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MCI-154, a Ca^{2+} sensitizer, increases survival in cardiomyopathic hamsters

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Abstract

To assess the long-term efficacy of a Ca^{2+} sensitizer MCI-154, 6-[4-(4′-pyridylamino)phenyl]-4,5-dihydro-3(2*H*)-pyridazinone hydrochloride trihydrate, on chronic heart failure, we studied the effects of the agent on the life span of cardiomyopathic hamsters of the BIO-14.6 strain. At approximately 150 days of age, 210 male hamsters were randomly divided into three groups: MCI-154 0.1 mg kg^{-1} day^{-1} (MCI-154-low), MCI-154 1 mg kg^{-1} day^{-1} (MCI-154-high), and control group. The median survival time in control, MCI-154-low and MCI-154-high groups was 227, 243 and 260 days after the start of treatment, respectively. Final survival rate at 284 days in control, MCI-154-low and MCI-154-high groups was 0, 17.1 and 38.6%, respectively. The cumulative survival times in the two MCI-154 treated groups were significantly prolonged in comparison with that in the control group ($P < 0.0001$). Thus, the present study clearly showed that MCI-154 prolonged the life span of cardiomyopathic hamsters, suggesting that long-term therapy with MCI-154 would be promising in the treatment of congestive heart failure.

Keywords: MCI-154; Ca^{2+}-sensitizer; Heart failure; Cardiomyopathic hamster; Survival rate

1. Introduction

MCI-154, 6-[4-(4′-pyridylamino)phenyl]-4,5-dihydro-3(2*H*)-pyridazinone hydrochloride trihydrate, is a positive inotropic agent that has peripheral vasodilator properties (Narimatsu et al., 1987). The main mechanism responsible for the inotropic action of MCI-154 is considered to be an increase in the Ca^{2+} sensitivity of cardiac contractile protein systems (Kitada et al., 1987; Liao and Gwathmey, 1993; Abe et al., 1996). This Ca^{2+}-sensitizing action of MCI-154 results in some advantages over conventional inotropic agents by avoiding the problems associated with Ca^{2+}-loading such as arrhythmias and by having an energetically more favorable way of producing the inotropic effect. In fact, previous studies clearly have shown that MCI-154 does not aggravate canine ventricular arrhythmias (Eto et al., 1996) and decreases the oxygen cost of contractility in beating canine whole hearts (Onishi et al., 1997). Thus, MCI-154 is expected to be an alternative to conventional inotropic agents (Mori et al., 1997). Although the acute favorable hemodynamic effect of MCI-154 is well established (Narimatsu et al., 1987; Abe et al., 1991, 1993; Teramura et al., 1997), it is still unresolved whether long-term treatment with MCI-154 is beneficial for the prognosis of heart failure or detrimental to the already depressed or damaged myocardium. To answer the unresolved question, in the present study the effects of chronic MCI-154 therapy from the age of approximately 150 days on survival time were assessed in hereditary cardiomyopathic Syrian hamster of the BIO 14.6 strain, which is well established as one of the representative models of congestive heart failure (Gertz, 1972; Factor et al., 1982).

2. Materials and methods

2.1. Animals

Male cardiomyopathic hamsters of the BIO-14.6 strain were purchased from Charles River (Japan) at 30 days of age. Until the initiation of the study protocol, the animals were housed in an animal room at a temperature of 22°C ± 3°C with a light/dark cycle of 12/12 h and were fed on normal chow and tap water ad libitum. Throughout the following experiments, MCI-154 was dissolved in the

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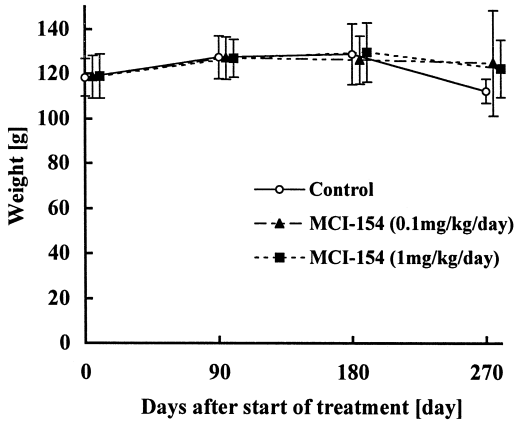

Fig. 1. Graph showing changes in body weight in cardiomyopathic Syrian hamsters of the BIO 14.6 strain. Symbols and vertical bars indicate means and S.D., respectively. There were no significant differences among groups measured at 0, 90 or 180 days after the start (determined by one-way analysis of variance). When the experiment reached 270 days, statistical analysis was not undertaken because the number of animals in the control group was too small. At 270 days, the number of surviving animals in each group was $n = 2$, 18 or 29 in control, 0.1 or 1 mg kg^{-1} day^{-1} of MCI-154-treated group, respectively.

drinking water. The animals were treated in accordance with the *Guide for the care and use of laboratory animals* published by US National Institutes of Health and extra care was taken to avoid animal suffering.

2.2. Protocol

At 149–153 days of age, 210 male cardiomyopathic hamsters were randomly divided into three groups of 70 animals. The hamsters in the first group (MCI-154-low group) were treated with 0.1 mg kg^{-1} day^{-1} of MCI-154 via the drinking water, and those in the second group (MCI-154-high group) were treated with 1 mg kg^{-1} day^{-1} of MCI-154. The third group (control group) was untreated, receiving tap water instead. When MCI-154 was given to the BIO-14.6 hamsters via the drinking water at doses of 0.1–1 mg kg^{-1} day^{-1}, the plasma concentrations were in a range of 0.01–0.1 μM. Because the Ca^{2+}-sensitizing action of MCI-154 was observed at concentrations higher than 0.01 μM (Kitada, 1997), we used the doses of MCI-154 as 0.1 and 1 mg kg^{-1} day^{-1}. The mean volume of water drunk (ml kg^{-1} day^{-1}) was estimated from the averaged body weight (g) and averaged volume drunk (ml day^{-1}) of randomly selected hamsters ($n = 105$) in the last 3 days just before the start of the study, and the concentrations of drug solution were determined. The mean volume of water drunk was repeatedly checked every 90 days during the study protocol. Drinking water was supplied ad libitum. The experiment was terminated when the survival rate of the control group was 0% (i.e., all the untreated hamsters had died).

2.3. Statistical analysis

The results for body weight were expressed as means ± S.D. and were analyzed statistically by analysis of variance at each time point. If the difference among groups reached a statistically significant level, the analysis was continued by using Dunnett statistics. The survival curve for each treatment was determined using the Kaplan–Meier method. Comparisons of the survival distributions between groups were performed by a log-rank test with Bonferroni's correction (Rosner, 1995). The difference was considered to be significant when the P value was less than 0.05.

3. Results

When the treatment period started, the mean volume of water drunk was estimated as 53.9 ml kg^{-1} day^{-1} from the results of averaged body weight and the averaged volume drunk for 105 cardiomyopathic hamsters of the BIO-14.6 strain. There were few changes in the volume of water drunk throughout the experiment and also few changes among groups (all in a range of 51.9–54.8 ml kg^{-1} day^{-1}). Fig. 1 shows changes in the body weight of cardiomyopathic hamsters in the untreated control group or MCI-154-treated groups. The treatment with MCI-154 (0.1 and 1 mg kg^{-1} day^{-1}) from approximately 150 days of age did not affect the body weight of hamsters. The days when the first animal died, the median survival time, and

Table 1
Effect of MCI-154 on the prognosis of cardiomyopathic hamsters

Treatment group	First death		Median survival time		Final survival	
	Days after the start (day)	Prolongation (day)	Days after the start (day)	Prolongation (day)	Number of hamsters (n/N)	Rate (%)
Untreated control	50	–	227	–	0/70	0.0
MCI-154						
0.1 mg kg^{-1} day^{-1}	68	+18	243	+16	12/70	17.1
1 mg kg^{-1} day^{-1}	82	+32	260	+33	27/70	38.6

Untreated control hamsters received tap water. Prolongation of time to the first death or the median survival time was expressed as the difference between the MCI-154-treated group and the control group, respectively. Final survival was determined when all animals in control group had died (i.e., 284 days after the experimental period started). Final survival rate was estimated in each group as the percentage of the number of animals that survived (n) out of the total number of hamsters (N).

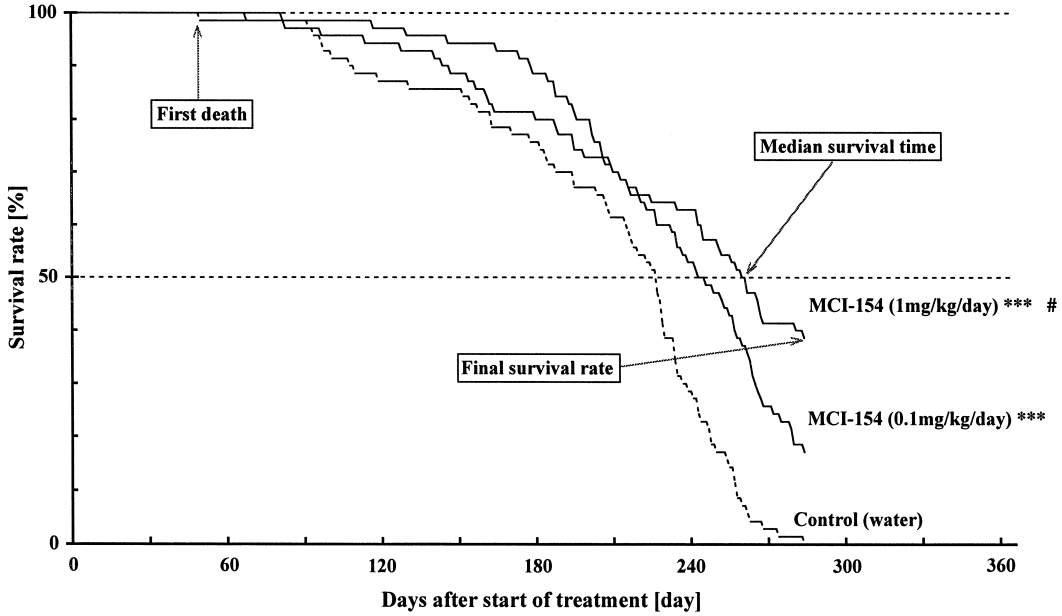

Fig. 2. Graph showing survival curves for untreated and MCI-154-treated cardiomyopathic hamsters of the BIO 14.6 strain. The initial number of animals in all the experimental groups was 70. The treatment with MCI-154 (0.1 and 1 mg kg^{-1} day^{-1}) in the drinking water started at approximately 150 days of age. Control animals received tap water. *** $P < 0.0001$ vs. control group by log-rank test with Bonferroni's correction. # $P < 0.05$ vs. MCI-154 (0.1 mg kg^{-1} day^{-1}) group by log-rank test with Bonferroni's correction.

the number of hamsters surviving when the study was terminated are summarized in Table 1. In the control group, the day when the first animal died was 50 days after the start of treatment (at 199–203 days of age), the median survival time was 227 days (at 376–380 days of age), and all 70 animals died after 284 days (at 433–437 days of age). The treatments with MCI-154 at the doses of 0.1 and 1 mg kg^{-1} day^{-1} delayed the day when the first animal died and increased the median survival time. At 284 days after the start of treatment, when all the hamsters in the control group had died, 12 and 27 of 70 hamsters were still alive in MCI-154-low and MCI-154-high groups, respectively (leading to 17.1 and 38.6% increase in survival rate, respectively). Fig. 2 shows the survival curves for untreated and MCI-154-treated cardiomyopathic hamsters of the BIO-14.6 strain. There were significant differences between control and MCI-154-low groups ($P < 0.0001$) and between control and MCI-154-high groups ($P < 0.0001$). And there was also a significant difference between MCI-154-low and MCI-154-high groups ($P < 0.05$). The cumulative percent survival curves clearly showed that MCI-154 dose dependently increased the probability of survival of cardiomyopathic hamsters.

4. Discussion

To investigate whether MCI-154 could improve survival, especially in the late stage of the disease, in the present study we started the treatment with MCI-154 at approximately 150 days of age, at which time the heart is already hypertrophic and dilated. We demonstrated that long-term treatment with MCI-154 for over 40 weeks increased the probability of survival of the hamsters. Before considering the results obtained with MCI-154, we should first think of the prognosis of untreated cardiomyopathic hamsters used in the present study, since changes in the time course of the disease process have been noted after generations of inbreeding (Gertz, 1972). It has been shown that hamsters of the BIO-14.6 strain spontaneously develop cardiac focal necrosis beginning at 30–35 days of age and continuing to 90–120 days, and that this is followed by ventricular hypertrophy and dilatation. In the final stage of the disease at 240–360 days, ventricular dilatation continues and clinical signs of congestive heart failure appear (Gertz, 1972; Factor et al., 1982). In the present experiments, the median survival time in the control group was 376–380 days. The results were in agreement with those of previous studies showing the beneficial effects of late treatment with angiotensin-converting enzyme inhibitors, in which the median survival time of untreated the BIO-14.6 hamsters was 340–440 days (Haleen et al., 1991; Narita et al., 1996). Thus, we conclude that MCI-154, when administered in the late stage of the disease, prolongs the life span of cardiomyopathic hamsters of the BIO-14.6 strain.

To estimate the long-term clinical efficacy in congestive heart failure, some investigators have examined whether positive inotropic agents can prolong the life span of cardiomyopathic hamsters. It was reported that a phosphodiesterase III inhibitor milrinone and a β_1-adrenoceptor agonist denopamine failed to prolong the total survival time of cardiomyopathic hamsters of the CHF 147 and the BIO-14.6 strains, respectively (Desjardins et al., 1989; Kurosawa et al., 1996). Pimobendan has been shown to

prolong the life span of cardiomyopathic hamsters of the BIO-14.6 strain, when treatment with the agent was started at quite a young age of 30–40 days (Van Meel et al., 1989). To our knowledge, the present study demonstrated for the first time that late treatment with a positive inotropic agent prolonged the life span of cardiomyopathic hamsters without exception, and therefore it is suggested that MCI-154 might be a possible candidate drug that could improve the prognosis of patients with congestive heart failure.

What mechanism(s) underlie the prolongation of life span by MCI-154? In general, an increase in cardiac contractility by inotropic stimulation may aggravate the energy deficit of the failing heart. The inotropic action mediated by MCI-154, however, has been reported to provide an energetic advantage over that of conventional inotropic agents in isolated canine heart (Onishi et al., 1997) and in diseased human heart (Mori et al., 1997). The vasodilatation by MCI-154 might decrease the ventricular volume, and hence offset the increase in myocardial oxygen demand. Therefore, it could be considered that the energetically favorable actions of MCI-154 contribute to the total improvement of the prognosis in the present study. Moreover, Eto et al. (1998) recently have reported that MCI-154 has anti-arrhythmic properties through an inhibitory action on the delayed rectifier K^+ current. Kumamoto et al. (1999) have demonstrated that treatment with MCI-154 induced angiogenesis of small vessels and thereby preserved the cardiac function of BIO-53.58 cardiomyopathic hamsters.

In conclusion, our present experiments clearly demonstrated that MCI-154, when given for over 40 weeks in the late stage of the disease, improved survival in cardiomyopathic hamsters of the BIO-14.6 strain. These data suggest that long-term therapy with MCI-154 may have clinical benefits for the prognosis of patients with advanced heart failure.

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