MET-88: SR Ca\(^{2+}\)-Uptake Stimulator for Treating Chronic Heart Failure

Tsukasa Kirimoto, Naomasa Asaka, Yukio Hayashi, *Toshimatsu Maeda, †Kenji Irimura, and Naosuke Matsuura

Pharmacological Research Laboratories, *Pharmacokinetics Research Laboratories, †Drug Safety Laboratories, Taiho Pharmaceutical Co., Ltd., Tokushima, Japan

Key Words: SR Ca\(^{2+}\) uptake—Glycolysis—MET-88—Myocardial function—Remodeling—Myocardial energy metabolism.

INTRODUCTION

Dysfunction of the sarcoplasmic reticulum (SR) and a decrease in SR Ca\(^{2+}\) ATPase protein levels have been reported in patients with myocardial infarction (MI) and congestive heart failure (CHF) (6,7,18). Contraction and relaxation of cardiac myocytes result from the sequential activation and inactivation of the contractile elements by the alternating rise and fall of the cytosolic Ca\(^{2+}\) concentration. The SR plays a crucial role in the regulation of cytosolic Ca\(^{2+}\) cycling. Ca\(^{2+}\) uptake by the cardiac SR occurs through SR Ca\(^{2+}\)-ATPase, the activity of which seems to be maintained by adenosine triphosphate (ATP) produced through glycolysis (11,24). Therefore, compounds that have the ability to regulate the activity of SR Ca\(^{2+}\)-ATPase would seem to be beneficial for the therapy of chronic heart failure.

MET-88 [3-(2,2,2-trimethylhydrazinum) propionate] was synthesized by the Institute of Organic Synthesis (Riga, Latvia) as an inhibitor of \(\gamma\)-butyrobetaine hydroxylase, the enzyme that catalyzes the synthesis of carnitine from \(\gamma\)-butyrobetaine in the liver (23). MET-88 lowers the intercellular level of free carnitine and thus suppresses fatty acid oxidation and facilitates glycolysis (23).

MET-88 has the ability to regulate the activity of SR Ca\(^{2+}\) uptake in rats with heart failure following myocardial infarction. It also has a beneficial effect on cardiac function in rats with experimental MI and in conscious dogs with CHF produced by pulmonary artery constriction and tricuspid valve avulsion.

MET-88 has cardioprotective effects on energy metabolism in the ischemic canine heart (16) and on the contractile function and energy metabolism of isolated perfused rat hearts in the hypoxic condition (2). MET-88, unlike a vasodilator or a positive inotropic agent, ameliorates cardiac dysfunction by modulating myocardial energy metabolism. Thus,
MET-88 appears to be a unique cardioprotector that stimulates the Ca\(^{2+}\) uptake by the cardiac SR that occurs via the SR Ca\(^{2+}\)-ATPase, the activity of which seems to be maintained by ATP produced through glycolysis. This article reviews the chemistry, pharmacology, pharmacokinetics, and toxicology of MET-88 in animals and clinical findings in humans.

**CHEMISTRY**

The chemical structure of MET-88, \([3-(2,2,2\text{-trimethylhydrazinium})\text{ propionate}]\), is shown in Fig. 1. The compound was synthesized by the Institute of Organic Synthesis (Riga, Latvia) as an inhibitor of \(\gamma\)-butyrobetaine hydroxylase, the enzyme that catalyzes the synthesis of carnitine from \(\gamma\)-butyrobetaine in the liver (23). It has a molecular weight of 182.22. It is freely soluble in water, glacial acetic acid, and methyl alcohol, is soluble in ethanol, and is slightly soluble in ether. Its melting point is 85–90°C.

**PHARMACOLOGY**

**Effects on Myocardial Energy Metabolism**

Kirimoto et al. (16) were the first to report that MET-88 attenuates the derangement of the energy metabolism in the ischemic myocardium. In dogs pretreated orally with MET-88 (50, 100, or 200 mg/kg/d) or a placebo for 10 d, the left anterior descending coronary artery (LAD) was occluded for 60 min. The myocardium was then taken from the ischemic area for metabolic analysis. LAD occlusion decreased the tissue levels of ATP, adenosine diphosphate (ADP), and creatine phosphate (CrP), increased the tissue level of adenosine monophosphate (AMP) and lactate, and decreased the value of the energy charge potential. These metabolic alterations, induced by occlusion of the LAD, were attenuated by MET-88 in a dose-dependent manner (Fig. 2). In addition, Asaka et al. (3) demonstrated previously that MET-88 administered (100 mg/kg/d p.o.) for 10 d significantly improved the reduction in left ventricular pressure (LVP) and high-energy phosphate (ATP and CrP) during 30 min of hypoxia in the isolated perfused working rat heart.

![FIG. 1. Chemical structure of MET-88.](https://example.com/fig1.png)
Aoyagi et al. (1) reported that MET-88, previously administered p.o. for 10 d at a dose of 100 mg/kg, improved the left ventricular dysfunction induced by brief ischemia (15 min of anoxia and acidosis) in the perfused isovolumic rat heart. In addition, Dahr et al. (4) demonstrated that treatment with MET-88 (100 mg/kg/d p.o.) for 10 d significantly improved mitochondrial respiratory function as measured in isolated guinea-pig hearts after 60 min of hypoxic perfusion and a subsequent 20 min of reoxygenation. In addition, the effect of MET-88 on changes in myocardial pH and infarct size during open-chest coronary artery occlusion and reperfusion in anesthetized dogs was examined by Kirimoto et al. (15). MET-88 (50 and 100 mg/kg) significantly facilitated the recovery of myocardial pH during reperfusion, and tended to reduce the infarct size induced by repeated ischemia and reperfusion. Furthermore, MET-88 reduced ischemia and reperfusion-induced fibrillation in dogs. Another study was carried out to investigate whether or not increased glucose oxidation could attenuate hypoxic damage in isolated perfused rat hearts (2). MET-88 antagonized the depression of cardiac contractility (+dP/dt) and aortic flow during the hypoxic state (Fig. 3). It also prevented the decrease in high-energy phosphate and the increase in long-chain acylcarnitine. In addition, MET-88 showed no significant effect on substrate oxidation in the case of normoxic perfusion. However, the drug significantly increased the steady state of glucose oxidation in hypoxic perfused rat hearts (Fig. 4).

These results indicate that MET-88 may be beneficial during myocardial ischemia and hypoxia because it facilitates glucose utilization and prevents the accumulation of fatty
acid metabolites (long-chain acylcarnitine). This interpretation is supported by the reports of Lopaschuk et al. (20) and Lopaschuk and Spafford (19), which indicated that the antiischemic action of carnitine acyltransferase I inhibitors did not correlate with the tissue level of long-chain acylcarnitine. Moreover, the intracellular increase in long-chain acylcarnitine during an almost zero myocardial flow was found not to be critical for sarcoplasmic sodium and calcium permeability and SR pumping activity (17). According to Lopaschuk et al. (20), carnitine acyltransferase I inhibitors exert their anti-ischemic action by reducing the \( \beta \)-oxidation of free fatty acids in mitochondria. Inhibition of the \( \beta \)-oxidation results in an increase in glucose utilization, and a decrease in the myocardial oxygen demand, both of which are important in attenuating ischemic damage (17,20). Simkovich et al. (23) reported that MET-88 also reduced the \( \beta \)-oxidation of free fatty acids that resulted from the inhibition of carnitine synthesis. Therefore, inhibition of \( \beta \)-oxidation may be involved in the mechanism of the protective action of MET-88 against the ischemia-induced metabolic derangement of the myocardium.

**Effects on Experimental Heart Failure**

Hayashi et al. (9) demonstrated that MET-88 protected against left ventricular (LV) dysfunction and ventricular remodeling in chronic myocardial ischemia. Myocardial infarction (MI) was induced by ligating the LAD in male Sprague-Dawley (SD) rats (12,22). MET-88 (50, 100 mg/kg) was orally administered to the rats 2 d after surgery, and the
treatment was then continued for 20 d. Hemodynamic studies were performed under the basal condition, preload stress (saline i.v. infusion), and afterload stress (aortic occlusion) (12,22) at the end of the twenty-two day study period. In addition, the LV volume was measured as an index of remodeling. MET-88 reduced the augmentation of right atrial pressure (RAP) and slightly increased the reduced LV peak +dP/dt in CHF rats (Fig. 5). In addition, hemodynamic studies were performed under volume or pressure loading. MET-88 improved hemodynamics in MI rats. MET-88 (100 mg/kg) significantly reduced the expansion of LV hypertrophy measured in terms of ventricular volume (Fig. 6), and lung weight increase, whereas no change in LV weight was observed. The Ca^{2+} uptake activity was determined by incubating SR with $^{45}$Ca$^{2+}$ and measuring the incorporation of radioactivity into the SR. The difference in the radioactivity measured in the presence or absence of ATP was defined as the Ca$^{2+}$ uptake due to Ca$^{2+}$-ATPase. The $K_d$ and $V_{max}$ were calculated from Lineweaver-Burke plot analysis. The rat LV myocardium, at 22 d after surgery for MI induction, showed an increase in the Ca$^{2+}$ uptake that was $p$Ca$^{2+}$ dependent. The activity in the heart failure group was significantly lower than that in the sham group. The Ca$^{2+}$ uptake activity in the MET-88 (100 mg/kg) group was significantly higher than that in the control group. MET-88 did not change the $K_d$ but increased the $V_{max}$ (Fig. 7). By 22 d after MI induction in the rat LV myocardium, the SR Ca$^{2+}$ uptake had decreased, and the administration of 100 mg/kg of MET-88 improved this

FIG. 4. Effect of MET-88 on steady-state rates of glucose oxidation during either normoxia or hypoxia. Substrate: $[^{14}C]$glucose, measured: $[^{14}C]$CO$_2$. The results are expressed as the mean ± S.E.M. (n = 3–9), **P < 0.01 vs control group (Student’s t-test).
FIG. 5. Effects of MET-88 on RAP under volume-loading and LV $+dP/dt$ under pressure-loading in rats with heart failure following myocardial infarction. MET-88 50: MET-88 50 mg/kg; MET-88 100: MET-88 100 mg/kg. RAP: right atrial pressure. The results are expressed as the mean ± S.E.M. (n) = number. *P < 0.05, **P < 0.01 vs control group (Dunnett’s multiple analysis).

FIG. 6. Effect of MET-88 on left ventricular volume in the rats with heart failure following myocardial infarction. S: sham; C: control; MET-88 50: MET-88 50 mg/kg; MET-88 100: MET-88 100 mg/kg; Capt: captopril, 20 mg/kg. The results are expressed as the mean ± S.E.M. (n) = number. *P < 0.05, **P < 0.01 vs control group (Dunnett’s multiple analysis).
decrease. In addition, analysis of the $K_d$ and $V_{max}$ showed that the effect of MET-88 was to inhibit the decrease in $Ca^{2+}$-ATPase. Thus, the mechanism of MET-88 in improving the cardiac function is improvement of the intracellular $Ca^{2+}$ kinetics mediated through improvement in the myocardial SR function. In addition, the cardiac SR $Ca^{2+}$-ATPase protein level was reduced in the MI group by 27%, but not in rats with MI given MET-88. Simultaneously, the cardiac hexokinase protein level and the glycogen synthase protein level were reduced after MI, but not in rats with MI given MET-88 (data not shown).

Kirimoto et al. (14) studied the effect of MET-88 on systemic and cardiac hemodynamics, and on heart weight in conscious dogs with right-heart failure (RHF) (8,10). MET-88 (100 mg/kg, p.o. for 10 d) improved the right ventricular (RV) dysfunction (peak RV $+dp/dt$, RV $-dp/dt$ and cardiac output without a significant effect on the heart rate or mean aortic pressure) induced by pulmonary artery constriction and tricuspid valve avulsion in conscious dogs with RHF (Fig. 8). Simultaneously, MET-88 reduced the absolute weight of the heart, and the ratio of right-side heart weight to left-side heart weight. These results indicate that MET-88 may improve cardiac dysfunction induced by MI, pulmonary artery constriction, and tricuspid valve avulsion in CHF by improving the $Ca^{2+}$-ATPase activity in the SR and the postinfarction LV enlargement and hypertrophy.

FIG. 7. Effects of MET-88 on $Ca^{2+}$ uptake activity of cardiac sarcoplasmic reticulum (SR) in rats with heart failure following myocardial infarction. S: sham n = 16; C: control n = 16; MET-88 50: MET-88, 50 mg/kg, n = 17; MET-88 100: MET-88, 100 mg/kg, n = 15; Capt: captopril, 20 mg/kg, n = 16. The results are expressed as the mean ± S.E.M. (n) = number. *P < 0.05, **P < 0.01 vs control group (Dunnett’s multiple analysis).
Effects on Ventricular Hypertrophy

Kirimoto et al. (13) demonstrated the beneficial effects of MET-88 on cardiac hypertrophy induced by monocrotaline in rats. MET-88 was orally administered to Wistar rats for 10 d, beginning 3 w after the injection of monocrotaline (2%, 40 mg/kg, s.c.). The ratio of right-sided heart weight to body weight was 2.6 times higher in monocrotaline-treated rats than in non-treated rats. MET-88 reduced right-sided cardiac hypertrophy in a dose-dependent manner (ED50: 34.7 mg/kg), as in the case of captopril (ED50: 20.4 mg/kg). Other metabolic modulators (L-carnitine, CoQ10, oxfenicine), however, had no effect (Fig. 9). In addition, four weeks after surgery for placement of an A-V shunt, the control group, compared with the sham group, clearly showed cardiac hypertrophy and dysfunction (5).

Nakano et al. (21) reported that MET-88, orally administered (25 and 50 mg/kg) for 10 d, significantly prevented LV hypertrophy and the increased left ventricular end-diastolic pressure (LVEDP) in rats with an A-V shunt. Captopril, at a dose of 20 mg/kg, also caused a decrease in LV weight in rats with A-V shunts. In in vitro studies, MET-88 had no effect on renin and angiotensin-converting enzyme (ACE) activity in the plasma of normal rats. In addition, Asaka et al. studied the effect of MET-88 on gene expression levels in the non-infarcted area of the LV following MI in rats. Gene expression levels of atrial natriuretic polypeptide (ANP), β-myosin heavy chain (β-MHC), and angiotensin II type 1 receptor (AT1) were increased at 7 d after MI. MET-88 significantly suppressed the
increases in expression levels of these three genes after the operation. These results indicate that MET-88 may improve ventricular remodeling and cardiac hypertrophy induced by MI as effectively as captopril, monocrotaline, and A-V shunt in rats by preventing qualitative and quantitative changes in the expression of various genes in the heart. Further studies are necessary to elucidate the mechanism of these favorable effects of MET-88 treatment.

**PHARMACOKINETICS**

The concentration of radioactivity in the blood increased rapidly after oral administration of [2,3-\(^{14}\)C] MET-88 (100 mg/kg) to male rats, and reached a \(C_{\text{max}}\) of 20.4 \(\mu\)g eq/mL (total radioactivity) at 1 h. The concentration of radioactivity in the blood increased rapidly after oral administration of [2,3-\(^{14}\)C] MET-88 (100 mg/kg) to male dogs, and showed a \(t_{\text{max}}\) of 0.58 h and a \(C_{\text{max}}\) of 54.7 \(\mu\)g/ml (unchanged MET-88 levels). At 4 h after oral administration of [2,3-\(^{14}\)C] MET-88 (100 mg/kg) to male rats, inspection of whole-body autoradiograms indicated that the radioactivity in the gastrointestinal contents and liver was the highest, followed by that in the kidney, medulla spinalis nodus, epididymis, lungs, and heart. The cumulative excretion of radioactivity in the urine and feces after oral

**FIG. 9.** Effects of MET-88, captopril, CoQ\(_{10}\), carnitine, and oxfenicine on monocrotaline-induced right-sided hypertrophy. N: normal; C: control; Capt: captopril; CoQ: CoQ\(_{10}\); Car: carnitine; Oxf: oxfenicine. RHW: right-sided heart weight; BW: body weight. The results are expressed as the mean ± S.E.M. *P < 0.05, **P < 0.01 vs control group (Dunnett’s multiple analysis).
administration of [2,3-14C] MET-88 (100 mg/kg) to male rats was approximately 56%, 14%, and 22% in the urine, feces, and exhaled air, respectively, within 168 h. MET-88 bound minimally (under 1%) to rat, human, and dog plasma proteins.

The blood radioactivity gradually increased during repeated oral administration, and reached a plateau on day 21. The concentration at 24 h after the 28th dose was 3.4 times higher than after the first dose. The concentration of radioactivity in most tissues at 24 h after repeated administration was 3–10 times higher than that after the first dose. Excretion of radioactivity in the urine, feces, and exhaled air was almost constant after the 7th dose.

The pharmacokinetics of MET-88 was also studied in normal human subjects. Each subject received MET-88 at a single oral dose of 25, 50, 100, 200, 400, 800, or 1500 mg. The observed Cmax and area under the time-concentration curve (AUC) of MET-88 increased proportionally to the dose. The tmax was 1–2 h, and the t1/2 was about 4 h. The urinary excretion rate of MET-88 increased with the dose up to 400 mg, but it showed almost the same value above 400 mg. Food intake delayed the tmax but did not affect the Cmax and AUC at a single oral dose of 400 mg. With multiple oral dosing of MET-88 (twice daily for seven days and once on the eighth day) at a dose of 400–800 mg/day, the plasma trough levels of MET-88 reached steady state 72–96 h after the first dosing.

TOXICOLOGY

Acute Toxicity Studies

Single-dose toxicity studies with MET-88 were carried out in Sprague Dawley rats and male beagle dogs (unpublished observations). The approximate lethal doses of MET-88 were >5000 mg/kg for rats and male dogs. The values indicated that acute toxicity of MET-88 was similar in rats and dogs and that there were no sex-related differences.

Subacute Toxicity Studies

A 13-week repeated-dose toxicity study of MET-88 was carried out in Sprague Dawley rats to obtain the non-toxic dose level (unpublished observations). MET-88 was given to rats (n = 10 each) in daily doses up to 1600 mg/kg for 13 w. The non-toxic dose level of MET-88 was estimated to be 25 mg/kg/d for female rats and 100 mg/kg/d for male rats. A 13-w repeated dose toxicity study of MET-88 was also carried out in beagle dogs to obtain the non-toxic dose level in these animals. MET-88 was given to dogs (male, n = 4; female, n = 4 each) in daily doses up to 1600 mg/kg for 13 w. The non-toxic dose level of MET-88 was estimated to be 100 mg/kg/d for both male and female dogs.

Chronic Toxicity Studies

A 52-w repeated-dose toxicity study of MET-88 was carried out in beagle dogs to obtain the non-toxic dose level (unpublished observation). MET-88 was given to dogs (n = 3 each) at daily doses ≤400 mg/kg for 52 w. The non-toxic dose level of MET-88 with chronic administration was found to be 25 mg/kg/d for both male and female dogs.

Fertility Study

A fertility study with MET-88 was carried out in Sprague Dawley rats. Rats were given MET-88 orally at doses ≤1600 mg/kg (n = 48 each males and females). Male rats were
given the compound for 63 d before mating and during mating. Female rats received the drug for 14 d before mating to the end of the lactation period. MET-88 had no adverse effects on parental reproductive function or on the fetuses. The non-toxic dose level of MET-88 was 400 mg/kg/d for general toxicity in parent animals (soft feces: only in males, 1600 mg/kg/d), 1600 mg/kg/d for reproductive function in parent animals, and >1600 mg/kg/d for the development of their fetuses.

Teratology Study

A teratology study of MET-88 was carried out in Sprague Dawley rats (unpublished observations). Pregnant rats were given MET-88 ≤5000 mg/kg from days 7–17 of gestation to study the effect of the compound on fetal development (n = 35–37, each) (unpublished observations). MET-88 had no adverse effect on parental delivery or nursing behavior or on the fetuses or on the offspring of their progeny. In the next generation (F1), MET-88 had no teratogenic, lethal, or growth retardation effects in any dosage group. The non-toxic dose levels of MET-88 were 200 mg/kg/d for general toxicity in dams, >5000 mg/kg/d for reproductive function of dams, and >5000 mg/kg/d for the development of their fetuses or the subsequent generation.

Mutagenicity Studies

MET-88 was studied for mutagenicity by the Ames method, in vitro cytogenetics, and the micronucleus test. MET-88 had no mutagenic potential.

CLINICAL STUDIES

Phase I Studies

Safety, pharmacological action, and pharmacokinetics of MET-88 were assessed in 16 healthy male volunteers after oral administration of a single dose in the range of 25–1200 mg. While no change in clinical laboratory parameters were found, the only adverse reactions reported were diarrhea and orthostatic hypotension, which occurred only at doses of MET-88 above 50 mg. In a multiple-dose study in which 12 healthy adult volunteers were given MET-88 orally at 400, 600, or 800 mg, b.i.d. for 8 d, there was no change in clinical laboratory parameters, but side effects including abdominal pain, loose stools, headache, orthostatic hypotension, twilight state, and diarrhea were reported.

SUMMARY

MET-88, an inhibitor of γ-butyrobetaine hydroxylase, can be characterized as a unique cardioprotective agent for the treatment of CHF with an ability to regulate the activity of SR Ca^{2+}-ATPase. MET-88 protected the hypoxic and ischemic myocardium by modulating the myocardial metabolism and improving cardiac remodeling and hypertrophy as effectively as captopril. MET-88 also restored Ca^{2+}-ATPase activity in the SR, possibly by increasing ATP synthesis through glycolysis. On the basis of these effects, MET-88 may be expected to improve mortality, prognosis, and exercise intolerance in CHF patients. Thus, MET-88 may be a useful drug for the treatment of CHF.
REFERENCES

1. Aoyagi T, Sugiura S, Eto Y, Yonekura K, et al. Inhibition of carnitine synthesis protects against left
synthesis, and insulin during hypoxia in isolated perfused rat hearts. Fundam Clin Pharmacol 1998;12:
158–163.
Eur Heart J 1994;93(Suppl):93.
4. Dahm PK, Grupp IL, Schwartz A, et al. Reduction of carnitine content by inhibition of its biosynthesis results
in protection of isolated guinea pig hearts against hypoxic damage. J Cardiovasc Pharmacol Ther 1996;
5. Garcia R, Diebold S. Simple, rapid, and effective method of producing aortocaval shunts in the rat.
8. Hashimoto K, Yabuuchi Y, Yamashita S, et al. Positive inotropic effect of 3,4-dihydro-6-[4-(3,4-
dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinolinone (OPC-8212) in the dog with experimentally-induced
ventricular remodeling in rats with chronic heart failure secondary to myocardial infarction. Jpn J Phar-
15. Kirimoto T, Hayashi Y, Miyake H, et al. The beneficial effects of MET-88 on changes in myocardial pH
and infarct size during coronary artery occlusion and reperfusion in dogs. Jpn J Pharmacol 1996;71(Suppl
1):P–349.
17. Lamers JMJ, Jonge-Stinis JT, Verdouw PD, et al. On the possible role of long chain acylcarnitine accu-
mulation in producing functional and calcium permeability changes in membranes during myocardial
18. Linck B, Eschenhage T, Scholz H, et al. Messenger RNA expression and immunological quantification of
phospholamban and SR-Ca(2+)-ATPase in failing and nonfailing human hearts. Cardiovasc Res 1996;31:
625–632.
19. Lopaschuk GD, Spafford M. Response of isolated working hearts to fatty acids and carnitine palmitoyl-
transferase I inhibition during reduction of coronary flow in acutely and chronically diabetic rats. Circ Res
hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine. Circ Res
22. Selye H, Bajusz E, Grasso S, et al. Simple techniques for the surgical occlusion of coronary vessels in the
202.
24. Xu KY, Zweier JL, Becker LC. Functional coupling between glycolysis and sarcoplasmic reticulum Ca$^{2+}$