—Review—

MET-88: Sarcoplastic Reticulum Ca^{2+}-Uptake Stimulator for Treating Chronic Heart Failure

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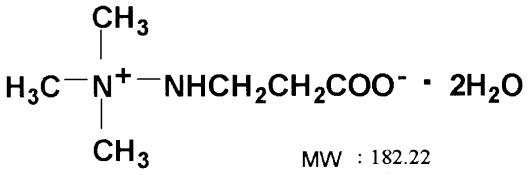
Abstract: MET-88, an inhibitor of γ-butyrobetaine hydroxylase, can be characterized as a unique cardioprotective agent for the treatment of congestive heart failure (CHF) with an ability to regulate the activity of SR Ca^{2+}-ATPase. MET-88 protected the hypoxic and ischemic myocardium due to the modulation of myocardial metabolism and improved cardiac remodeling and hypertrophy as effectively as captopril. MET-88 also increased the failed Ca^{2+}-ATPase activity in the Sarcoplastic reticulum (SR), which increase might have resulted from ATP synthesis through glycolysis. These effects of MET-88 may be expected to improve mortality, prognosis, and exercise intolerance in CHF patients. In summary, MET-88 may be a useful drug for the treatment of CHF.

Keyphrases: SR Ca^{2+} uptake、Glycolysis、MET-88、Myocardial function、Remodeling、Myocardial energy metabolism

Introduction

Dysfunction of the sarcoplastic 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.



3-(2,2,2-trimethylhydrazinium)propionate dihydrate

FIG. 1. Chemical structure of MET-88.

MET-88 [3-(2,2,2-trimethylhydrazinum) propionate] was synthesized by the Institute of Organic Synthesis (Riga, Latvia) as an inhibitor of γ-butyrobetaine hydroxylase, the enzyme that catalyzes the synthesis of carnitine from γ-butyrobetaine in the liver (23). This compound 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 myocardial infarction 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 due to the modulation of myocardial energy metabolism. Thus, MET-88 appears to be a unique cardioprotector that stimulates Ca^{2+} uptake by the cardiac SR occurring via 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-trimethylhydrazinium) propionate], is shown in Fig. 1. The compound was synthesized by the Institute of Organic Synthesis (Riga, Latvia) as an inhibitor of γ-butyrobetaine hydroxylase, the enzyme that catalyzes the synthesis of carnitine from γ-butyrobetaine in the liver (23). It has a

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molecular weight of 182.22; is freely soluble in water, glacial acetic acid, or methyl alcohol; is soluble in ethanol; and is slightly soluble in ether. Its melting point is 85 -90℃.

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/day) or a placebo for 10 days, the left anterior descending coronary artery (LAD) was occluded for 60 min; and 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 dose-dependently attenuated by MET-88 (Fig. 2). In addition, Asaka et al. (3) demonstrated that previous p.o. application of MET-88 (100 mg/kg/day) for 10 days 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. Aoyagi et al. (1) reported that MET-88, previously administered p.o. for 10 days 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, after 60 min of hypoxic perfusion and a subsequent 20 min of reoxygenation in the guinea pig isolated heart, previous p.o. application of MET-88 (100 mg/kg/day) for 10 days significantly improved the reduction in the mitochondrial respiratory function. In addition, the effect of MET-88 on changes in myocardial pH and infarct size during coronary artery occlusion and reperfusion in anesthetized open-chest 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 it tended to reduce the infarct size induced by repeated ischemia/reperfusion. Furthermore, MET-88 reduced ischemia and reperfusion-induced fibrillation in the anesthetized open-chest 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 decreased the extent of the depression of cardiac contractility (+dP/dt) and aortic flow during the hypoxic state (Fig. 3). It also prevented the

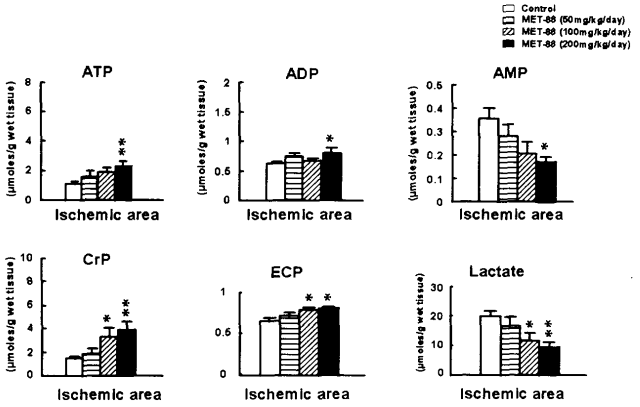
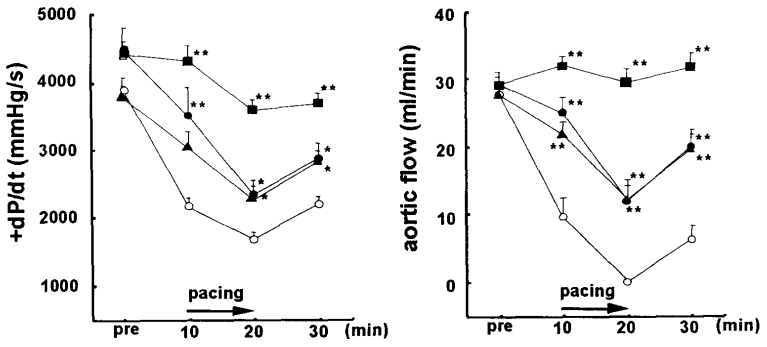


FIG. 2. Effects of oral pretreatment with MET-88 on the changes in tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP), energy charge potential (ECP), and lactate induced by occlusion of the left anterior descending coronary artery (LAD) for 60 min. Myocardial samples were taken from the ischemic area after the occlusion. Each value represents the mean ± S.E.M. (n=4-10). *P < 0.05, **P < 0.01 vs control group (Dunnett's multiple analysis).



○: Control, ▲: MET-88 100 mg/kg, ●: Insulin, and ■: MET-88 + Insulin.

Group	n	ATP (μ mol/g wet wt)	CrP (μ mol/g wet wt)	Long-chain acylcarnitine (n mol/g wet wt)
Normal	5	3.64 ± 0.15 **	5.97 ± 0.11 **	68 ± 9 **
Control	7	2.45 ± 0.14	2.75 ± 0.22	470 ± 21
MET-88	7	3.02 ± 0.13 **	3.99 ± 0.18 **	223 ± 11 **
Insulin	7	2.38 ± 0.07	3.76 ± 0.13 **	472 ± 10
MET-88 + Insulin	7	2.86 ± 0.04 *	4.01 ± 0.14 **	244 ± 18 **

FIG. 3. Effects of MET-88 and insulin on cardiac function, high-energy phosphate, and long-chain acylcarnitine in isolated perfused working hearts. The results are expressed as the mean ±S.E.M. (n = 4-10). *P < 0.05, **P < 0.01 vs. control group (Dunnett's multiple analysis).

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 is supported by the reports of Lopaschuk et al. (20) and Lopaschuk and Spafford (19), which indicated that the anti-ischemic action of carnitine

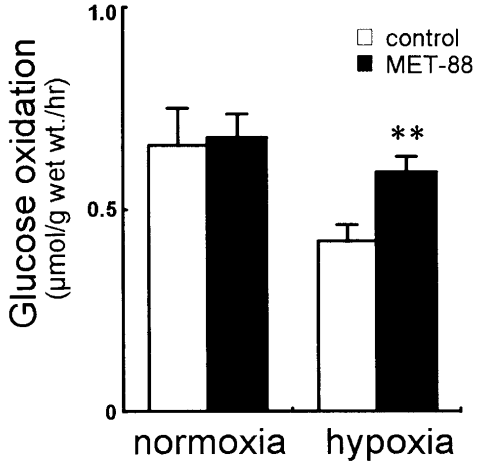


FIG. 4. Effect of MET-88 on steady-state rates of glucose oxidation during either normoxia or hypoxia. Substrate: [^{14}C]glucose, measured: [^{14}C]CO_2. The results are expressed as the mean ± S.E.M. (n = 3-9). **P < 0.01 vs control group (Student's t-test).

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 to be not critical for sarcolemmal sodium and calcium permeability and SR pumping activity (17). According to Lopaschuk et al. (20), carnitine acyltransferase I inhibitors have an anti-ischemic action through reduction of the β-oxidation of free fatty acids in mitochondria. Inhibition of the β-oxidation results in an increase in glucose utilization, and a decrease in 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 β-oxidation of free fatty acids, which reduction resulted from inhibition of carnitine synthesis. Therefore, inhibition of β-oxidation may be involved in the mechanism of the protective action of MET-88 against the ischemic-induced metabolic derangement.

Effects on experimental heart failure

Hayashi et al. (9) demonstrated that MET-88 protected against left ventricular dysfunction and ventricular remodeling in chronic myocardial ischemia. MI was induced by ligation of the LAD in male Sprague-Dawley (SD) rats (12,22). MET-88 (50, 100 mg/kg) was orally administered to the rats two days after surgery, and the treatment was then continued for twenty days. Hemodynamic studies were performed under the basal condition, preload stress (saline iv. infusion), and afterload stress (aortic occlusion) (12,22) at the end of the twenty-two day study period. In addition, left ventricular cavity volume as an index of remodeling was measured. MET-88 deceased the augmentation of right atrial pressure (RAP) and slightly

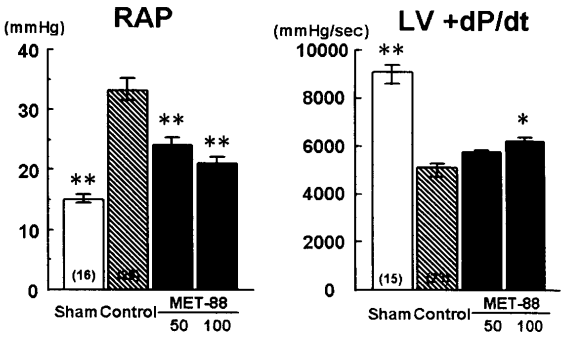


FIG. 5. Effects of MET-88 on RAP under volume-loading and LV+*dp/dt* under pressure-loading in rats with heart failure following myocardial infarction. MET-88 50: MET-88 50 mg/kg, MET-88 100: MET-88 100 mg/kg. RAP: right atrial pressure. The results are expressed as the mean ± S.E.M.(n) = number. *P < 0.05, **P < 0.01 vs control group (Dunnett's multiple analysis).

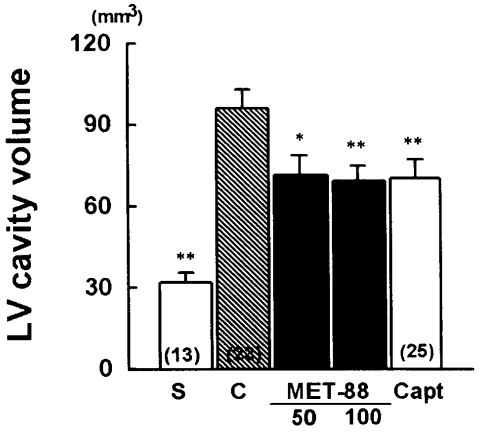


FIG. 6. Effects of MET-88 on left ventricular volume in the rats with heart failure following myocardial infarction. S: sham; C: control; MET-88 50: MET-88, 50 mg/kg; MET-88 100: MET-88, 100 mg/kg. Capt: captopril, 20 mg/kg. The results are expressed as the mean ± S.E.M.(n) = number. *P < 0.05, **P < 0.01 vs control group (Dunnett's multiple analysis).

increased the reduced LVpeak+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 left ventricular hypertrophy

measured in terms of cavity volume (Fig.6), and lung weight increase, whereas no change in left ventricular 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. Kd and Vmax were calculated from Lineweaver-Bark plot analysis. The rat left ventricular myocardium at 22 days after surgery for induction of myocardial infarction showed an increase in the Ca^{2+} uptake in the SR that was pCa 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 Kd but increased the Vmax (Fig. 7). At 22 days after induction of myocardial infarction in the rat left ventricular myocardium, the SR Ca^{2+} uptake decreased; and 100 mg/kg of MET-88 improved this decrease. In addition, analysis of Kd and Vmax 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 via improvement in the myocardial SR function. In addition, the cardiac SR Ca^{2+}-ATPase protein level was reduced by MI 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 by MI, but not in rats with MI given MET-88 (data not shown).

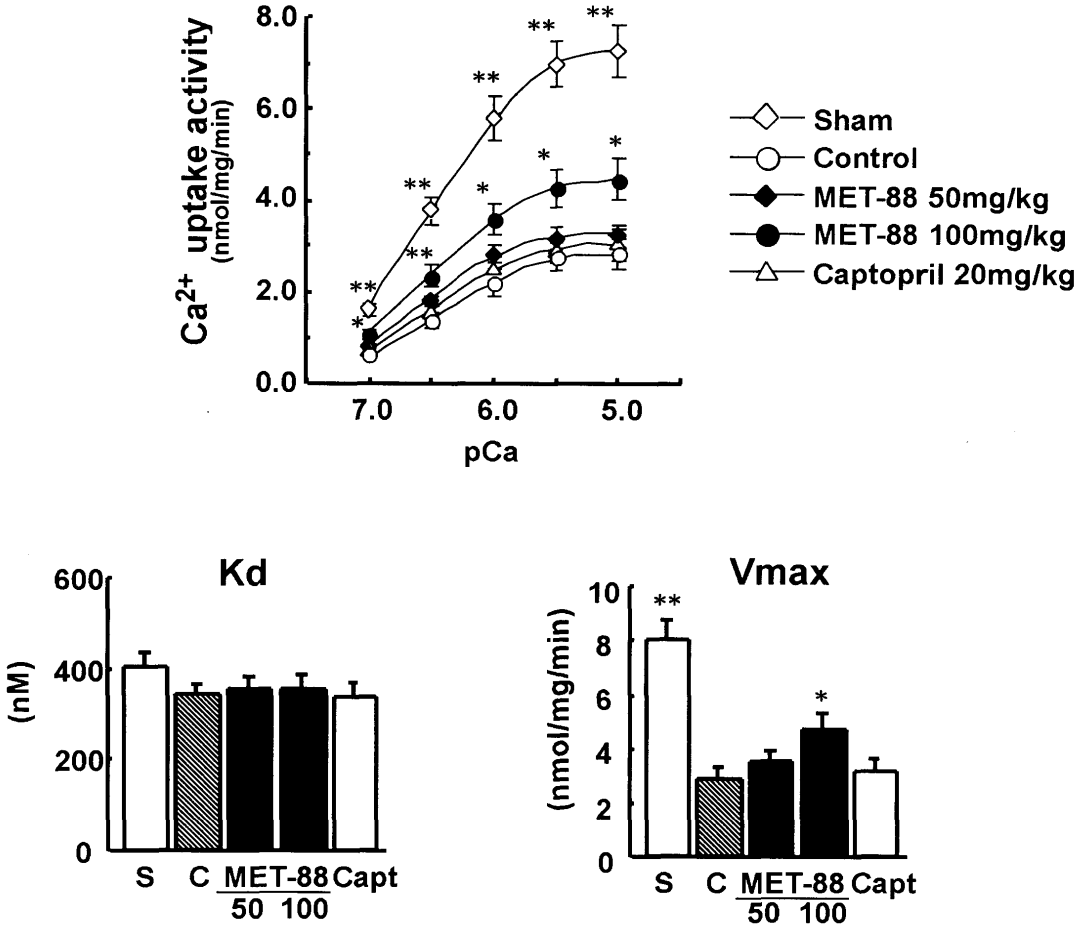


FIG. 7. Effects of MET-88 on Ca^{2+} uptake activity of cardiac sarcoplasmic reticulum (SR) in rats with heart failure following myocardial infarction. S: sham n = 16; C: control n = 16; MET-88 50: MET-88, 50 mg/kg, n = 17; MET-88 100: MET-88, 100 mg/kg, n = 15; Capt: captopril, 20 mg/kg, n = 16. The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

Kirimoto et al. (14) studied the effect of MET-88 on systemic and cardiac hemodynamics, and on heart weight in conscious right-heart failure (RHF) dogs (8,10). MET-88 (100 mg/kg, p.o. for 10 days) improved the right ventricular dysfunction (improved the 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 right-sided congestive heart failure (Fig. 8). Simultaneously, MET-88 reduced the absolute weight, 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 and pulmonary artery constriction and tricuspid valve avulsion in CHF because it may improve the failed Ca^{2+}-ATPase activity in the SR and postinfarction LV enlargement and hypertrophy.

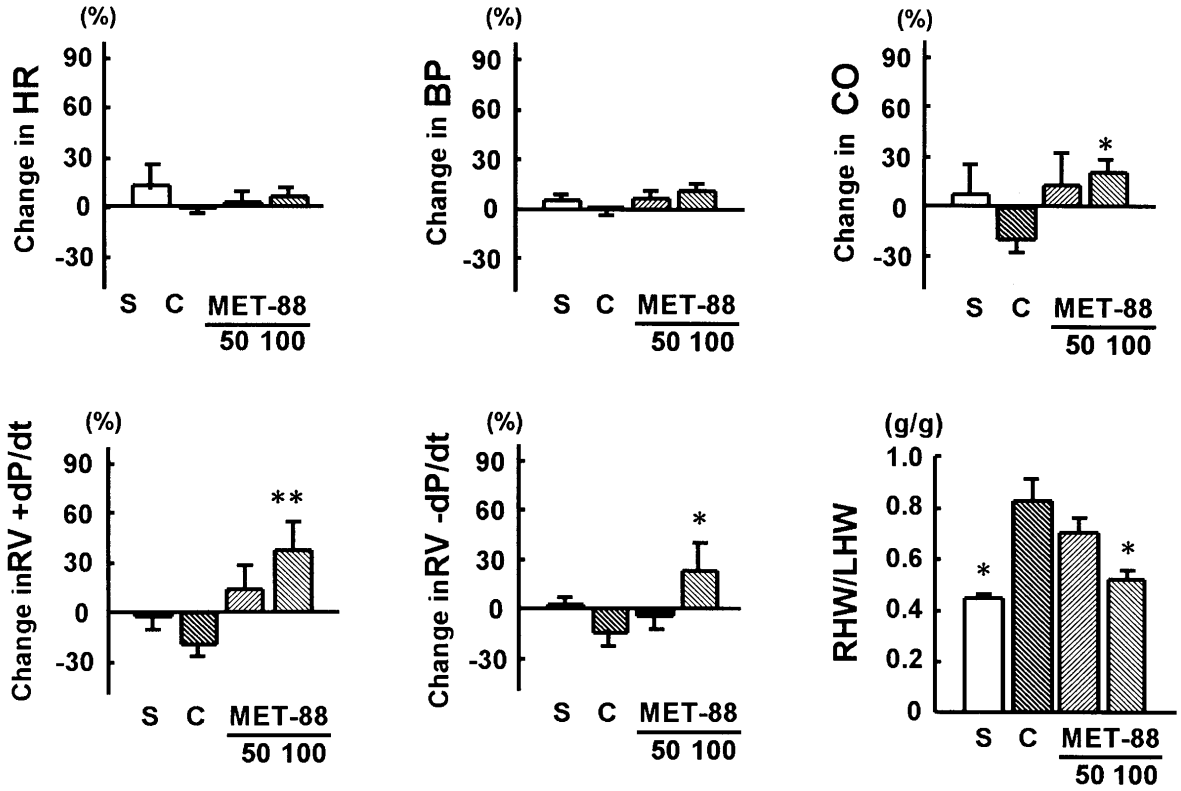
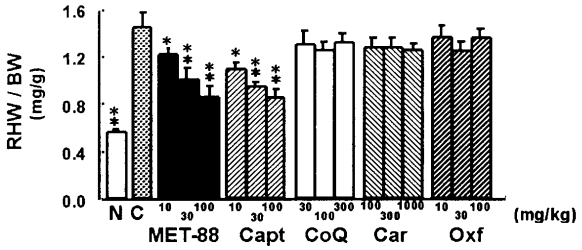


FIG. 8. Effects of MET-88 on cardiac function in conscious dogs with CHF produced by pulmonary artery constriction and tricupsid valve avulsion. S: sham n = 3; C: control n = 8; MET-88 50: MET-88, 50 mg/kg, n = 6; MET-88 100: MET-88, 100mg/kg, n = 6. RHW: right-sided heart weight; LHW: left-sided heart weight. The results are expressed as the mean ± S.E.M. *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 days from 3 weeks after the injection of monocrotaline (2%, 40 mg/kg, s.c.). The ratio of right-sided heart weight to body weight was 2.6 time higher in monocrotaline-treated rats than in non-treated rats. MET-88 dose-dependently reduced right-sided cardiac hypertrophy (ED_{50}: 34.7 mg/kg), as in the case of captopril (ED_{50}: 20.4 mg/kg). However, other metabolic modulators (L-carnitine, CoQ10, oxfenicine) had no effect (Fig. 9). In addition, four weeks after surgery for placement of an A-V shunt, the control group compared to 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 days, 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 A-V shunt rats. In *in vitro* studies, MET-88 had no effect on renin and angiotensin-converting enzyme 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, β-myosin heavy chain, and angiotensin II type 1 receptor were increased at 7 days 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



	ED_{50} (mg/kg)	Limits 95% (mg/kg)
MET-88	34.7	20.4-64.1
Captopril	20.4	9.4-33.2

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).

A-V shunt in rats because it may protect against 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 Cmax of 20.4 μg 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 Tmax of 0.58 h and a Cmax of 54.7 μg /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 administration of [2,3-^{14}C] 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 on day 21, it reached a plateau. The concentration at 24 h after the 28th dose was 3.4 times higher than that 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 C_{max} and AUC of MET-88 increased proportionally to the dose. The T_{max} was 1-2 h, and the $T_{1/2}$ 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 T_{max} but did not affect the C_{max} and AUC at a single oral dose of 400 mg. With multiple oral dosing of MET-88(twice daily for 7 days and once on the 8th day) at a dose of 400-800 mg/day, the plasma trough levels of MET-88 really reached the steady state 72 or 96 h after the first dose.

TOXICOLOGY

Acute Toxicity Studies

Single-dose toxicity studies on MET-88 were carried out in SD rats and male beagle dogs in order to obtain the potential of single-dose administration (unpublished observations). The approximate lethal doses of MET-88 were suggested to be >5000 mg/kg for rats and male dogs. The values indicated that the acute toxicity of MET-88 was similar in rats and dogs and that there were no sex-related differences in the acute toxicity.

Subacute Toxicity Studies

A 13-week repeated dose toxicity study of MET-88 was carried out in SD rats in order 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 weeks. The non-toxic dose level of MET-88 was suggested to be 25 mg/kg/day for female rats and 100 mg/kg/day for male rats. A 13-week repeated dose toxicity study of MET-88 was also carried out in beagle dogs in order to obtain the non-toxic dose level in these animals. MET-88 was given to the dogs (male: n = 4, female: n = 4 each) in daily doses up to 1600 mg/kg for 13 weeks. The non-toxic dose level of MET-88 was suggested to be 100 mg/kg/day for both male and female dogs.

Chronic Toxicity Studies

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

Fertility Study

A fertility study of MET-88 was carried out in SD rats. Rats were given MET-88 orrally at doses up to 1600 mg/kg (n = 48 each). Male rats were given the compound for 63 days before mating and during mating. Female rats received the drug for 14 days 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/day for general toxicity in parent animals (soft feces: only in males, 1600 mg/kg/day), 1600 mg/kg/day for reproductive function in parent animals, and > 1600 mg/kg/day for development of their fetuses.

Teratology Study

A teratology study of MET-88 was carried out in SD rats (unpublished observations). Pregnant rats were given MET-88 up to 5000 mg/kg from days 7 to 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 their fetuses and their offspring. 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 was 200 mg/kg/day for general toxicity in dams, > 5000 mg/kg/day for reproductive function of dams, and > 5000 mg/kg/day for development of their fetuses and offsprings of theses fetuses.

Mutagenicity Studies

MET-88 was studied for mutagenicity by use of 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 following adverse reactions, only after 50 mg of MET-88, were reported: diarrhea and orthostatic hypotension. In a multiple-dose study on 12 healthy adult volunteers, MET-88 at an oral dose of 400, 600, or 800 mg, 2 times a day for 8 days, caused no change in clinical laboratory parameters, but had side effects including abdominal pain, loose stools, headache, orthostatic hypotension, twilight state, and diarrhea.

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