in a concentration-dependent manner (Fig. 1B). Compared with a stimulated control of 242.1 ± 43.1 pg/mL, denopamine alone, in a concentration of 1 μmol/L decreased TNF-α levels by 50.6 ± 5.9% ($p < 0.05$ vs control). Addition of metoprolol in increasing concentrations of 1, 5, and 10 μmol/L increased the TNF-α levels compared to denopamine alone by 64.2 ± 3.1%, 76.0 ± 8.0%, and 96.6 ± 9.1%, respectively (n = 4 each). At the highest drug concentration of metoprolol (10 μmol/L), TNF-α levels were significantly higher than in the denopamine alone culture stimulated by LPS ($p < 0.05$) and comparable to values measured in controls.

In vivo study. *Pharmacokinetic study.* The plasma concentration of denopamine was 13.1 ± 1.9 nmol/L at 1 h, 4.3 ± 0.9 nmol/L at 2 h, 1.8 ± 0.5 nmol/L at 3 h, and <0.6 nmol/L at 5 h after its administration. A single 14 μmol/kg dose of denopamine in mice produced a peak level at 1 h. The peak plasma concentration was comparable to that achieved with a single, oral 10 mg daily dose associated with therapeutic efficacy in humans (27). The half-life of the drug in plasma was 0.71 h.

Protocol 1. At day 14, the survival rate of 57.1% (16 of 28 mice) in the denopamine group was significantly higher than the 33.3% (10 of 30 mice) survival rate in the control group ($p < 0.05$, Fig 2A). The survival rate from day 6 to day 14 was also significantly improved in the denopamine group (69.6%; 16 of 23 mice) versus the control group (45.5%; 10 of 22 mice, $p < 0.05$). By day 7, compared with an infected control of 1,258.5 ± 247.0 pg/mg of heart (n = 11), denopamine, in a concentration of 14 μmol/kg, had decreased TNF-α levels by 58.5 ± 6.6% ($p < 0.05$ vs control, n = 17). This statistically significant difference was found in the absence of a significant difference in heart weight-to-body weight ratio (HW/BW) ratio on day 7 (Table 1) between the treated group (7.8 ± 0.3 ×

Table 1. Effects of Denopamine on HW/BW and Myocardial Histology on Day 7

	n	HW/BW ($\times 10^{-3}$)	Histologic Score	
			Infiltration	Necrosis
Control	11	8.5 ± 0.5	1.7 ± 0.2	2.0 ± 0.3
Denopamine 14 μg/kg	17	7.8 ± 0.3	1.9 ± 0.2	1.9 ± 0.2

HW/BW = heart weight-to-body weight ratio. Values are means ± SE. The differences between the 2 groups are not statistically significant.

10^{-3}, n = 17) and the control group (8.5 ± 0.5 × 10^{-3}, n = 11). Likewise, no significant differences were found between the two groups in histologic scores for cellular infiltration (1.9 ± 0.2, n = 17 in the treated group versus 1.7 ± 0.2, n = 11 in the control group) or in the scores for myocardial necrosis (1.9 ± 0.2 versus 2.0 ± 0.3, Table 1). The effects of denopamine on TNF-α levels in the heart and myocardial histology at day 14 were examined in separate experiments. Compared with an infected control of 43.5 ± 11.3 pg/mg of heart (n = 6), denopamine significantly decreased TNF-α levels by 54.5 ± 6.2% ($p < 0.05$ vs control, n = 7, Fig 2B). In contrast to day 7, a significant difference in HW/BW ratio was found on day 14 (Table 2) between the treated group (5.5 ± 0.2 × 10^{-3}, n = 7, $p < 0.05$) and the control group (6.6 ± 0.4 × 10^{-3}, n = 6). Likewise, significant differences were found between the two groups in histologic scores for cellular infiltration (1.4 ± 0.2, n = 7 in the treated group vs 2.3 ± 0.2, n = 6 in the control group, $p < 0.05$) and in the scores for myocardial necrosis (1.1 ± 0.3 vs 2.0 ± 0.3, $p < 0.05$, Table 2 and Fig. 3).

Protocol 2. At day 14, the survival rates were 56% (14 of 25 mice) in the group treated with denopamine alone, 40% (4 of 10 mice) in the group treated with denopamine (14 μmol/kg) and metoprolol (42 μmol/kg), 25% (5 of 20 mice) in the group treated with denopamine (14 μmol/kg) and metoprolol (84 μmol/kg), and 20% (5 of 25 mice) in the control group (Fig. 4A). The survival rate was significantly higher in the denopamine-alone-treated group than in either the group treated with denopamine (14 μmol/kg) and high-dose metoprolol (84 μmol/kg) or the control group ($p < 0.05$).

No statistically significant differences were found among the four groups in the HW/BW ratio, histologic scores for cellular infiltration or in the scores for myocardial necrosis (Table 3). The HW/BW ratio was 8.5 ± 0.4 × 10^{-3} in the group treated with denopamine alone, 9.0 ± 0.7 × 10^{-3} in the denopamine with low-dose metoprolol group, 9.1 ± 0.2 × 10^{-3} in the denopamine with high-dose metoprolol group and 8.8 ± 0.2 × 10^{-3} in the control group (n = 5 each). Histologic scores for cellular infiltration were 2.6 ± 0.4, 2.8 ± 0.2, 2.8 ± 0.2, and 2.6 ± 0.2, respectively, and those for myocardial necrosis were 2.6 ± 0.4, 2.8 ± 0.2, 3.0 ± 0.3, and 2.8 ± 0.4, respectively (n = 5 each).

Table 2. Effects of Denopamine on HW/BW and Myocardial Histology on Day 14

	n	HW/BW ($\times 10^{-3}$)	Histologic Score	
			Infiltration	Necrosis
Control	6	6.6 ± 0.4	2.3 ± 0.2	2.0 ± 0.3
Denopamine 14 μg/kg	7	5.5 ± 0.2*	1.4 ± 0.2*	1.1 ± 0.3*

HW/BW = heart weight-to-body weight ratio. $p < 0.05$ vs control. Values are means ± SE.

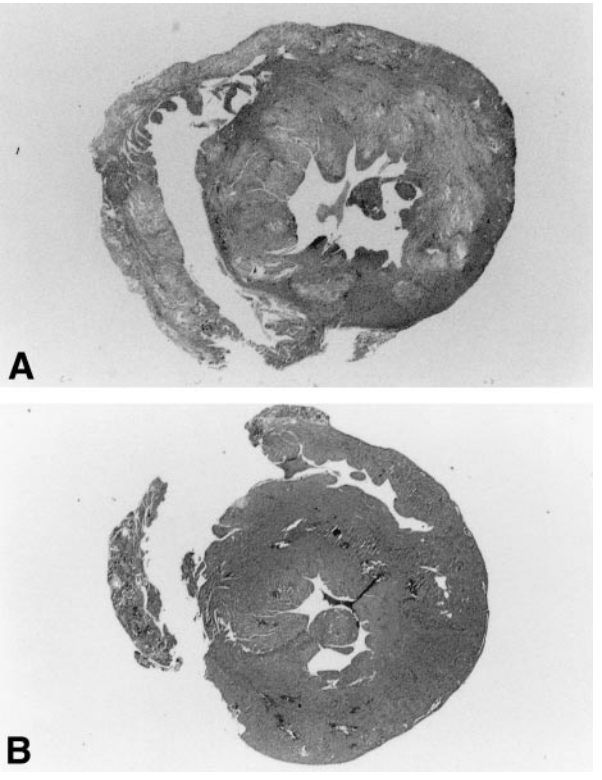


Figure 3. Effects of denopamine on histopathologic changes at day 14. Myocardial lesions in mice treated with denopamine were significantly less than in control animals. **(A)** control animals. **(B)** denopamine-treated animals. Hematoxylin-eosin stain; original magnification: ×12.

At day 6, denopamine alone suppressed TNF-α levels in the heart and metoprolol reversed the suppression of TNF-α production in a concentration-dependent manner (Fig. 4B). Compared with a control value of 113.5 ± 15.1 pg/mg of heart, denopamine alone, in a concentration of 14 μmol/kg, decreased TNF-α levels by 58.5 ± 6.6% ($p < 0.05$ vs control). Addition of metoprolol in concentrations of 42 and 84 μmol/kg increased the TNF-α levels by 73.3 ± 15.3% and 91.9 ± 12.1%, respectively (n = 5 each), compared to denopamine alone. At the highest concentration of metoprolol (84 μmol/L), TNF-α levels were significantly higher than in the denopamine alone group ($p < 0.05$, Fig 4B).

The relation between TNF-α levels in the heart on day 6 and mortality from day 6 to day 14. Mortality from day 6 to day 14 was 33.3% (7 of 21 mice) in the group treated with denopamine alone, 50% (4 of 8 mice) in the group treated with denopamine and low-dose metoprolol, 68.8% (11 of 16 mice) in the group treated with denopamine and high-dose metoprolol, and 73.7% (14 of 19 mice) in the control group (Table 4).

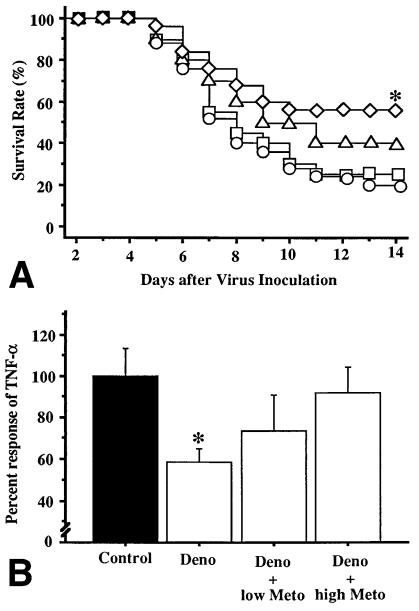


Figure 4. (**A**) Effects of denopamine and metoprolol on survival of mice to day 14 after EMCV inoculation. Denopamine significantly improved the survival; the effect was significantly blocked by metoprolol at a concentration of 84 μmol/kg. ○ = control group (n = 25); □ = denopamine, 14 μmol/kg + metoprolol, 84 μmol/kg treatment group (n = 20); △ = denopamine, 14 μmol/kg + metoprolol, 42 μmol/kg treatment group (n = 10); ◆ = denopamine alone, 14 μmol/kg treatment group (n = 25). *p < 0.05 versus control, or versus denopamine, 14 μmol/kg + metoprolol, 84 μmol/kg treatment group. (**B**) Effect of denopamine and metoprolol on TNF-α levels in the heart on day 6. TNF-α in the hearts of mice treated with denopamine was significantly lower than in those of control animals. The effect of denopamine was significantly blocked by metoprolol at a concentration of 84 μmol/kg. Values are means ± SE. N = 5 in each group. Deno = denopamine, 14 μmol/kg; low Meto = metoprolol, 42 μmol/kg; high Meto = metoprolol, 84 μmol/kg. *p < 0.05 versus control or 14 μmol/kg denopamine with 84 μmol/kg metoprolol treatment group.

A significant linear relationship was found (Fig. 5) between second week mortality and TNF-α levels in the heart measured on day 6 (R = 0.98, n = 4, p < 0.05). The same strong relationship (R = 0.98, n = 4, p < 0.05) was observed on day 14.

Table 3. Effects of Denopamine and Metoprolol on HW/BW and Myocardial Histology on Day 6

	n	HW/BW ($\times 10^{-3}$)	Histologic Score	
			Infiltration	Necrosis
Control	5	8.8 ± 0.2	2.6 ± 0.2	2.8 ± 0.4
Deno + high Meto	5	9.1 ± 0.2	2.8 ± 0.2	3.0 ± 0.3
Deno + low Meto	5	9.0 ± 0.7	2.8 ± 0.2	2.8 ± 0.2
Deno	5	8.5 ± 0.4	2.6 ± 0.4	2.6 ± 0.4

HW/BW = heart weight-to-body weight ratio; high Meto = metoprolol 84 μg/kg; low Meto = metoprolol 42 μg/kg; Deno = denopamine 14 μg/kg; Values are means ± SE. The differences among the 4 groups are not statistically significant.

Table 4. Mortality from Day 7 to Day 14

	Number of Survivors		
	Day 6	Day 14	Mortality
Control	19	5	73.7%
Deno + high Meto	16	5	68.8%
Deno + low Meto	8	4	50.0%
Deno	21	14	33.3%*

High Meto = metoprolol 84 μg/kg; low Meto = metoprolol 42 μg/kg; Deno = denopamine 14 μg/kg

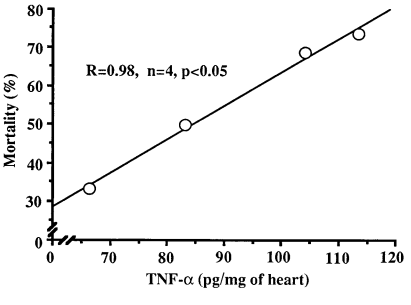
*p < 0.05 vs control or Deno + high Meto. Values are means ± SE.

Discussion

The goals of heart failure management are to improve quality of life and increase patient survival. Although positive inotropic agents have favorable initial hemodynamic effects and offer symptomatic relief in heart failure patients, their impact on survival has been disappointing (5,28). These drugs are divided into three main groups: digitalis glycosides, phosphodiesterase inhibitors, and sympathomimetic amines. Digitalis glycosides, which increase sodium intracellular concentrations by inhibiting sodium-potassium ATPase (29), have occupied a prominent place in the management of CHF, but their clinical use is limited by a narrow therapeutic window (30). Phosphodiesterase inhibitors, which increase intracellular cyclic AMP by inhibiting phosphodiesterase III, are in the spotlight as new inotropic agents (31), although their efficacy has not been firmly established (32). Catecholamines and other sympathomimetic amines, which increase intracellular cyclic AMP by stimulating β-adrenergic receptors, have been used for many years to treat severe CHF. However, their use is limited by various undesirable chronotropic, vasoconstrictor or vasodilator, and arrhythmogenic effects (33,34). It has also been suggested that increased intracellular concentration of cyclic AMP, by stimulating the β-adrenergic receptors, may accelerate the rate of cell death, and that calcium overload may induce arrhythmias and myocardial injury (35,36). Furthermore, clinical trials have observed an increase in mortality associated with their long-term use (37).

General properties of denopamine. Denopamine was developed and synthesized in the hope of eliminating or reducing

Figure 5. Significant, linear relationship between mortality from day 6 to day 14 and TNF-α levels measured on day 6 (R = 0.98, n = 4, p < 0.05).



these limitations. In Japan, the drug has been used in approximately 60,000 patients since 1988, and several reports have confirmed its efficacy in the treatment of chronic CHF (27,38–40). Denopamine has little effect on heart rate (41) and is less proarrhythmic than ouabain or isoproterenol (42). Minimal or no changes have been measured in left ventricular systolic pressure and myocardial oxygen consumption (43). Tolerance of β-adrenergic receptors to its inotropic action has not been observed after its administration for 14 days (44). In addition, denopamine causes peripheral vascular smooth muscle relaxation mediated by the blocking effect of α1-adrenoceptors (45).

Denopamine, cytokine production, and beta adrenergic receptors. Several studies have shown an integration of neuroendocrine hormones into the immune response. Adrenergic agents, in particular, have been shown to influence cytokine production (46–50). Similarly, the present study found that denopamine directly suppressed LPS-induced TNF-α production from murine spleen cells. In splenic tissue, TNF-α is mainly synthesized by macrophages and lymphocytes (51). Agents that act via β1-adrenoceptors inhibit LPS-induced TNF-α production from the promonocytic leukemia cell line THP-1 by increasing intracellular cyclic AMP levels (46). On the other hand, lymphocytes have β2- but no β1-adrenergic receptors (52). Because denopamine does not increase cyclic AMP via β2-adrenergic receptors even in a dose of 100 μmol/L (7), it probably does not act on lymphocytes through their β2-receptors. To determine whether the effect of denopamine on TNF-α was mediated by β1-adrenoceptors, the selective β1-antagonist metoprolol was administered with denopamine (53). Metoprolol significantly blocked the effect of denopamine. Thus, it is suggested that denopamine inhibits LPS-induced TNF-α production by macrophages via β1-adrenoceptors in murine spleen cells.

The present study also showed that denopamine inhibited TNF-α production in the heart. In our murine model of viral myocarditis, immunohistochemical studies showed that TNF-α immunostaining in the heart was localized to macrophages, lymphocytes, and endothelial cells (22). To date, no report has described the effects of β-adrenergic agonists on TNF-α production by endothelial cells. We also examined the effects of denopamine combined with metoprolol in our model and found that metoprolol is able to block the action of denopamine in vitro and in vivo. As discussed above, the effect of denopamine on TNF-α production in heart tissue homogenates is probably due to inhibition of TNF-α production by macrophages through β1-adrenoceptors.

Effects of denopamine on short-term survival. Denopamine prolonged the survival of mice during the acute stage of viral myocarditis. In our animal model of CHF due to viral myocarditis, myocardial necrosis and mononuclear cellular infiltration appear 4 to 5 days after viral inoculation, with some mice dying as early as day 5, and others developing severe CHF after day 7 (9,10). Denopamine significantly improved survival rates past day 7, despite no significant improvement in the extent of myocardial injury on day 6 or 7. In contrast, denopamine significantly attenuated myocardial lesions on day 14. Thus, it exerted its primary effect at a stage when the majority of deaths were caused by CHF. Recent reports have emphasized the importance of cytokines, TNF-α in particular, in the pathophysiology of CHF (14–20). Severe CHF was caused in transgenic mice with myocardial expression of TNF-α (19) and the infusion of TNF-α led left ventricular dysfunction and remodeling in the experimental model (20). Circulating levels of TNF-α are increased in patients with CHF (25,54–57) and its direct and indirect negative inotropic effects have been described (57–59). These observations lead us to hypothesize that TNF-α is one of the factors that exacerbate heart failure in its acute phase. In this study, denopamine suppressed TNF-α production in vitro and in vivo. These findings suggest that denopamine may improve inotropy, attenuate myocardial damage, and prolong survival in CHF due to viral myocarditis by inhibiting TNF-α, a hypothesis supported by the significant linear relationship that was found between second-week mortality and TNF-α levels in the heart. Because most cytokines act in an autocrine or paracrine manner, circulating levels of cytokines may not accurately reflect their effects on target organs (15). Furthermore, there is no significant correlation between the level of TNF-α in the myocardium and its level in plasma, probably because of its multiple sites of production in CHF (25). For this reason, TNF-α levels in this study were measured in heart tissue homogenates and a correlation was found between the level of TNF-α in the heart and the prognosis of CHF in its acute phase.

Denopamine treatment and myocardial injury. Studies from several laboratories have shown that TNF-α exacerbates myocardial injury (11–13). In this study, the extent of myocardial damage was not attenuated in the denopamine-treated group on day 6 or 7, despite the inhibition of TNF-α production in the heart, an observation that seems to contradict these other reports. However, we have reported that anti-TNF-α antibody treatment attenuated myocardial lesions in the same murine model if the onset of treatment is postponed until day 1 and administered between day 1 and day 4, instead of between day 0 and day 4 (12). In this study denopamine treatment was started on day 0 until day 14. Thus, myocardial lesions may not have been attenuated on day 6 or 7, despite inhibition of TNF-α production. In contrast, denopamine did attenuate the extent of myocardial lesions and inhibited TNF-α production at day 14. Past day 7, at the stage of CHF, it exerted its protective effects through its inhibitory action on TNF-α production.

Conclusions. Denopamine prolonged survival, attenuated myocardial lesions, and inhibited TNF-α production in a murine model of CHF induced by viral myocarditis. These results suggest that denopamine may exert its beneficial effects partially through the suppression of TNF-α production.

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