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Hemodynamic characterization of left ventricular function in experimental coxsackieviral myocarditis: effects of carvedilol and metoprolol

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Abstract

Background: Carvedilol, a vasodilating nonselective β-adrenoceptor antagonist, but not metoprolol, a selective β_1-adrenoceptor antagonist, has been shown to increase the production of cardiac antiinflammatory cytokines in experimental myocarditis. However, the hemodynamic consequences of these differences had not been investigated until today. Therefore, we determined the effects of carvedilol and metoprolol on left ventricular function in a murine model of coxsackievirus B3 (CVB3)-induced myocarditis. *Methods*: BALB/c mice were inoculated with the coxsackie-B3 virus. Four and 10 days after infection, left ventricular function was investigated using a conductance micromanometer system. Additional groups were treated starting 24 h after infection using equipotent doses of carvedilol and metoprolol and studied on day 10. *Results*: On day 4, infected mice manifested increased afterload-enhanced contractility and abnormal diastolic function. On day 10, contractile function of untreated mice was impaired. Carvedilol significantly improved cardiac index and most systolic indices, whereas metoprolol was substantially less effective. Diastolic dysfunction was not influenced by either of the β-adrenoceptor antagonists. *Conclusions*: These hemodynamic data indicate that not only β_1-adrenoceptor blockade but also pleiotropic effects are involved in the cardioprotective effects of carvedilol on the pathophysiology of acute viral myocarditis.

Keywords: Myocarditis; β-Adrenoceptor; Conductance; Pressure–volume loops

1. Introduction

Enteroviruses, particularly coxsackievirus B (CVB), are the most common viruses in the etiology of viral myocarditis (Pauschinger et al., 1999; Seong et al., 2001). Viral infection of the myocardium produces myocardial necrosis and inflammatory cellular infiltration that can cause acute heart failure. Activation of the cytokine network combined with extracellular matrix remodeling and myocyte dysfunction is considered a hallmark in the progression of cardiac damage (Li et al., 2002). In addition, neuroendocrine activation, especially catecholamines, can participate in this inflammation. Thus, inhibition of sympathetic stimulation by β-adrenoceptors in heart failure includes cardiac protection from β-adrenoceptor overstimulation, antiarrhythmic effects, reduction in heart rate and positive energetic effects as well as a reduction of proinflammatory cytokines (Bohm and Maack, 2000). Recently, it was shown that selective β_1-adrenoceptor antagonists like metoprolol and the nonselective vasodilating β-adrenoceptor antagonist, carvedilol, also reduce mortality in heart failure (Eichhorn and Bristow, 2001; MERIT-HF-Group, 1999; Poole-Wilson et al., 2003). However, carvedilol has additional unique properties compared to selective β-adrenoceptor antagonists like metoprolol. It is the only β-adrenoceptor antagonist blocking β_1-, β_2- and α_1-adrenoceptors without an intrinsic sympathomimetic effect, such as an increased cardiac norepinephrine release or β-adrenoceptor upregulation (Bristow, 2000). The result is a more comprehensive degree of adrenergic inhibition. Furthermore, carve-

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dilol and several of its metabolites are potent antioxidants (Yue et al., 1992). Recently, Nishio et al. (2003) showed that carvedilol, but not metoprolol or propanolol, increases the production of antiinflammatory cytokines in an experimental myocarditis model. However, the hemodynamic consequences of these differences in acute myocarditis are unknown. Therefore, this study was performed to compare the hemodynamic effects of carvedilol and metoprolol in a murine model of acute coxsackieviral myocarditis using a conductance micromanometer system.

2. Materials and methods

2.1. Experimental infection

As previously described, 8-week-old inbred male BALB/c (H-2d) mice (Robert von Ostertag Institute Berlin, Germany; $n=40$) were infected by an intraperitoneally injection of 5×105 plaque-forming units (PFU) of coxsackie B3 virus (CVB3, strain Nancy) (Huber et al., 1994). Uninfected mice served as controls ($n=10$).

2.2. Treatment protocol

Serial hemodynamic measurements were made on days 4 and 10 after infection and compared with controls ($n=10$/group). Starting 24 h after infection or vehicle application additional groups of mice were treated by gavage with carvedilol (10 mg/kg/24 h) or metoprolol-succinat (30 mg/kg/24 h) ($n=10$/group). In agreement with Nishio et al. (2003), the doses were chosen to have nearly equal β_1-adrenoceptor blocking effects for both drugs (Sponer et al., 1987). All drugs were administered for nine consecutive days and measurements were performed on day 10. After recording hemodynamic data, the mice were sacrificed, the hearts removed, and body and heart weighed.

2.3. Surgical procedures and hemodynamic measurements

The animals were anesthetized with thiopental (125 μg/g; ip), intubated and artificially ventilated (200 strokes/min; tidal volume 8 μl/g bodyweight; FiO_2: 21%). A 1.4-F microconductance pressure catheter (ARIA SPR-719, Millar Instruments, Houston, TX, USA) was positioned in the left ventricular via the right carotid artery for continuous registration of left ventricular pressure–volume loops (Georgakopoulos et al., 1998). Calibration of the recorded volume signal was obtained by hypertonic saline (10%) wash-in technique (Steendijk and Baan, 2000). All measurements were performed while ventilation was turned off momentarily.

Indices of systolic and diastolic cardiac performance were derived from ventricular pressure–volume data

Table 1
Hemodynamic effects of carvedilol and metoprolol in myocarditic mice

	Baseline	Infected day 4	Infected day 10	Infected carvedilol day 10	Control carvedilol day 10	Infected metoprolol day 10	Control metoprolol day 10
BW (g)	27.0 ± 0.8	22.5 ± 0.4*	20.5 ± 0.4*	21.0 ± 0.4*	27.8 ± 0.8**,***	20.6 ± 0.5*	26.8 ± 0.8**,***
HW (mg)	128.9 ± 2.6	126.0 ± 3.2	115.3 ± 2.8*	113.6 ± 5.6*	130 ± 2***	116.4 ± 2.5*	132 ± 3***
HW/BW (g/mg)	4.8 ± 0.08	5.6 ± 0.1*	5.6 ± 0.1*	5.4 ± 0.3*	4.6 ± 0.1**,***	5.6 ± 0.1*	4.7 ± 0.2**,***
HR (bpm)	463 ± 12	409 ± 7*	292 ± 7*,**	320 ± 14*,**	402 ± 18*	303 ± 11*,**	410 ± 10*
CI (μl/min/g)	529 ± 35	826 ± 52*	330 ± 26*,**	540 ± 41**,***	485 ± 60	401 ± 42*,**	444 ± 48
ESP (mm Hg)	90.6 ± 3	107.5 ± 3*	59.0 ± 3*,**	73.6 ± 1.3*,**,***	90.2 ± 4**,***	68.3 ± 3*,**	86.8 ± 2**,***
d*P*/d*t* max (mm Hg/s)	7707 ± 358	7942 ± 355	3376 ± 243*,**	5241 ± 325*,**,***	7596 ± 421***	4456 ± 380*,**	6778 ± 498***
Ees (mm Hg/μl)	2.0 ± 0.2	2.3 ± 0.4	1.3 ± 0.1*,**	1.7 ± 0.2*	1.8 ± 0.2	2.1 ± 0.2***	1.9 ± 0.2
PRSW (mm Hg)	79.5 ± 3.8	68.6 ± 15	53.6 ± 3.5	72.0 ± 2.5***	76 ± 10.9***	58.0 ± 6.0	82 ± 6.5***
EF (%)	49.5 ± 3	58.8 ± 3	57.5 ± 2	53.3 ± 3	50 ± 4	54.0 ± 5	55 ± 1.5
SV (μl)	30.4 ± 2.7	33.0 ± 4.4	23.0 ± 1.5*	29.0 ± 3.5	34 ± 2***	26.0 ± 2.5	29.2 ± 3.9***
ESV (μl)	37.1 ± 4.0	24.1 ± 2.1*	17.1 ± 2.0*	29.0 ± 2.2	37 ± 4***	24.2 ± 2.2*	36.1 ± 2.4***
EDV (μl)	68.0 ± 4.9	56.1 ± 4.3*	38.0 ± 3.1*	56.9 ± 4.1*	64 ± 4***	48.0 ± 2.2*	65 ± 5.9***
EDP (mm Hg)	6.3 ± 0.7	6.2 ± 1.2	4.4 ± 0.8	5.4 ± 0.5	6.2 ± 0.9	5.0 ± 0.5	7 ± 1.2
Tau (ms)	9.3 ± 0.2	11.2 ± 0.3*	11.6 ± 0.5*	10.9 ± 0.4*	9.6 ± 0.8***	10.6 ± 0.3*	9.9 ± 1.1***
PHT (ms)	5.4 ± 0.2	6.2 ± 0.3*	6.9 ± 0.5*	6.2 ± 0.2*	5.3 ± 0.3***	6.2 ± 0.2*	5.6 ± 0.4***
d*P*/d*t* min (mm Hg/s)	− 6306 ± 267	− 7144 ± 367*	− 3230 ± 272*,**	− 4503 ± 203*,**	− 6356 ± 366***	− 4051 ± 409**	− 6245 ± 293***
Stiffness constant *b* (μl^{-1})	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.04	0.09 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Ea (mm Hg/μl)	2.8 ± 0.2	3.7 ± 0.7*	2.6 ± 0.1**	2.7 ± 0.3	2.7 ± 0.3	2.7 ± 0.3	3 ± 0.1

Body weight (BW), heart weight (HW), heart rate (HR), cardiac index (CI), end-systolic pressure (ESP), end-systolic elastance (Ees), preload recruitable stroke work (PRSW), ejection fraction (EF), stroke volume (SV), left ventricular end-systolic volume (ESV), left ventricular end-diastolic volume (EDV), end-diastolic pressure (EDP), time constant of isovolumic pressure relaxation (Tau), pressure half-time (PHT), afterload (Ea).

* $P<0.05$ vs. baseline.

** $P<0.05$ vs. day 4.

*** $P<0.05$ vs. untreated day 10.

obtained both at steady state and during transient preload reduction induced by direct occlusion of the inferior abdominal vena cava. Cardiac preload was indexed as left ventricular end-diastolic pressure and as left ventricular end-diastolic volume index. Cardiac afterload was defined as left ventricular end-systolic pressure divided by stroke volume.

Myocardial contractility was quantified by the peak rate of rise in left ventricular pressure (dP/dt max). In addition, left ventricular inotropy was determined by the slope [left ventricular end-systolic elastance (Ees)] of the end-systolic pressure–volume relationship and by the stroke work–end-diastolic volume relation (preload recruitable stroke work), as relative load-independent parameters of systolic function.

Diastolic performance was measured by peak dP/dt min, the time constant of isovolumic pressure relaxation (tau), pressure half-time, and the diastolic stiffness constant b determined from a monoexponential fit to the end-diastolic pressure–volume points (Georgakopoulos et al., 1998).

Furthermore, left ventricular end-systolic volume index, cardiac index, ejection fraction and heart rate were determined by customized software (IOX V 1.5, Emka, France).

2.4. Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was performed by two-way analysis of variance, followed by Student's t-tests. Differences were considered significant with $P < 0.05$.

3. Results

3.1.1. Basal characteristics

Four and 10 days after infection, untreated mice showed a reduction in BW and HW and an increase in BW/HW ratio. No differences in BW or HW were found between untreated and carvedilol or metoprolol treated mice (Table 1).

Time course of hemodynamic function in untreated infected mice.

3.1.2. Day 4

At day 4, the untreated infected mice showed increased systolic function evidenced by significantly increase in Ees, end-systolic pressure, dP/dt max, cardiac index and ejection fraction (Table 1). The value of dP/dt min was more negative; however, this is presumably largely due to the increased afterload. Tau, which is less dependent on afterload, and pressure half-time were significantly prolonged indicating a depressed early relaxation. Parameters describing late diastole, diastolic stiffness and end-diastolic pressure, were unchanged. Heart rate was found to be slightly reduced compared to baseline (Table 1).

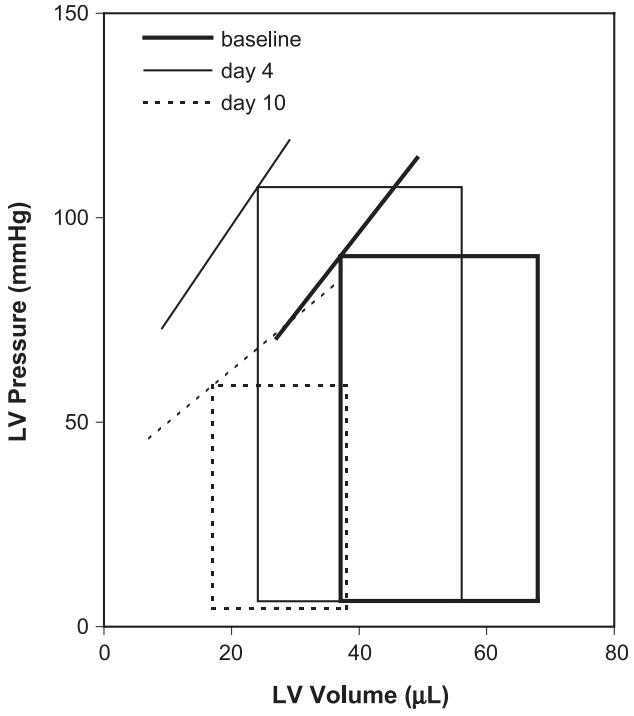

Fig. 1. Schematic left ventricular pressure volume loops (based on average end-systolic pressure, end-systolic volume, end-diastolic pressure and end-diastolic volume) showing the changes in untreated mice 4 and 10 days after induction of myocarditis compared to baseline.

3.1.3. Day 10

Whereas mice were hypercontractile at day 4, left ventricular function was depressed at day 10 (Fig. 1) indicated by a reduction in cardiac index, end-systolic pressure, dP/dt max, Ees, preload recruitable stroke work, stroke volume and a less negative dP/dt min (Table 1). The degree of early diastolic relaxation, characterized by a comparable prolongation of Tau, pressure half-time, did not differ compared to infected mice on day 4. In contrast, although values for end-diastolic pressure were unchanged, analysis of the diastolic pressure–volume relationship (stiffness constant b) resulted in an increase in left ventricular stiffness compared to control animals. Finally, afterload was unchanged compared to baseline but reduced compared to mice infected on day 4. Heart rate was further reduced compared to baseline (Table 1).

3.2. Hemodynamic function of carvedilol versus metoprolol treated mice 10 days after infection

3.2.1. Carvedilol treatment

On day 10, despite a reduction in heart rate, left ventricular function of carvedilol-treated control animals did not differ to untreated controls.

Contractility of carvedilol-treated animals was significantly improved compared to untreated infected mice (Fig. 1), indicated by significant improvements in cardiac index (+63%), end-systolic pressure (+23%), d*P*/d*t* max (+55%), preload recruitable stroke work (+36%) and in d*P*/d*t* min. (+37%) and stroke volume (+26%). Ees also increased by approximately 30%, but this effect did not reach statistical significance (Table 1). Afterload and the degree of diastolic dysfunction (Tau, pressure half-time, stiffness constant *b*) did not differ compared to untreated mice with myocarditis (Table 1).

3.2.2. Metoprolol treatment

Despite a reduction in heart rate, left ventricular function of metoprolol-treated control animals did not differ to untreated controls In infected mice, metoprolol treatment led to no significant improvement in cardiac index (+24%), end-systolic pressure (+15%), d*P*/d*t* max. (+13%) and preload recruitable stroke work (+8%) (Table 1; Fig. 1). However, there was a significant increase in Ees. The decrease in heart rate and the impairment of diastolic function (Tau, pressure half-time, stiffness constant *b*) did not differ compared to untreated or carvedilol-treated mice with myocarditis (Table 1).

4. Discussion

This study demonstrates that acute CVB3-induced myocarditis causes different phases of hemodynamic changes, leading to left ventricular systolic and diastolic dysfunction 10 days after infection. Carvedilol but not metoprolol significantly improves systolic function. The impairment in early diastolic relaxation and the increase in left ventricular stiffness were not affected by the investigated β-adrenoceptor antagonists.

4.1. Four days after infection

Virus infections are known to exhibit stress-like endocrine and neurochemical changes. Similar to findings in murine autoimmune myocarditis (Afanasyeva et al., 2002), untreated animals developed an early hyperdynamic phase 4 days after infection evidenced by increased afterload and increased systolic pressures, reduced systolic and diastolic volumes, and increased cardiac index (Nishio et al., 2002). The increase in cardiac index was due to reduced body weight because stroke volume was unchanged and heart rate was reduced. The finding that stroke volume was maintained despite the increased afterload while end-diastolic volume was not increased clearly indicates improved systolic function. The phenomenon of enhanced contractile performance due to increased afterload, also called homeometric autoregulation may play a role (Alvarez et al., 1999). This causes positive inotropy but also negative lusitropy, leading to an impaired diastolic relaxation as also indicated in our model. In agreement with others (Herzum et al., 1995), we found a significant decrease in heart rate, which may be baroreceptor mediated. Thus, given the known heart rate dependence of Tau and pressure half-time, the impairment in early diastolic relaxation may be partly also explained as secondary to the reduction in heart rate.

However, it has been shown that mice with viral myocarditis display an injury of myocardial structural proteins already 1 day after infection (Shioi et al., 1996). Thus, the changes may be directly related to injury of myocytes including pacemaker cells. Negative lusitropy may be caused also by viral activity or by immune mediators, including CVB3-induced cytokines. This would be in agreement with findings showing that proinflammatory cytokines like interleukin 1β and tumor necrosis factor α can cause an impairment of the sarco/endoplasmic reticulum Ca^{2+} ATPase, which regulates particularly diastolic relaxation (Yokoyama et al., 1999). Thus, several mechanisms could be involved in the hemodynamic changes found in the early period after induction of viral myocarditis.

4.2. Ten days after infection

Ten days after infection, left ventricular function of untreated animals was depressed compared to baseline and to mice 4 days after infection, characterized by a reduction in cardiac index, left ventricular contractility and systolic pressure (Fig. 2). After the acute phase of myocarditis, there are still direct cytotoxic virological effects causing myocyte necrosis with consecutive reparative fibrosis (Li et al., 2002). The host immune response may damage tissue by protective removal of virus-infected myocytes or cause cardiac injury, mainly by sensitized T-lymphocytes. This is characterized by focal myocytolysis, necrotic myocardial foci with infiltration of immune cells and the existence of interstitial edema leading to left ventricular depression already 1 week after viral myocarditis and in addition, impaired contractility may also be caused indirectly by negative inotropic effects of cytokines and nitric oxide (Huber and Pfaeffle, 1994). In contrast to the described changes in systolic function, we found no progression in the impairment of early diastolic relaxation, despite a further decrease in heart rate. However, left ventricular stiffness raised, which is related to cardiac extracellular remodeling, characterized by changes in metalloproteinases activity and their inhibitors (Li et al., 2002). Consistent with Huber (1997), also using CBV-infected BALB/c mice, no chamber dilatation was observed.

4.3. Therapeutic effects of metoprolol and carvedilol 10 days after infection

Already mild cardiac failure is associated with a reduction in myocardial β-adrenoceptor density. Similarly, in experimental murine myocarditis, a downregulation of

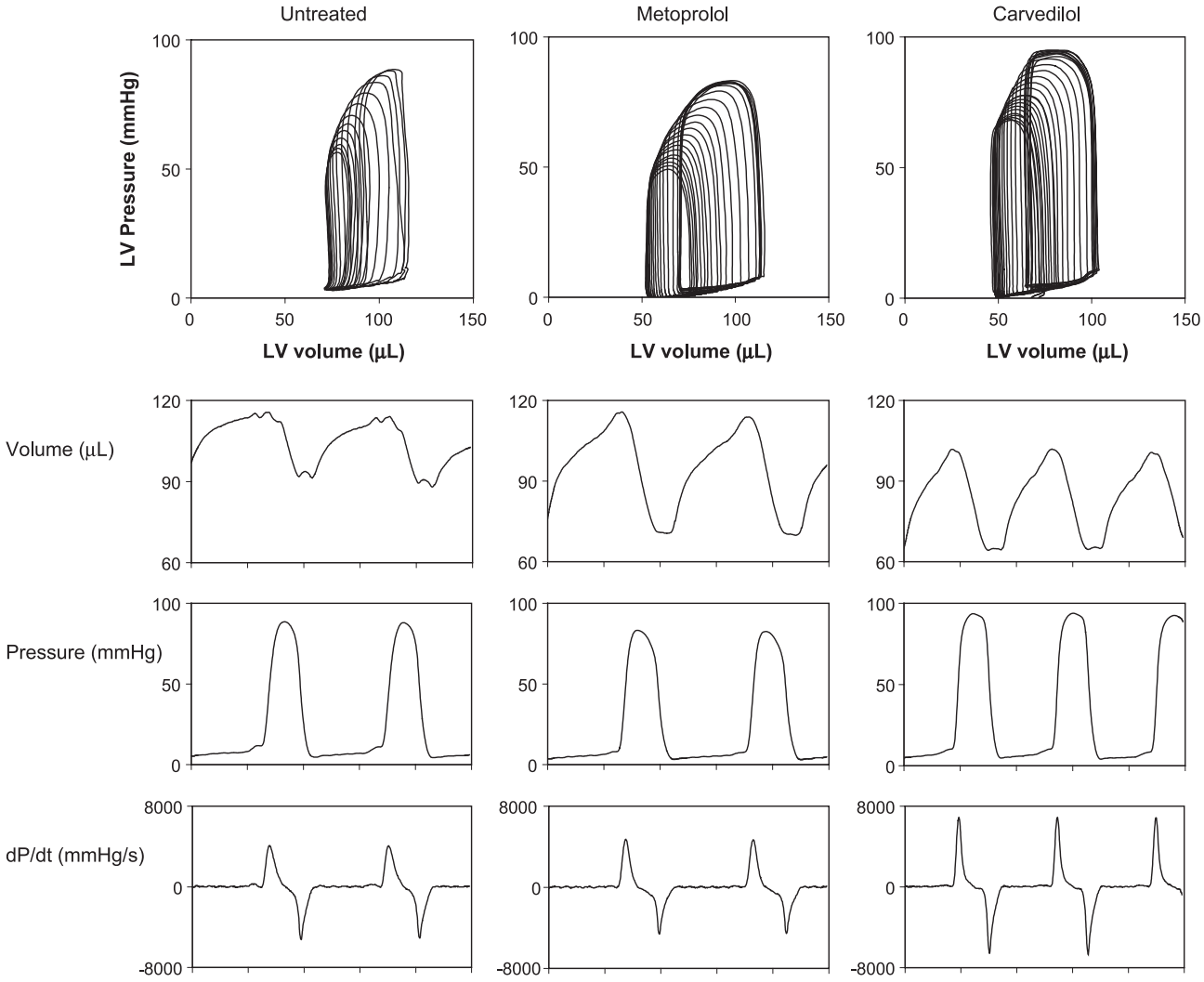

Fig. 2. Representative pressure–volume loops, left ventricular volume, left ventricular pressure and d*P*/d*t* curves in untreated, metoprolol-treated and carvedilol-treated mice 10 days after infection. Tick marks on the axes indicate 100 ms.

myocardial β_1-adrenoceptor density has been found on day 10 after infection, correlating with decrease in heart rate, followed by an increase in β_2-adrenoceptor density (Kanda et al., 1994). Hemodynamic consequences of the dynamics in β-adrenoceptor regulation in acute murine myocarditis by an intervention with the selective β_1-adrenoceptor antagonist metoprolol and the nonselective adrenoceptor antagonist carvedilol had not been studied before. In contrast to metoprolol, carvedilol also leads to additional β_2- and α-adrenoceptor blockade and has antioxidative properties. Despite theses differences, both drugs were very effective in reducing mortality in patients with chronic heart failure, although the COMET study revealed an improvement of carvedilol by comparison with metoprolol in patients with chronic heart failure (Poole-Wilson et al., 2003).

Investigating the effect of β-adrenoceptor antagonists in myocarditis, Popovic et al. (1998) reported an improvement in the acute left ventricular hemodynamic effects of metoprolol in myocarditis patients. Long-term consequences, however, have not been studied. In our study, using equivalent doses of carvedilol and metoprolol, cardiac index, systolic pressures, preload recruitable stroke work and d*P*/d*t* max were increased only in the carvedilol-treated group compared to untreated animals 10 days after infection. As an exception, Ees was increased more with metoprolol. However, previous studies indicate that preload recruitable stroke work is a more consistent indicator of changes in the inotropic state than Ees (Kass et al., 1987). Thus, taking into account the effects on the various parameters, we conclude that carvedilol has a greater therapeutic benefit than metoprolol in our model.

Progression of cardiac failure in our model may depend not only on β_1-adrenoceptor stimulation but also on adrenergic stimulation of β_2- and/or α-adrenoceptors for the progression of cardiac failure in our model. The α-adrenoceptor blocking effect of carvedilol causes vasodilation and a reduction in afterload. However, because afterload did not differ in the two β-adrenoceptor antagonist-treated groups,

this may indicate that α-adrenoceptor-dependent hemodynamic changes are not dominant in our model.

What could be the mechanisms by which carvedilol but not metoprolol had the greatest therapeutic benefit in our model? It is suggested, that the antioxidant properties of carvedilol contributes to a reduction in stress-activated protein kinases and polymorphonuclear neutrophil granulocytes induced apoptosis (Feuerstein, 2001). Similar findings are reported by Nishio et al. (2003) in murine myocarditis. Furthermore, carvedilol, but not metoprolol induced the production of antiviral cytokines like interleukin 12 and interferone β, which is controlled by the β_2-adrenoceptor pathway (Panina-Bordignon et al., 1997). Thus, it is reasonable to suggest that carvedilol may not only provide more comprehensive protection against adrenergic overstimulation than metoprolol but may also activate endogenous cell-mediated immunity and host defense during viral infection, preventing the progressive loss of myocardial cells and the rise in myocardial fibrosis, as also shown already in other models of experimental myocarditis (Watanabe et al., 2002). In conclusion, the pleiotropic effects of carvedilol are of decisive importance especially in viral myocarditis and may explain its superiority over metoprolol.

The role of adrenoceptor blockade is controversially discussed with respect to diastolic dysfunction. Some authors report an improvement in diastolic function in patients with dilated cardiomyopathy after treatment with different β-adrenoceptor antagonists including metoprolol (Andersson et al., 1996; Eichhorn et al., 1990). However, others found no improvement in Tau, peak filling rate or the time to peak filling rate after chronic treatment with carvedilol or metoprolol. In our model, both β-adrenoceptor antagonists did not affect diastolic dysfunction. Thus, diastolic abnormalities in murine CBV-induced myocarditis are caused by mechanisms that may differ from those leading to systolic dysfunction and are resistant to adrenoceptor blockade at the investigated time window.

In conclusion, our study indicates that carvedilol has a greater therapeutic benefit than metoprolol in murine CBV3-induced myocarditis. Thus, not only β_1-adrenoceptor blockade but also pleiotropic properties are involved in the cardioprotective effects of carvedilol on the pathophysiology of acute viral myocarditis.

References

Afanasyeva, M.R.N., 2002. Immune mediators in inflammatory heart disease: insights from a mouse model. Eur. Heart J. 2002, I31–I36.

Alvarez, B.V., Perez, N.G., Ennis, I.L., Camilion de Hurtado, M.C., Cingolani, H.E., 1999. Mechanisms underlying the increase in force and Ca(2+) transient that follow stretch of cardiac muscle: a possible explanation of the Anrep effect. Circ. Res. 85, 716–722.

Andersson, B., Caidahl, K., di Lenarda, A., Warren, S.E., Goss, F., Waldenstrom, A., Persson, S., Wallentin, I., Hjalmarson, A., Waagstein, F., 1996. Changes in early and late diastolic filling patterns induced by long-term adrenergic beta-blockade in patients with idiopathic dilated cardiomyopathy. Circulation 94, 673–682.

Bohm, M., Maack, C., 2000. Treatment of heart failure with beta-blockers. Mechanisms and results. Basic Res. Cardiol. 95 (Suppl 1), I15–I24.

Bristow, M.R., 2000. What type of beta-blocker should be used to treat chronic heart failure? Circulation 102, 484–486.

Eichhorn, E.J., Bristow, M.R., 2001. The Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) trial. Curr. Control. Trials, Cardiovasc. Med. 2, 20–23.

Eichhorn, E.J., Bedotto, J.B., Malloy, C.R., Hatfield, B.A., Deitchman, D., Brown, M., Willard, J.E., Grayburn, P.A., 1990. Effect of beta-adrenergic blockade on myocardial function and energetics in congestive heart failure. Improvements in hemodynamic, contractile, and diastolic performance with bucindolol. Circulation 82, 473–483.

Feuerstein, G.Z., 2001. Apoptosis—new opportunities for novel therapeutics for heart diseases. Cardiovasc. Drugs Ther. 15, 547–551.

Georgakopoulos, D., Mitzner, W.A., Chen, C.H., Byrne, B.J., Millar, H.D., Hare, J.M., Kass, D.A., 1998. In vivo murine left ventricular pressure–volume relations by miniaturized conductance micromanometry. Am. J. Physiol. 274, H1416–H1422.

Herzum, M., Weller, R., Jomaa, H., Wietrzychowski, F., Pankuweit, S., Mahr, P., Maisch, B., 1995. Left ventricular hemodynamic parameters in the course of acute experimental coxsackievirus B3 myocarditis. J. Mol. Cell. Cardiol. 27, 1573–1580.

Huber, S.A., 1997. Coxsackievirus-induced myocarditis is dependent on distinct immunopathogenic responses in different strains of mice. Lab. Invest. 76, 691–701.

Huber, S.A., Pfaeffle, B., 1994. Differential Th1 and Th2 cell responses in male and female BALB/c mice infected with coxsackievirus group B type 3. J. Virol. 68, 5126–5132.

Huber, S.A., Polgar, J., Schultheiss, P., Schwimmbeck, P., 1994. Augmentation of pathogenesis of coxsackievirus B3 infections in mice by exogenous administration of interleukin-1 and interleukin-2. J. Virol. 68, 195–206.

Kanda, T., Adachi, H., Ohno, T., Suzuki, T., Murata, K., 1994. Myocardial beta-receptor and cardiac angiotensin alterations during the acute and chronic phases of viral myocarditis. Eur. Heart J. 15, 686–690.

Kass, D.A., Maughan, W.L., Guo, Z.M., Kono, A., Sunagawa, K., Sagawa, K., 1987. Comparative influence of load versus inotropic states on indexes of ventricular contractility: experimental and theoretical analysis based on pressure–volume relationships. Circulation 76, 1422–1436.

Li, J., Schwimmbeck, P.L., Tschope, C., Leschka, S., Husmann, L., Rutschow, S., Reichenbach, F., Noutsias, M., Kobalz, U., Poller, W., Spillmann, F., Zeichhardt, H., Schultheiss, H.P., Pauschinger, M., 2002. Collagen degradation in a murine myocarditis model: relevance of matrix metalloproteinase in association with inflammatory induction. Cardiovasc. Res. 56, 235–247.

MERIT-HF-Group, 1999. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 353, 2001–2007.

Nishio, R., Sasayama, S., Matsumori, A., 2002. Left ventricular pressure–volume relationship in a murine model of congestive heart failure due to acute viral myocarditis. J. Am. Coll. Cardiol. 40, 1506–1514.

Nishio, R., Shioi, T., Sasayama, S., Matsumori, A., 2003. Carvedilol increases the production of interleukin-12 and interferon-gamma and improves the survival of mice infected with the encephalomyocarditis virus. J. Am. Coll. Cardiol. 41, 340–345.

Panina-Bordignon, P., Mazzeo, D., Lucia, P.D., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C., Sinigaglia, F., 1997. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. J. Clin. Invest. 100, 1513–1519.

Pauschinger, M., Bowles, N.E., Fuentes-Garcia, F.J., Pham, V., Kuhl, U., Schwimmbeck, P.L., Schultheiss, H.P., Towbin, J.A., 1999. Detection of adenoviral genome in the myocardium of adult patients with idiopathic left ventricular dysfunction. Circulation 99, 1348–1354.

Poole-Wilson, P.A., Swedberg, K., Cleland, J.G., Di Lenarda, A., Han-

rath, P., Komajda, M., Lubsen, J., Lutiger, B., Metra, M., Remme, W.J., Torp-Pedersen, C., Scherhag, A., Skene, A., 2003. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. Lancet 362, 7–13.

Popovic, Z., Miric, M., Vasiljevic, J., Sagic, D., Bojic, M., Popovic, A.D., 1998. Acute hemodynamic effects of metoprolol ± nitroglycerin in patients with biopsy-proven lymphocytic myocarditis. Am. J. Cardiol. 81, 801–804.

Seong, I.W., Choe, S.C., Jeon, E.S., 2001. Fulminant coxsackieviral myocarditis. N. Engl. J. Med. 345, 379.

Shioi, T., Matsumori, A., Sasayama, S., 1996. Persistent expression of cytokine in the chronic stage of viral myocarditis in mice. Circulation 94, 2930–2937.

Sponer, G., Bartsch, W., Strein, K., Muller-Beckmann, B., Bohm, E., 1987. Pharmacological profile of carvedilol as a beta-blocking agent with vasodilating and hypotensive properties. J. Cardiovasc. Pharmacol. 9, 317–327.

Steendijk, P., Baan, J., 2000. Comparison of intravenous and pulmonary artery injections of hypertonic saline for the assessment of conductance catheter parallel conductance. Cardiovasc. Res. 46, 82–89.

Watanabe, K., Takahashi, T., Nakazawa, M., Wahed, M.I., Fuse, K., Tanabe, N., Kodama, M., Aizawa, Y., Ashino, H., Tazawa, S., 2002. Effects of carvedilol on cardiac function and cardiac adrenergic neuronal damage in rats with dilated cardiomyopathy. J. Nucl. Med. 43, 531–535.

Yokoyama, T., Arai, M., Sekiguchi, K., Tanaka, T., Kanda, T., Suzuki, T., Nagai, R., 1999. Tumor necrosis factor-alpha decreases the phosphorylation levels of phospholamban and troponin I in spontaneously beating rat neonatal cardiac myocytes. J. Mol. Cell. Cardiol. 31, 261–273.

Yue, T.L., Cheng, H.Y., Lysko, P.G., McKenna, P.J., Feuerstein, R., Gu, J.L., Lysko, K.A., Davis, L.L., Feuerstein, G., 1992. Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J. Pharmacol. Exp. Ther. 263, 92–98.