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Improved survival with simendan after experimental myocardial infarction in rats

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Abstract

This study compared the effects of simendan, a calcium sensitizer, with those of milrinone and enalapril on survival of rats with healed myocardial infarction. Seven days after ligation-induced myocardial infarction, the rats were randomized to control, milrinone, enalapril, or simendan groups. All compounds were administered via the drinking water for 312 days, at which time there was 80% mortality in the control group—the study's primary endpoint. The infarct sizes were similar across all groups. At endpoint, the mortality rates were: 63% (milrinone), 56% (enalapril) and 53% (simendan); the risk reductions were 25% ($P = 0.04$ vs. control) and 28% ($P = 0.02$ vs. control) with enalapril and simendan, respectively. Milrinone had no statistically significant effect on the survival rate. These findings suggest that, like enalapril, simendan improved survival in rats with healed myocardial infarction.

Keywords: Ca^{2+} sensitizer; Simendan; Myocardial infarction; Mortality

1. Introduction

Myocardial infarction can lead to rapid deterioration of left ventricular function. Early after myocardial infarction, increased neurohormone activation (Cohn, 1981; McAlpine et al., 1988) and left ventricle dilatation (Pfeffer and Braunwald, 1990) are among the compensatory mechanisms that aim to restore the heart's functional capacity. Sustained increases in neurohormonal activation and progressive dilatation are, however, both associated with poor prognosis (Pfeffer et al., 1991; Vantrimpont et al., 1998), leading to conditions such as heart failure that are associated with an increased risk of morbidity and mortality (Pool, 1998). Early use of aspirin, thrombolytic therapies, β-adrenoceptor antagonists and angiotensin-converting enzyme inhibitors have all been shown to reduce morbidity and mortality after acute myocardial infarction (Domanski et al., 1999; Gottlieb et al., 1999; Hall et al., 1997; Yusuf et al., 1985). Long-term, both β-adrenoceptor antagonists and angiotensin-converting enzyme inhibitors have been shown to reduce mortality and morbidity in heart failure, irrespective of aetiology (Garg and Yusuf, 1995; Lechat et al., 1998).

Simendan is a calcium sensitizer that improves cardiac contractility with coronary and peripheral vasodilatation. It is a racemic mixture from which the more active enantiomer, levosimendan, has been developed for short-term intravenous use in acute decompensated heart failure (Folláth et al., 1999; Moiseyev et al., 1999; Slawsky et al., 2000). The primary mechanism of action for both simendan and levosimendan is through Ca^{2+} sensitization of contractile proteins (Levijoki et al., 2000). Levosimendan does not affect the intracellular Ca^{2+} concentrations and, therefore, does not have any effect on relaxation (Haikala et al., 1995; Pagel et al., 1994). The vasodilator effects (Harkin et al., 1995) of both simendan and levosimendan are mediated through the opening of the ATP-sensitive K^+ channels (Haikala et al., 1997; Yokoshiki et al., 1997).

Experimental models of myocardial infarction following coronary ligation have shown that levosimendan decreased myocardial infarct size (Kersten et al., 2000). Levosimendan, in addition to improving cardiac haemodynamics in patients with acute heart failure due to myocardial infarction (Moiseyev et al., 1999), has also been shown to decrease neurohormonal activation (measured as atrial natriuretic peptide and endothelin-1 serum levels) in this patient population (Nicklas et al., 1999).

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This study compared the effects of simendan with those of enalapril and milrinone on survival, left ventricular hypertrophy and dilatation in animals with healed myocardial infarction.

2. Materials and methods

2.1. Experimental myocardial infarction

The study was conducted with written permission from the Provincial State Office of South Finland, in accordance with Finnish law and government regulations complying with the European Community guidelines for the use of experimental animals.

Male Wistar rats (Möllergaard Breeding Center, Denmark, 300–350 g) were pre-treated with atropine (0.5 mg/kg, s.c.), anaesthetised using sodium pentobarbital (50 mg/kg, i.p.), and ventilated with room air. A thoracotomy was performed at the fifth intercostal space, the pericardium was opened and the left anterior descending coronary artery was ligated as described by Selye et al. (1960). The ligation was made using a non-absorbable (4-0 TI CRON, Davis + Geck) suture. After surgery, the rats received a 5-day course of buprenorphine (0.2 mg/kg, s.c. once daily) for pain relief.

After 7 days, the rats were randomized into four treatment groups: control, milrinone, enalapril and simendan. Each group contained 32 animals. The concentrations of the compounds in the drinking water (given ad libitum) were 40, 20 and 40 mg/l for milrinone, enalapril, and simendan, respectively. The average daily doses (mean ± S.E.M.) were 2.4 ± 0.09, 1.5 ± 0.09 and 2.5 ± 0.07 mg/kg for milrinone, enalapril and simendan, respectively. The doses of milrinone and enalapril were selected according to a previous study (Sweet et al., 1988), in which the long-term survival of rats with healed myocardial infarction and congestive heart failure treated with milrinone and enalapril was investigated. The protocol of the present study was planned so as to be consistent with the aforementioned publication. The primary endpoint was the time taken to reach a mortality rate higher than 80% in the control group. Sham-operated (non-ligated) untreated rats were also studied.

2.2. Left ventricular pressure–volume curves

The left ventricle pressure–volume relationship was determined as described by Fletcher et al. (1981). Cardiac arrest was induced by intravenous KCl injection in rats surviving the 312-day treatment period; within 10 min of arrest, pressure–volume curves were measured over a pressure range of 0–30 mm Hg. A double-lumen catheter was inserted via the aorta into the left ventricle, isolated by ligation of the atrioventricular groove; the right ventricle was incised to eliminate any possible compressive effect. Saline was pumped through the inner lumen (PE 50) of the catheter and the pressure was measured via the outer lumen (PE 200). The pressure–volume curves were fitted into the exponential function of $p = be^{kV}$, where p, b, k, and V represent pressure, intercept, slope and volume, respectively. The slopes of the overall compliance curve were determined.

2.3. Histopathology of the left ventricle

Hearts from all study animals were excised, flushed and fixed with 10% phosphate-buffered formalin pending infarct size determination. Ventricles of eight sham-operated rats were also included in the histopathological analysis. The left ventricle, the septum and the right ventricle were weighed. The ventricular weight of each rat was normalized by dividing it by the rat's body weight. Infarct sizes were determined by Life Science Research Laboratories, England.

The left ventricle was cut into four sections at standard levels, dehydrated in alcohol and embedded in paraffin wax. A section of approximately 5-μm thickness was cut from each of the four samples, and Masson's Trichrome stain was applied. Image analysis was performed using a true-colour image analyser for the determination of the left ventricle infarct size. The external and internal perimeters of the whole and infarcted part of the left ventricle were measured. Infarct size was expressed as a percentage of infarcted portion of perimeter to whole perimeter of the left ventricle.

2.4. Statistical analyses

The log-rank test was used to compare the Kaplan–Meier survival curves. A one-way analysis of variance was used to compare infarct sizes between the groups. The Bonferroni/Dunn procedure was used as a post hoc test to test multiple, between-group comparisons when required. The slopes of the pressure–volume curves, normalized against body weights, were used to assess the stiffness of the left ventricular chamber. The overall slope of pressure–volume curves were compared between the groups using the non-parametric Kruskal–Wallis test. The critical level of statistical significance was $P < 0.05$.

3. Results

3.1. Survival

The study was discontinued on day 312 of treatment when the mortality in the control group was 81%. At this time, the mortality rates were: milrinone 63%; enalapril

56%; simendan 53%. Comparison with control Kaplan–Meier plots showed that this improvement in survival was significant with both enalapril ($P = 0.04$) and simendan ($P = 0.02$; Fig. 1), but not with milrinone. The 6-month mortality rates were: control 44%; milrinone 38%; enalapril 28%; simendan 19%.

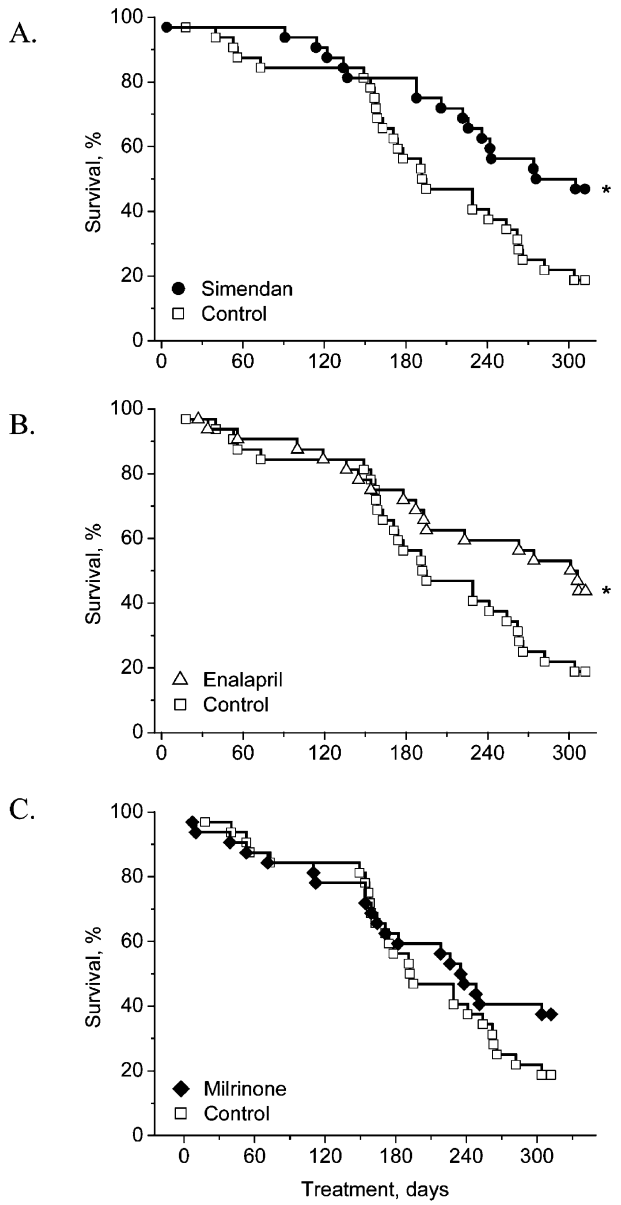

Fig. 1. Survival curves for rats with healed myocardial infarction treated with simendan (A), enalapril (B) or milrinone (C) as compared to those for untreated myocardial infarction rats (control). None of the sham-operated animals died. The number of animals at the beginning of the study was 32 per group. The respective concentrations of the compounds in drinking water (given ad libitum) were 40, 20 and 40 mg/l for milrinone, enalapril and simendan, respectively. The average daily doses (mean ± S.E.M.) during the study were 2.4 ± 0.09, 1.5 ± 0.09 and 2.5 ± 0.07 mg/kg for milrinone, enalapril, and simendan, respectively. The log rank test was used to compare the Kaplan–Meier survival curves to each other. * $P < 0.05$ vs. control group.

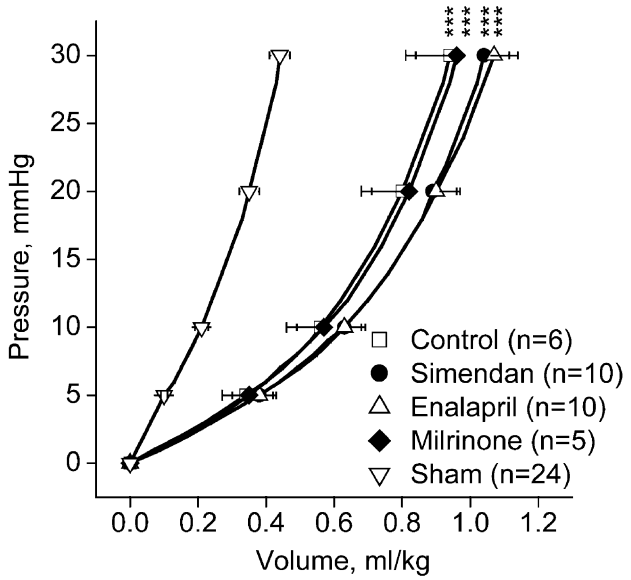

Fig. 2. Left ventricular pressure–volume relationship in surviving rats with healed myocardial infarction and sham-operated rats. The overall slopes of pressure–volume curves were compared among groups, using the non-parametric Kruskal–Wallis test. *** $P < 0.001$ (sham vs. any of the myocardial infarction groups).

3.2. Pressure–volume curves

Pressure–volume curves were similar across all myocardial infarction groups (Fig. 2). The slopes of the curves for all the myocardial infarction groups differed significantly ($P < 0.001$) from those of the sham-operated group.

3.3. Histopathology

All coronary-ligated rats were confirmed to have an infarct. The infarct sizes (expressed as percentages of infarcted portion of perimeter to whole perimeter) were similar for all animals in the control (46 ± 0.9), milrinone (44 ± 1.6), enalapril (46 ± 1.2) and simendan (43 ± 1.4) groups. Furthermore, no difference in infarct size was seen in those rats who survived beyond the study endpoint, irrespective of their treatment groups. Concurrently, the ratios of left and right ventricular weights to body weight of the surviving myocardial infarction rats were also similar across all groups.

The left ventricles were dilated in all myocardial infarction rats. The average cross-sectional infarct area was similar across all myocardial infarction groups (Table 1). The external and internal boundaries of the infarct, the ratio of cross-sectional infarcted area to that of the whole area of the left ventricle, and the extent of fibrosis in the infarct did not differ between myocardial infarction groups, irrespective of treatment. Moreover, the perimeter values of non-infarcted myocardium were similar for all myocar-

Table 1
Histological characteristics of the infarct area of all rats
Data are means ± SD.

Parameter [*n*]	Control [32]	Simendan [32]	Enalapril [32]	Milrinone [32]	Sham [8]
External normal boundary (mm)	11.6 ± 2.0	11.9 ± 1.7	11.6 ± 1.9	11.8 ± 1.5	10.3 ± 1.2
Internal normal boundary (mm)	7.6 ± 2.0	7.6 ± 2.0	7.7 ± 2.0	7.7 ± 1.7	3.9 ± 1.1
External infarct boundary (mm)	7.7 ± 4.1	7.4 ± 4.4	8.5 ± 4.0	7.8 ± 4.3	0.0 ± 0.0
Internal infarct boundary (mm)	6.4 ± 3.5	6.2 ± 3.7	7.2 ± 3.5	6.5 ± 3.6	0.0 ± 0.0
Whole area (mm^2)	52.2 ± 14.0	60.7 ± 15.8	53.0 ± 14.3	56.0 ± 15.1	65.7 ± 14.0
Normal area (mm^2)	44.0 ± 13.7	52.4 ± 15.7	44.3 ± 14.2	47.5 ± 15.9	65.7 ± 14.0
Infarct area[a] (mm^2)	8.1 ± 4.9	8.2 ± 5.5	8.6 ± 4.7	8.4 ± 5.4	0.0 ± 0.0
Fibrous replacement in left ventricle	11.6 ± 3.8	10.8 ± 4.5	12.1 ± 4.5	11.5 ± 4.3	n/a
Fibrous replacement in infarct area	6.0 ± 3.5	5.1 ± 3.6	5.5 ± 3.4	5.0 ± 3.5	n/a

[a] Excluding papillary muscle.

dial infarction groups and were significantly greater than those in the sham-operated group.

4. Discussion

Our major finding was that simendan, like enalapril, improved the survival rate in rats with healed myocardial infarction. In contrast, milrinone did not significantly affect mortality. The data also showed that none of the drug treatments altered right and left ventricular hypertrophy or dilatation. All treatments reduced the collagen content—seen as a reduction in fibrosis—of the left ventricle compared with the control group.

The phosphodiesterase inhibitor, milrinone, has been shown to improve cardiac function by increasing intracellular Ca^{2+} concentrations (Alousi et al., 1983; Gwathmey and Morgan, 1985). A long-term study in rats showed a mortality benefit with milrinone (Sweet et al., 1988), but the findings of the current study failed to reproduce these results. Any mortality benefits attributable to milrinone based on animal models have not been supported in humans (Packer et al., 1991; Thackray et al., 2000).

Simendan, which is the racemic mixture of levosimendan and dextrosimendan, has been tested in Phase I clinical studies (Lilleberg et al., 1994a,c) and proved to be as effective as levosimendan (Lilleberg et al., 1994b), after the presence of the dextro enantiomer had been taken into account. Dextrosimendan, at the total plasma concentrations reached during administration of simendan (0.2 μM), is not active as positive inotrope either in papillary muscle preparations ($EC_{50} > 3$ μM) or in perfused heart preparations (non-detectable effect under 1 μM), nor has it any marked vasorelaxant effect on histamine-induced transient contractions in guinea-pig aorta ($EC_{50} > 30$ μM).

The active enantiomer, levosimendan, has a strong positive inotropic effect (EC_{50} 45 ± 8 nM in perfused heart preparations). However, critically, levosimendan does not affect Ca^{2+} concentrations (Hasenfuss et al., 1998; Lancaster and Cook, 1997) because it acts, at therapeutic concentrations, primarily as a Ca^{2+} sensitizer (Haikala et al., 1997). In addition, the mechanism of action of levosimendan differs from that of the early Ca^{2+} sensitizers such as (+)-5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl)-6-methyl-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-one (EMD 57033) or (+)-(5-methyl-6-phenyl)-1,3,5,6-tetrahydro-3,6-methano-1,5-benzodiazocine-2,4-dione (CGP 48506), which slowed the rate of relaxation (Hajjar et al., 1997; Neumann et al., 1996; Webster et al., 1993; Zimmermann et al., 1996), thereby increasing the risk of arrhythmias (Evans et al., 1995). Levosimendan has a specific Ca^{2+}-dependent binding to troponin C and does not bind to the contractile apparatus during diastole, thus enhancing cardiac contractility without disturbing relaxation (Haikala et al., 1995).

Based on the mechanism of action alone, our findings suggest that while simendan and milrinone both improved cardiac performance, simendan's unique mechanism of action may confer an additional mortality benefit compared to the effect of milrinone

There is an accepted correlation between infarct size, worsening of the left ventricular function after myocardial infarction (Fletcher et al., 1981) and increased mortality. In addition, clinical trials have also shown that infarct size, the extent of fibrosis and left ventricular hypertrophy are all known to influence outcome of patients (Ho et al., 1993). In the present study, however, infarct size, as well as the extent of fibrosis and hypertrophy, were consistent across all myocardial infarction groups at study endpoint. Thus, we concluded that they were unlikely to have influenced the different survival outcomes.

There are earlier reports that angiotensin-converting enzyme inhibition reduced left ventricular hypertrophy (Schieffer et al., 1994) and dilatation (Ali et al., 1998) in myocardial infarction in rats, and remodelling early after myocardial infarction in humans (Bonarjee et al., 1993). These findings contrasted with those from the present study, in which enalapril did not reduce either hypertrophy or dilatation. One possible explanation for this difference may be the duration of follow-up. In the earlier studies, treatment was started early after myocardial infarction (Ali et al., 1998) and the assessments were made after a

relatively short time (Schieffer et al., 1994). The longer-term follow-up from the present study may mean that enalapril and simendan had favourable inhibitory effects on the left ventricular dilatation early in the study. However, as there was no difference in left ventricular dimensions between treated and untreated animals, our findings suggest that any favourable effect that either agent may have had on slowing or preventing hypertrophy in the short-term had disappeared.

Levosimendan is currently licensed for intravenous use in hospitalized patients with acute heart failure. The favourable outcomes for simendan in this study confirmed the utility of levosimendan in the management of patients with acute cardiac illness. This use may extend to the myocardial infarction patients, irrespective of their heart failure status, with treatment periods longer than the 24-h infusion that is currently indicated for levosimendan.

In conclusion, simendan improved the survival rate in rats with healed myocardial infarction. This beneficial effect is not thought to be mediated through inhibition of ventricular hypertrophy, dilation or alterations in late-phase healing of the infarct, but primarily through Ca^{2+} sensitization of the contractile proteins.

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