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Influence of Enalapril Therapy Schedule on the Progression of the Disease in Dilated Cardiomyopathic Syrian Hamsters (Bio TO-2 strain)

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Abstract

The influence of enalapril therapy schedule on the progression of the disease in terms of hemodynamics, cardiac remodeling and survival was assessed in cardiomyopathic Syrian hamsters (CMHs). CMHs (Bio TO-2 dilated strain) were treated orally with enalapril at 20 mg/kg/day from 60 to 200 (early treatment), from 120 to 200 (late treatment) or 120 to 250 (prolonged treatment) days of age. In survival studies, CMHs were treated until 90% of controls are death. Early, late and prolonged treatments significantly decreased mean arterial blood pressure (-23%, -27% and -22%, respectively), but had no effects on cardiac output and stroke volume. Late and prolonged treatments significantly decreased total peripheral resistance (-38% and -24%, respectively), but not early treatment. Only, prolonged treatment significantly decreased left ventricle (LV) end diastolic blood pressure (-60%). Early, late and prolonged treatments significantly decreased LV collagen density (-44%, -31% and -54%, respectively) and LV cavity area (-26%, -21% and -29%, respectively). Survival was significantly improved when enalapril was administered from 120 days of age, but not significantly when administered earlier. In conclusion, enalapril exerted beneficial effects (improvement of cardiac function and prolonged survival) more marked when the treatment was begun late in the evolution of congestive heart failure.

Keywords: Therapy schedule; Enalapril; Cardiac hemodynamics; Systemic hemodynamics; Cardiac remodeling; Cardiomyopathic hamsters; Survival

Introduction

Animal models remain widely used to improve the understanding of the pathophysiology of congestive heart failure (CHF) and to evaluate new drugs or new therapeutic strategies in this area. None of the available models reproduce completely the progression of the natural disease observed in human because each model has its own limitations and interest. Among them, the model of cardiomyopathic Syrian hamsters has been used for many years as an animal model of CHF [1,2]. Two groups of strains were successively derived from the initial polymyopathic line Bio 1.50. The first group (e.g. Bio 14.6, UM-X7.1, CHF 146 and CHF 147 strain) is mainly characterized by a significant hypertrophy of the cardiac muscle whereas the second group (e.g. Bio 53.58 and Bio TO-2 strain) shows a wide dilation of ventricles without wall hypertrophy [1,3]. The cardiomyopathic hamsters do not show any clinical or histological signs of the disease before 30 days of age. Three successive phases are then generally observed during the disease: 1) a phase of necrosis which extends usually from 30 to 120 days of age, followed by 2) a phase of healing with fibrosis, moderate cardiac hypertrophy and/or dilation between 120 and 250 days of age and, 3) a terminal phase of heart failure [1]. Because the dilated strain of Syrian cardiomyopathic hamsters mimics many clinical features of dilated cardiomyopathy in humans, typically exhibiting alteration of the cardiac function associated with a complex process of cardiac remodeling [4], this model is widely used for pharmacological studies [5-7].

The efficacy of angiotensin I-converting enzyme (ACE) inhibitors in the treatment of chronic CHF is well documented and ACE inhibitors have become an integral component of the treatment of CHF. Indeed, ACE inhibitors have been demonstrated to improve cardiac function and reduce morbidity and mortality in CHF patients [8-11]. Such beneficial effects have also been reported in experimental models of CHF [12-16]. Although the treatment is often administered soon after myocardial infarction in the model of coronary artery ligation in the rat [17], the most appropriate time for starting the treatment is not clearly defined in the model of dilated cardiomyopathy in the hamster which develops gradually the disease over time. Indeed, in the works published on this animal model, the choice of starting treatment during the disease, as well as the doses regimen and the duration of the treatment vary widely among authors.

The aim of the present work was to evaluate the influence of the therapy schedule on the progression of the disease in cardiomyopathic Syrian hamsters (Bio TO-2 dilated strain). This was undertaken by using enalapril, an ACE inhibitor for which the effects are well established in the context of CHF. Enalapril was given at a fixed dose of 20 mg/kg/day, once-daily, oral administration. In the first experiment, treatment with enalapril was initiated early during the course of the disease (early treatment), i.e. starting at the age of 60 days (stage of necrosis) up to the age of 200 days, i.e. for 140 days. In the second and the third experiments, treatment with enalapril was initiated later, i.e. starting at the age of 120 days (phase of healing) up to the age of 200 (late treatment) or 250 (prolonged

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treatment) days, i.e. for 80 or 130 days, respectively. Cardiac and systemic hemodynamic parameters, cardiac remodeling and survival were investigated.

Materials and Methods

Animals

Male cardiomyopathic hamsters (Bio TO-2 dilated strain), 69 - 95 g body weight range and 60 day-old at the beginning of treatment (early treatment), 80 - 132 g body weight range and 120 day-old at the beginning of treatment (late and prolonged treatment) were used for hemodynamic and histomorphometric evaluation and survival study. They were supplied by Bio Breeders Inc., Fitchburg, MA, U.S.A. No healthy-control animals were included in this study whose aim was primarily to assess the influence of enalapril therapy schedule in the process of heart failure. All animal interventions were performed in accordance with the European Directive number 86/609, and the rules of the "Guide for the Care and Use of Laboratory Animals", 7th edition, 1996, Institute for Laboratory Animal Research (ILAR), Washington, DC.

Animals were delivered to the laboratory at least 5 days before the first day of administration, during which time they were acclimatized to the laboratory conditions. They were housed in groups of 4 in macrolon cages on wood litter with free access to food (code 113 - SAFE, 89290 Augy, France) and water until tested. The animal house was maintained under artificial lighting (12 hours light/dark cycle). Ambient temperature was 21 ± 3°C, and relative humidity ranged from 30 - 80%. Animals were weighed every week to allow for adjustment of the quantity of substance administered (treatment period) and immediately before being anesthetized (hemodynamic evaluation). The surviving hamsters were sacrificed at the end of the survival study by exposure to a mixture of O_2/CO_2 (20%/80%) followed by CO_2.

Treatment

Enalapril was administered orally by gastric gavage every morning at 20 mg/kg. Enalapril was dissolved in distilled water and administered as 5 ml/kg body weight. The control group was administered 5 ml/kg body weight of distilled water by gastric gavage. Enalapril was obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).

Three sub-experiments were performed in this study: evaluation of an early, late and prolonged treatment. The early treatment was started at 60 days of age and the variables of interest were measured at 200 days of age. The late treatment was started at 120 days of age and the variables of interest were measured at 200 days of age. The prolonged treatment was started at 120 days of age and the variables of interest were measured at 250 days of age.

Hemodynamic evaluation

Cardiomyopathic hamsters were anesthetized (ketamine 200 mg/kg i.p. and xylazine 5 mg/kg i.p.), and placed under artificial respiration. Mean, systolic and diastolic aortic blood pressure (MAP, SAP and DAP, mmHg), heart rate (HR, bpm), were monitored via a 2F micromanometer-tipped catheter introduced into the right carotid artery and advanced into the aorta. Left ventricular pressure (LVP, mmHg), was monitored via a 2F micromanometer-tipped catheter introduced into the right carotid artery and advanced into the left ventricle. Left ventricular end diastolic blood pressure (LVEDP, mmHg) was calculated from LVP measurements. Cardiac output (CO, ml/min), was measured via a perivascular flow probe placed around the abdominal aorta (just below the diaphragm). Total peripheral resistance (TPRes, mmHg/min/ml), stroke volume (SV, ml, = CO (ml/min)/HR (bpm), and $+dP/dt_{max}$, the first derivative of LVP (mmHg/s), were calculated from the measurements mentioned above. The parameters to be measured were allowed to stabilize for a period of at least 10 minutes.

Histomorphometric evaluation

After the recording of hemodynamic parameters, the animals were sacrificed. Heart, lungs and liver were rapidly removed, rinsed and blotted dry. The heart was divided into atria, left ventricle (including the septum) and right ventricle. These organs were immediately weighed (mg). Each organ weight was normalized by body weight to estimate cardiac hypertrophy and CHF of target organs (mg/g body weight). The left ventricle was immediately immersed into 10% formaldehyde solution. A few days later, the tissue sample was prepared for light microscopy. The tissue sample was cut perpendicularly to the apex-to-base axis into two sections. The sample was then dehydrated and embedded in paraffin. Each specimen was cut into 3-μm-thick slices and subjected to Sirius red.

Histomorphometric analyses were performed using a computer-assisted image analyzer (Histolab and Saisam, Microvision Instruments, Evry, France) as previously described [4,5]. Left ventricle dilation was estimated by measuring left ventricle cavity area (mm^2). Cardiac collagen density was evaluated in the subepicardial region of the left ventricle on Sirius red-stained slides. For each field, collagen density was expressed as the ratio of collagen area to field area (%). Perivascular collagen was excluded from these measures. Left ventricle wall thickness was also assessed. For each parameter, fifteen measures per slide were performed to determine a mean value.

Survival evaluation

In the survival studies, the hamsters were treated from Day 60 (early treatment) or from Day 120 (late treatment). For the therapy initiated from 120 days of age, two survival evaluations were performed (the first in the context of the "late treatment" sub-experiment and the second in the context of the "prolonged treatment" sub-experiment). The hamsters are treated until 90% of control hamsters are death and did not undergo any investigation.

Statistics

Results are means ± s.e.m. of 8 to 12 (hemodynamic evaluation), 11 to 12 (histomorphometric evaluation) and 30 (survival study) animals per group. All statistical calculations were performed using commercial software (Microsoft® Excel and GB-Stat version 6.5). All differences were considered statistically significant when the null hypothesis could be rejected at a risk α of less than 0.05. Inter-group comparison was performed using a one-way analysis of variance (ANOVA) with group as factor. Cumulative survival distributions for the two groups were constructed using the Kaplan-Meier method, and differences between survival curves were globally tested for significance with the log rank test.

Results

Hemodynamic evaluation

During the hemodynamic and histomorphometry study (early treatment), one enalapril-treated hamster spontaneously died before the end of the treatment period (at 115 days of age).

	Early treatment from 60 to 200 days of age				Late treatment from 120 to 200 days of age				Prolonged treatment from 120 to 250 days of age		
	Vehicle	Enalapril 20 mg/kg/day	(1)		Vehicle	Enalapril 20 mg/kg/day	(1)		Vehicle	Enalapril 20 mg/kg/day	(1)
MAP (mmHg)	52 ± 2	40 ± 2	**		55 ± 2	40 ± 1	***		49 ± 1	38 ± 2	***
SAP (mmHg)	68 ± 1	61 ± 3	*		72 ± 2	61 ± 2	***		63 ± 1	56 ± 2	**
DAP (mmHg)	44 ± 2	30 ± 2	***		48 ± 2	30 ± 2	***		42 ± 1	30 ± 2	***
HR (bpm)	180 ± 9	157 8	NS		197 ± 7	180 ± 4	*		193 ± 5	186 ± 9	NS
CO (ml/min)	9.9 ± 0.8	8.2 0.6	NS		5.7 ± 0.7	6.2 ± 0.5	NS		9.8 ± 0.4	10.0 ±0.5	NS
SV (ml)	0.056 ± 0.005	0.054 0.004	NS		0.029 ± 0.003	0.034 ± 0.002	NS		0.051 ± 0.003	0.056 0.004	NS
TPRes (mmHg/min/ml)	5.6 ± 0.6	5.0 ± 0.3	NS		10.8 ± 1.6	6.7 ± 0.4	*		5.1 ± 0.3	3.9 ± 0.3	**

MAP : Mean Aortic Blood Pressure ; SAP : Systolic Aortic Blood Pressure ; DAP : Diastolic Aortic Blood Pressure ; HR : Heart Rate ; CO : Cardiac Output ; SV : Stroke Volume ; TPRes : Total Peripheral Resistance.
Data are means ± s.e.m.
(1): **One-way ANOVA** (with group as factor): NS = Not Significant ; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 1: Effects of enalapril (20 mg/kg/day) on mean, systolic and diastolic aortic blood pressure, heart rate, cardiac output, stroke volume and total peripheral resistances in cardiomyopathic hamsters (n = 8 to 12 per group).

	Early treatment from 60 to 200 days of age				Late treatment from 120 to 200 days of age				Prolonged treatment from 120 to 250 days of age		
	Vehicle	Enalapril 20 mg/kg/day	(1)		Vehicle	Enalapril 20 mg/kg/day	(1)		Vehicle	Enalapril 20 mg/kg/day	(1)
BW (g)	110 ± 7	110 ± 3	NS		120 ± 3	111 ± 4	NS		122 ± 3	101 ± 3	***
AW/BW (mg/g)	0.35 ± 0.04	0.28 ± 0.03	NS		0.27 ± 0.02	0.21 ± 0.01	*		0.44 ± 0.03	0.29 ± 0.01	***
LVW/BW (mg/g)	2.54 ± 0.16	2.34 ± 0.03	NS		2.47 ± 0.07	2.37 ± 0.09	NS		2.55 ± 0.04	2.53 ± 0.03	NS
RVW/BW (mg/g)	0.67 ± 0.06	0.61 ± 0.03	NS		0.53 ± 0.02	0.56 ± 0.04	NS		0.68 ± 0.03	0.64 ± 0.01	NS
LiW/BW (mg/g)	31.05 ± 0.67	30.26 ± 0.74	NS		39.57 ± 1.81	36.68 ± 1.48	NS		28.69 ± 0.45	30.32 ± 0.63	*
LuW/BW (mg/g)	5.82 ± 0.43	6.18 ± 0.40	NS		4.42 ± 0.08	4.48 ± 0.12	NS		5.57 ± 0.31	5.20 ± 0.17	NS

BW : Body Weight ; AW : Atria Weight ; LVW : Left Ventricle Weight ; RVW : Right Ventricle Weight ; LiW : Liver Weight ; LuW : Lungs Weight.
Data are means ± s.e.m.
(1): **One-way ANOVA** (with group as factor): NS = Not Significant ; * = $p < 0.05$; *** = $p < 0.001$.

Table 2: Effects of enalapril (20 mg/kg) on body weight and atria, left ventricle, right ventricle, liver and lungs normalized weights in the cardiomyopathic hamsters (n = 10 to 12 per group).

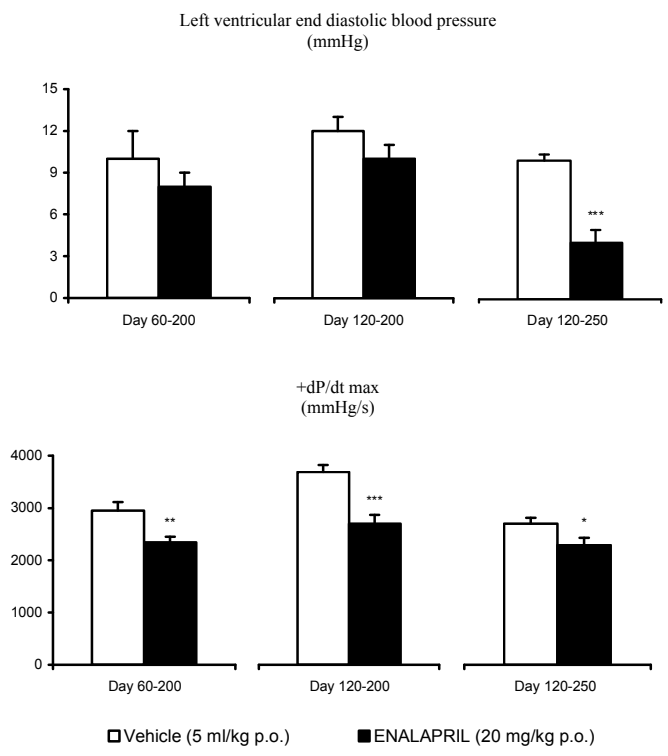

Figure 1: Effects of enalapril (20 mg/kg/day) on left ventricular end diastolic blood pressure and maximum positive dP/dt in cardiomyopathic Bio TO-2 dilated hamsters. Early treatment was administered from 60 to 200 days of age, late treatment from 120 to 200 days of age and prolonged treatment from 120 to 250 days of age. Mean ± s.e.m. (n = 10 to 12). Significantly different from corresponding control values using ANOVA (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Cardiac hemodynamics: Early and late enalapril treatment slightly decreased LVEDP and $+dP/dt_{max}$, but the effect reached statistical significance only for $+dP/dt_{max}$ (LVEDP: -20% and -17%, NS for each and $+dP/dt_{max}$: -21%, $p < 0.01$ and -27%, $p < 0.001$, respectively). In contrast, prolonged treatment significantly decreased LVEDP (-60%, $p < 0.001$) and $+dP/dt_{max}$ (-15%, $p < 0.05$) (Figure 1).

Systemic hemodynamics: Early, late and prolonged enalapril treatment significantly decreased SAP, DAP and MAP of the same amplitude (MAP: -23%, $p < 0.01$, -27%, $p < 0.001$ and -22%, $p < 0.001$, respectively). Late enalapril treatment also significantly decreased HR (-9%, $p < 0.05$) whereas early or prolonged enalapril treatment did not significantly modify this variable (Table 1). It should be noted that the arterial blood pressure of the vehicle control group was relatively low (between 49 and 55 mmHg) but similar to the previously recorded blood pressure in this model with the same (ketamine/xylazine) or other anesthetics [7,18]. However, although the blood pressure of the control animals can be considered low, a significant effect of enalapril was clearly detected. This relatively low pressure is related to this model because in normal control hamsters evaluated under the same experimental conditions, the arterial blood pressure is higher (about 70 mmHg, internal data not shown).

Early enalapril treatment had no statistically significant effects on CO, SV and TPRes. In contrast, late and prolonged enalapril treatment significantly decreased TPRes (-38%, $p < 0.05$ and -24%, $p < 0.01$), but had no significant effects on CO and SV (Table 1). These effects are suggestive of a slight improvement of systemic hemodynamics and reflect a systemic vasodilation when the treatment was initiated from 120 days of age.

Morphometry

Early enalapril treatment had no significant effects on body weight, left ventricle, right ventricle, liver and lungs normalized

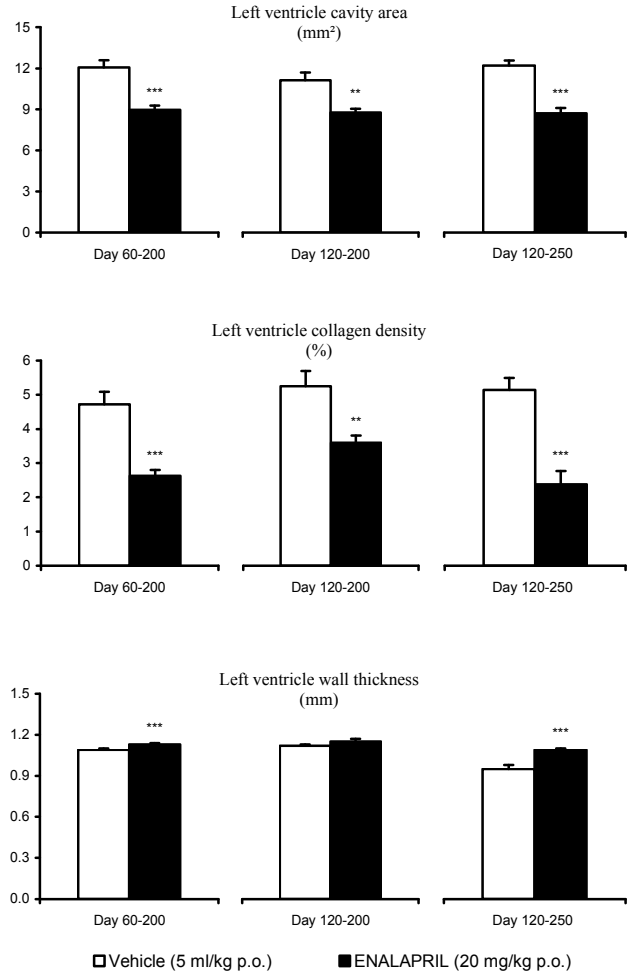

Figure 2: Effects of enalapril (20 mg/kg/day) on left ventricle cavity area, left ventricle collagen density and left ventricle wall thickness in cardiomyopathic Bio TO-2 dilated hamsters. Early treatment was administered from 60 to 200 days of age, late treatment from 120 to 200 days of age and prolonged treatment from 120 to 250 days of age. Mean ± s.e.m. (n = 11 to 12). Significantly different from corresponding control values using ANOVA (** = $p < 0.01$, *** = $p < 0.001$).

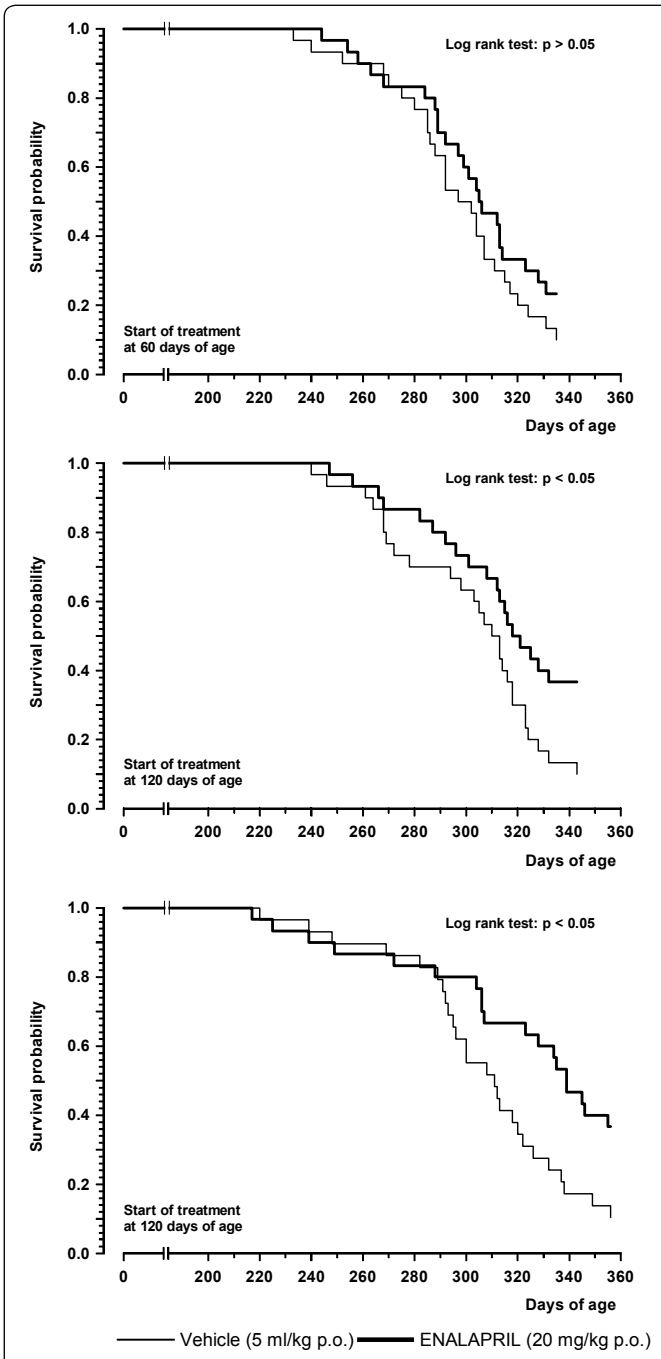

Figure 3: Effects of enalapril (20 mg/kg/day) on survival probability in cardiomyopathic Bio TO-2 dilated hamsters. Early treatment was administered from 60 days of age and late treatment was administered from 120 days of age. Mean ± s.e.m. (n = 30). Significantly different from corresponding control values using Log rank test (* = $p < 0.05$).

weights, a slight atria weight decrease was observed (-20%, NS) (Table 2). In contrast, late and prolonged enalapril treatment decreased body weight (-8%, NS and -17%, $p < 0.001$, respectively) and atria weight (-22%, $p < 0.05$ and -34%, $p < 0.001$, respectively), but had no effects on the other target organ weights although a slight liver weight increase was observed in the prolonged enalapril-treated group (+6%, $p < 0.05$) (Table 2). This slight increase of the normalized liver weight was mainly due to the fact that the body weight of the hamsters subjected to prolonged treatment with enalapril was markedly decreased. Indeed, if one considers only the weight of the liver not normalized by the body weight, it appears that enalapril had no substantial effects on the liver weight (3052 ± 85 mg in the enalapril treated group versus 3512 ± 126 mg in the control group).

Histomorphometric evaluation

Early, late and prolonged enalapril treatment significantly decreased left ventricle collagen density (-44%, $p < 0.001$, -31%, $p < 0.01$ and -54%, $p < 0.001$, respectively) and left ventricle cavity area (-26%, $p < 0.001$, -21%, $p < 0.01$ and -29%, $p < 0.001$, respectively) (Figure 2). These decreases were approximately of the same amplitude in the three experiments. Early, late and prolonged enalapril treatment also very slightly increased left ventricle wall thickness but the effect reached statistical significance for only the early and prolonged treatment (+0.04 mm, i.e. +4%, $p < 0.001$, +0.03 mm, i.e. +3%, NS and +0.14 mm, i.e. +4%, $p < 0.001$, respectively) (Figure 2). Nevertheless, it is to be noted that for the prolonged treatment, the statistical significance could be partly due to the lower left ventricle wall thickness measured in the control group. These effects

(mainly on collagen density and dilation decrease) are indicative of the cardiac remodeling improvement by enalapril whatever the stage of substance administration.

Survival evaluation

(Figure 3) shows the cumulative survival distributions for cardiomyopathic hamsters treated with vehicle and enalapril (20 mg/kg/day) from 60 or 120 days of age using the Kaplan-Meier method. When administered from 60 days of age (early treatment), enalapril had no significant effects on life expectancy and median survival time as compared with vehicle control group (upper panel). In contrast, when administered later (120 days of age), enalapril significantly improved survival as compared with vehicle control group (log rank test, $p < 0.05$ for each experiment). Indeed, as regards the treatment initiated from 120 days of age, two estimates of survival were performed: one for the late treatment (middle panel) and the second for the prolonged treatment (bottom panel) Nevertheless, it is to be noted that a slight mortality appeared earlier in the third experiment (bottom panel). Indeed, up to 290 days of age, mortality was similar between vehicle and enalapril-treated groups. Then, the two curves diverged, thereby confirming the beneficial effect of enalapril. In the previous experiment (middle panel), the beneficial effects of enalapril appeared 20 days earlier. In addition, enalapril significantly increased the median survival time as compared with vehicle control group, in the third experiment (+30 days, $p < 0.05$). The late treatment with enalapril modified survival of cardiomyopathic hamsters to an extent greater than when enalapril treatment was initiated earlier in the course of the disease. Indeed, survival improvement was approximately of the same amplitude in the two sub-experiments where treatment was started at 120 days of age (+379 and +441 days, respectively). In contrast, when administered earlier, the beneficial effect of enalapril was less pronounced (+203 days).

Discussion

The model of dilated cardiomyopathy, which progressively develops an alteration of the cardiac function, provides possibilities of studying the effects of pharmacological treatments on the disease progression. Indeed, as compared to other experimental models of CHF, the effect of a prolonged administration can be easily evaluated in the CMHs and the therapy can be initiated either at an early or late stage of the disease. To illustrate this purpose, the ACE inhibitor enalapril, was chosen for its well established effects in the context of CHF.

This study shows that when administered from 120 days of age, enalapril (20 mg/kg/day) increased survival in male cardiomyopathic Syrian hamsters (Bio TO-2 dilated strain) and exerted beneficial effects on systemic hemodynamics and cardiac remodeling whatever the treatment duration, i.e. from 120 to 200 days of age (late treatment) or from 120 to 250 days of age (prolonged treatment). In addition, prolonged treatment also improved cardiac hemodynamics. When administered earlier (60 days of age), enalapril exerted beneficial effects on cardiac remodeling, but did not significantly improve cardiac function and survival.

Early, late and prolonged treatment with enalapril reduced left ventricle collagen density and cavity area to a similar extent and very slightly increased left ventricular wall thickness. This slight increase could result from the marked antifibrosis effect of enalapril (which probably allowed a better architectural rearrangement of myocytes) and from a lower effect on myocyte volume. When the treatment was initiated early during the time course of the disease (60 day-old), the results obtained following administration of enalapril mainly reflected the ability of the compound to prevent (or to slow) the progression of the complex remodeling process (as assessed on the basis of the effect on ventricular dilation and collagen accumulation). In contrast, when administered later (120 day-old), i.e. in a context of established left ventricular dysfunction and cardiac remodeling, the measured effects can rather reflect the ability of the compound to reverse the pathophysiological syndrome. Nevertheless, in this study, the histomorphometric parameters were not measured at the beginning of the treatment (i.e. 120 days of age) and it was therefore difficult to conclude that the late treatment reversed the development of cardiac remodeling. However, whatever the treatment duration and the stage of treatment initiation, enalapril exerted beneficial effects on cardiac remodeling. The effects of enalapril on cardiac remodeling are similar to those previously reported in this model [7,19]. These beneficial effects result from the blockade of angiotensin II and aldosterone production and of bradykinin degradation [20]. Angiotensin II is known to promote cardiomyocyte hypertrophy and fibroblast growth [21] and to stimulate collagen synthesis [22]. Aldosterone is known to increase collagen type I mRNA levels and the subsequent deposition of collagen fibers [23]. The ventricular remodeling and left ventricle dilation are a process in which matrix metalloproteinases also play an essential role in the high turnover of collagen in the extracellular matrix [24,25]. ACE inhibitors appear to act on matrix metalloproteinases to inhibit their activation, thereby preventing the subsequent detrimental remodeling [26].

Early, late and prolonged treatment with enalapril significantly decreased arterial blood pressure and left ventricular dP/dtmax. In contrast, the significant LVEDP decrease was only observed when enalapril was administered from 120 days of age and for a period of 130 days (prolonged treatment). Although dP/dtmax does vary with myocardial contractility, it has been demonstrated for a long time that it is a complex function that is subject not only to intrinsically induced changes in contractility but is also affected by other factors including arterial blood pressure, LVEDP and HR [27-29]. It is therefore not surprising that dP/dtmax did not increase as it was associated with reduction in these three parameters. When administered at 120 days of age, enalapril also significantly decreased total peripheral resistance. This effect observed whatever the treatment duration results from systemic arteriolar vasodilation. Although the beneficial effects of enalapril on cardiac function have already been reported in this model [7,19,30], the influence of the onset of treatment or the duration of treatment on the disease process have not really been discussed. It is generally agreed that the beneficial effects of ACE inhibitors on the cardiovascular system is due to prevention of angiotensin II formation and bradykinin degradation which may decrease peripheral resistances and improve cardiac function. ACE inhibitors also inhibited the circulating plasma norepinephrine levels [31] and improved the cardiac noradrenaline uptake [32]. Indeed, activation of presynaptic angiotensin II type 1 receptors with angiotensin II facilitates the release of norepinephrine from sympathetic nerve terminals [33]. However, in our study, these beneficial vasodilator effects were not observed when the treatment was administered in 60 day-old animals. They contrast with the results obtained in other studies in which enalapril administered early in the time course of the disease showed beneficial effects on cardiac function [19,30]. We have no clear explanation on this result although it has been shown that in 60-day old cardiomyopathic hamsters, the ACE activity is low and similar to control hamsters and that enalapril had no effect on heart cell communication at this stage [34]. It has also been shown that cardiac ACE activity in

Bio TO-2 cardiomyopathic hamsters was significantly increased in the advanced stage of heart failure [35], thereby suggesting that the administration of enalapril at an advanced stage of the disease could be more appropriate. In addition, it has been established for a long time that, whatever the variety of abnormalities that lead to cause the human CHF syndrome, intrinsic compensatory mechanisms come into play [36,37]. They include (1) activation of the sympathetic nervous and renin-angiotensin-aldosterone systems, which increases contractility and heart rate in an attempt to maintain cardiac output; (2) cardiac muscle hypertrophy, which helps to maintain cardiac function; (3) a rise in left ventricle filling pressure, which can optimize performance according to the Frank-Starling mechanism; and (4) an increase in peripheral arterial-venous oxygen extraction, which maximizes the oxygen delivered for a given cardiac output. Although these mechanisms are initially helpful, they may become excessive afterwards. In our study, the late and prolonged treatment with enalapril, but not the early treatment decreased total peripheral resistances. Therefore, it may be hypothesized that, in this model of dilated cardiomyopathy (Bio TO-2 strain), the inhibition of the angiotensin II formation is perhaps not appropriate during the early stage of heart failure, i.e. when the compensatory mechanisms are still effective in an attempt to support cardiac output through enhanced heart contractility and heart rate.

The chronic administration of enalapril (20 mg/kg/day) from 120 days of age significantly prolonged lifespan expectancy of cardiomyopathic hamsters. These beneficial effects were confirmed in two successive experiments. The beneficial effects of chronic ACE inhibition (treatment from about 6 months of age) on survival have already been reported in this model [14,15,38]. In the present study, when administered earlier (60 days of age), enalapril did not significantly increase survival. In contrast, in previous studies, early therapy started in young animals (30 to 50 day-old) with perindopril (Bio 53.58 strain) [12], quinapril (CHF 146 strain) [39] or enalapril (Bio TO-2 strain) [40] improved survival of cardiomyopathic hamsters. This discrepancy could be related to differences in the strain studied (different evolution of the remodeling process) or in the dose administered (enalapril being evaluated at 30 mg/kg/day versus 20 mg/kg/day in the present study). In our study, hemodynamic improvement observed when the treatment was administered from 120 days of age likely explains the increase in survival observed in these enalapril-treated groups.

In conclusion, this study shows that when administered from 120 days of age, enalapril improved the life expectancy and exerted beneficial effects on cardiac function and cardiac remodeling in the male Syrian cardiomyopathic hamster (Bio TO-2 dilated strain). When administered earlier (60 days of age), enalapril only prevented the development of cardiac remodeling, but did not improve the life expectancy. Therefore, these findings suggest that enalapril exerted beneficial effects more marked when treatment was initiated late in the evolution of CHF. However, in any event, given that only enalapril was studied in this work, it would be presumptuous to want to extend the results obtained to other ACE inhibitors or other substances having different mechanisms of action.

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