Free Rad. Res. Comms., Vol. 17, No. 1, pp. 41–47
Reprints available directly from the publisher
Photocopying permitted by license only

EXTRACELLULAR-SUPEROXIDE DISMUTASE TYPE C (EC-SOD C) REDUCES MYOCARDIAL DAMAGE IN RATS SUBJECTED TO CORONARY OCCLUSION AND 24 HOURS OF REPERFUSION

GÖRAN WAHLUND, STEFAN L. MARKLUND and PER-OVE SJÖQUIST

Cardiovascular Pharmacology, Astra Hässle. S-431 83 Mölndal, Department of Clinical Chemistry, Umeå University Hospital, S-901 85 Umeå, Sweden

(Received February 1, 1992; in revised form April 3, 1992)

Extracellular-superoxide dismutase type C (EC-SOD C) is a secretory SOD isoenzyme which, in contrast to the intracellular CuZn SOD, has affinity to the endothelium and a long vascular half-life. In the present study, the effects of EC-SOD C and CuZn SOD on reperfusion-induced myocardial damage were determined in rats subjected to 10 min of left coronary artery ligation followed by 24 h of reperfusion. Recombinant human EC-SOD C (rh-EC-SOD C) or the corresponding volume of the vehicle was administered after completion of the coronary ligation. CuZn SOD was given in two equal doses, the first dose directly after ligation and the second one 6 h later. At the end of the reperfusion period the myocardial damage was quantified by measuring the creatine kinase concentration (CK) in the reperfused part of the left ventricular free wall (LVFW), and expressed as a percentage of the concentration in the non-ischemic septum. In the group given the vehicle, 47 ± 10 (mean ± SD) of the CK remained in the reperfused LVFW. In the rats receiving rh-EC-SOD C the corresponding values for each dose: 1.4, 4.2 and 12.6 mg/kg were 55 ± 12 (ns), 55 ± 12 (ns) and 65 ± 12% ($p < 0.05$, vs. vehicle, Dunnett's multiple comparison test), respectively. Administration of CuZn SOD (2 × 10 mg/kg) resulted in 58 ± 16% (ns) CK remaining in the LVFW.

It is concluded that rh-EC-SOD C, unlike CuZn SOD, significantly reduced myocardial damage associated with ischemia and reperfusion, and that the myocardial protection was sustained after 24 h of reperfusion.

KEY WORDS: Oxygen free radicals, myocardial ischemia, reperfusion, superoxide dismutase, infarct size

INTRODUCTION

Oxygen free radicals have been implicated as important mediators of ischemia/reperfusion-induced damage in several organs, including the heart.[1,2] Superoxide dismutase (SOD) has frequently been used in experimental studies as a free radical scavenger for treatment of reperfusion-induced myocardial damage. Positive effects have been reported in a variety of experimental models,[3–6] but results on final infarct sizes are conflicting.[7–9] Most studies have been performed with the intracellular low-molecular weight isoenzyme CuZn SOD, which is rapidly eliminated from the circulation owing to glomerular filtration.[10–12] Therefore, the difference in outcome of

Address for correspondence and reprint requests: P-O Sjöquist, PhD, Cardiovascular Pharmacology, Astra Hässle, S-431 83 Mölndal, Sweden. Telephone: +46 31676000.

these studies could reflect differences in dose regimes used or, in some cases, sub-optimal dosages of the enzyme.

Extracellular-superoxide dismutase C (EC-SOD C) is a secretory Cu- and Zn-containing glycoprotein.[13,14] The property that most clearly distinguishes this SOD isoenzyme from the other isoenzymes is its affinity for heparan sulfate, and in the body it is bound to heparan sulfate proteoglycan in the glycocalyx of cell surfaces and in the connective tissue matrix.[15,16] After intravenous injection, EC-SOD C is mainly sequestered by the vascular wall, forming an equilibrium with the plasma phase.[15] The half-life in the vasculature is long, being about 15 h in rabbits.[15] The pharmacokinetic properties, and especially the endothelium binding capacity, are thus distinctly different from those of CuZn SOD.

The aim of the present study was to evaluate the effects of i.v. administered recombinant human EC-SOD C on ischemia/reperfusion-induced myocardial injury and to compare the enzyme with the bovine CuZn SOD.

METHOD

Male Sprague-Dawley rats (275–372 g) were anesthetized with methohexital sodium (Brietal®, Lilly, Indianapolis, Ind., USA, 60 mg/kg i.p.) and placed on a heated operating table. The animals were intubated with a PE 190 catheter connected to an animal ventilator. After a left thoracotomy through the 4th or 5th intercostal space a 6-0 silk thread was sutured around the left coronary artery (LCA) 2–3 mm distal to its origin, and the LCA was ligated with a shoestring-knot. The air in the thorax was evacuated and the chest and skin were closed with silk sutures with one end of the occlusion suture exteriorized. During the occlusion and the first 5 min of reperfusion, artificial ventilation was maintained if necessary. Recombinant human EC-SOD C, bovine CuZn SOD or the corresponding vehicle (0.9% NaCl solution) was given as a bolus injection in the ventral tail vein directly after the LCA-ligation. The animals receiving CuZn SOD recieved an additional dose 6 h later. This dose regime of CuZn SOD significantly reduces CK-loss from ischemic and reperfused myocardium in rats,[17] and in rabbits 10 mg/kg is the optimal dose for reducing infarct size.[18] After 10 min of LCA occlusion, an ischemic time sufficient to produce myocardial infarction in the rat,[19] myocardial reperfusion was induced by pulling the exteriorized end of the occlusion suture, and the rats were then returned to their cages to recover. An analgesic drug (buprenorphinum, 0.2 mg/kg, Temgesic, Reckitt & Colman, Hull, UK) was given i.p. at 12-h intervals. Food and water were supplied ad libitum.

After 24 h of reperfusion the animals received an overdose of anesthetics, and their hearts were excised and placed in ice-cold 0.9% NaCl-solution. After removing the atria, connective tissue and the right ventricular free wall the left ventricle was sectioned into septum and left ventricular free wall (LVFW). The LVFW was further divided along the anterior papillary muscle giving the posterior part containing the area at risk (AR). The myocardial segments were then weighed, and the different parts were placed separately in a phosphate buffer solution containing 1 mM EDTA and 5 mM mercaptoethanol (pH 7.0) and homogenized in a glass–glass tissue homogenizer. The homogenates were centrifuged at 30 000 × *g* for 30 min at 2°C. The supernatants were decanted and creatine kinase (CK) activity was measured with a commercial reagent kit: Boehringer-Mannheim Gmbh, Cat. No 126349. The CK-content (U/g wet tissue) in the ventricular free wall was calculated as a percentage of the

TABLE 1
Effect of EC-SOD C and CuZn SOD on myocardial damage in rats after ischemia and 24 h reperfusion

	Dose (mg/kg)	n	CK activity		Mortality
			Septum (U/kg)	LVFM (% of septum)	
Vehicle	–	12	1918 ± 116	47 ± 10	(5/17)
EC-SOD	1.4	12	1746 ± 178	55 ± 12	(4/16)
EC-SOD	4.2	11	1783 ± 204	55 ± 12	(4/15)
EC-SOD	12.6	11	1763 ± 198	65 ± 12*	(3/14)
CuZn-SOD	2 × 10	12	1944 ± 163	58 ± 16	(6/18)

CK = creatine kinase, LVFM = left ventricular free wall, n = the number of rats surviving 24 h of reperfusion. All values are means ± standard deviations.
*$p < 0.05$ from vehicle. (Dunnett's multiple comparison test.)

content in the septum (not subjected to ischemia/reperfusion). Measurements of the depletion of tissue CK activity is a reliable and consistent method for evaluating myocardial ischemic damage, and it correlates well with histologic and morphometric measurements.[17,20]

In the present study the area at risk could not be measured, and consequently failure to occlude the LCA could not be established properly. In a small pilot study in 4 sham operated rats, CK content in the LVFW varied between 96 and 102% of that in the septum. To avoid including rats with semi-occluded coronary arteries, all rats with a CK content in the ischemic part of the LV exceeding 90% of the septum content were regarded as non-occluded individuals and excluded from this study. Two rats in the vehicle group, two in the CuZn-SOD group, and one in the 1.4 and 4.2 mg/kg EC-SOD groups, respectively, were excluded for this reason.

Recombinant human EC-SOD C[14] was obtained from Symbicom AB, Umeå, Sweden, and bovine CuZn-SOD was obtained from Boehringer-Mannheim Scandinavia, Bromma, Sweden. Three doses of rh-EC-SOD C were tested: 1.4 (n = 16), 4.2 (n = 15) and 12.6 mg/kg body weight (n = 14). CuZn-SOD was given twice in a dose of 2 × 10 mg/kg (n = 18). In the vehicle group a corresponding volume of 0.9% NaCl solution was administered (n = 17). The specific activity in the KO_2 assay[21] of the rh-EC-SOD C preparation was 116 000 U/mg, compared with 161 000 U/mg in the bovine CuZn-SOD preparation. 40 SOD units in the KO_2 assay correspond to one unit in the more commonly used xanthine oxidase—cyt C assay.[22]

Statistical analysis: Coronary artery ligation was performed in a total of 102 rats. Sixteen of the rats died during the ligation period and were not included in the study. The results are given as means ± SD in the text and table. Differences between groups were tested by Dunnett's multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The effect of rh-EC-SOD and CuZn-SOD on the degree of myocardial damage in rats subjected to ischemia and 24 h of reperfusion is summarized in Table I. There was a tendency towards a reduction of CK-loss in all groups receiving SOD treatment, but

it was possible to obtain statistical proof ($p < 0.05$) of the protective effect only for the group of animals receiving the highest dose of rh-EC-SOD C (12.6 mg/kg).

Of all the rats intended for this study, 16% died during the occlusion and these animals were not included in the mortality calculation. Twenty-two rats (27%) died during the reperfusion period. These animals, were evenly distributed among the different groups studied.

DISCUSSION

The present results show that rh-EC-SOD C given during occlusion significantly reduced the myocardial damage in rats subjected to coronary ligation and reperfusion, while CuZn SOD did not, although the rats in the latter group received a comparable amount of enzymic activity at occlusion and also an equal dose 6 h later. The difference in the degree of protection provided by the two SOD isoenzymes is probably related to differences in their pharmacokinetic properties. CuZn SOD has a plasma half-life in the rat of about 7 min[12] and will be present in significant amounts in the circulation for only limited periods during 24 h experiments, even if it is administered on two occasions as in this study. Intravenously injected EC-SOD C displays a half-life of 15 h in the rabbit vasculature[15] and has a similar kinetic profile in the rat (unpublished data). Furthermore, most of the injected EC-SOD C is sequestered by the vascular wall in equilibrium with the plasma phase.[15]

In vitro and *in vivo* studies on myocardial ischemia and reperfusion have shown that the peak formation of oxygen radicals occurs just after reperfusion.[23,24] However, in dogs subjected to 15 min of LAD occlusion and subsequent reperfusion, an increased formation of radicals was also demonstrated during the following 3 h observation period.[25] Thus, any salvaging effect of a scavenger with a short half-life might be insufficient to protect the heart for longer periods. In the reperfused myocardium, radicals are formed not only by cellular sources in the myocardium, such as xanthine oxidase,[26] but also by activated leukocytes that are attracted to the reperfused area.[27] SOD may protect the tissue by a direct effect on the radicals produced by the activated leucocytes and also by reducing the adhesion of leucocytes to the cardiac vasculature, which may be superoxide-dependent.[28,29] This adhesion phenomenon is likely to be operative for a long time. Myocardial salvage by CuZn SOD in the dog has mainly been shown after short reperfusion periods,[30–33] although failures have also been reported.[34] In contrast, studies with long observation times ($\geq$ 1 day) have often failed to demonstrate salvage.[11,35–39] The discrepancies may partially be due to sustained oxy-radical formation, and it has been reported that treatment with polyethyleneglycol-substituted CuZn SOD (PEG-SOD), which has a very long plasma half-life, could reduce the infarct size in a 4-day protocol.[40] However, in a recent similar study in the dog, PEG-SOD failed to salvage the myocardium,[41] and it is possible that the longer vascular half-life of EC-SOD *per se* is not the sole explanation for the efficacy of this enzyme compared with CuZn SOD in the present study.

A unique property of EC-SOD C is its affinity for heparan sulfate and after intravenous injection it will rapidly bind to heparan sulfate proteoglycanin the glycocalyx of the endothelium.[15] It is also possible that it is attracted to heparan sulfate deeper in the vascular wall and possibly to the cardiomycocytes. The local concentration of EC-SOD in these structures should be several orders of magnitude larger than in plasma at equilibrium.[16] This phenomenon may result in highly enhanced protection

of the cardiac vasculature and the myocardium. It has been shown in perfused organ models where CuZn–SOD or EC–SOD C is present in the medium during the entire experiments that EC–SOC C has higher protective efficacy than CuZn SOD at equal concentrations. EC–SOD was more efficient in reducing free radicals detectable by a spin trap in the reperfused rat heart,[24] and it appeared slightly more efficient in reducing injury in the reperfused rat heart.[42] It was also more efficient in reducing the increased post-ischemic vascular permeability in the hamster cheek pouch *in vivo* at a time when the plasma concentrations were similar for EC–SOD C and CuZn–SOD.[43] Finally, in a perfused rat heart model aimed specifically at probing the importance of vascular bound EC–SOD C, the enzyme was given only during a pre-ischemic period followed by a washout period with enzyme-free medium. At reperfusion following the ischemia, the pretreatment resulted in reduced CK loss to the effluent and a better preserved post-ischemic coronary perfusion.[44]

The discrepant results in the literature regarding the effects of SOD on *in vivo* infarct size reduction have been the subject of several recent reviews.[8,9] Several reasons for the disparate results have been put forward. The optimal observation time in the rat is not known, but we believe that our findings after 24 h of reperfusion do reflect true myocardial salvage. Much longer observation times may be complicated by resorption of injured tissue. The problems with rapid elimination of CuZn SOD, sustained oxy-radical formation and delay of injury vs true salvage have been discussed above. In the commonly used dog model, collateral flow varies considerably and some studies have failed to account for this problem. In the rat, the collateral flow is very low and homogeneous,[45] so this parameter should not have influenced our findings. The methods for estimation of injury have also been discussed. Depletion of tissue CK-activity has proved to be a reliable and consistent method for evaluation of ischemic damage and correlates well with histologic and morphometric measurements.[20] However, it should be noted that our findings differ from those of a previous almost identical study in the rat, in which CuZn SOD was found to significantly preserve the CK content of the myocardium.[17] In that study loss of CK in the controls was much less extensive (about 70% remaining). The favorable effect of CuZn SOD may be related to the less severe damage in that study.

To conclude, the present results show that EC–SOD C, unlike CuZn SOD, was able to protect the myocardium in a 10 min occlusion and 24 h reperfusion model. The positive effect of EC–SOD C may be related to both its long half-life in the circulation and its efficient binding to the vascular wall.

References

1. S.W. Werns, P.J. Simpson, J.K. Mickelson, M.J. Shea, B. Pitt and B.R. Lucchesi (1988) Sustained limitation by superoxide dismutase of canine myocardial injury due to regional ischemia followed by reperfusion. *Journal of Cardiovascular Pharmacology*, **11**, 36–44.
2. K.P. Burton (1988) Evidence of direct toxic effects of free radicals on the myocardium. *Free Radical Biology Medicine*, **4**, 15–24.
3. K. Ytrehus, S. Gunnes, R. Myklebust and O.D. Mjös (1987) Protection by superoxide dismutase and catalase in the isolated rat heart reperfused after prolonged cardioplegia: a combined study of metabolic, functional and morphometric ultrastructural variables. *Cardiovascular Research*, **21**, 492–499.
4. G. Ambrosio, L.C. Becker, G.M. Hutchin, H.F. Weisman and M.L. Weisfelt (1986) Reduction in experimental infarct size by recombinant human superoxide dismutase; insights into the pathophysiology of reperfusion injury. *Circulation*, **6**, 1424–1433.

5. K.P. Burton (1985) Superoxide dismutase enhances recovery following myocardial ischemia. *American Journal of Physiology*, **248**, H637–643.
6. S.R. Jolly, W.J. Kane, M.B. Bailie, G.D. Abrams and B.R. Lucchesi (1984) Canine myocardial reperfusion injury: its reduction by the combined administration of superoxide dismutase and catalase. *Circulation Research*, **54**, 277–285.
7. R. Engler and E. Gilpin (1989) Can superoxide dismutase alter infarct size? *Circulation*, **79**, 1137–1142.
8. R. Ferrari, C. Ceconi, S. Curello, S. Ghielmi and A. Albertini (1989) Superoxide dismutase: Possible therapeutic use in cardiovascular disease. *Pharmacological Research Communications*, **21**, suppl 2, 57–65.
9. R.A. Kloner, K. Przyklenk and P. Whittaker (1989) Deleterious effects of oxygen radicals in ischemia/reperfusion. *Circulation*, **80**, 1115–1127.
10. K.P. Gallagher, A.J. Buda, D. Pace, R.A. Gerren and M. Shlafer (1986) Failure of superoxide dimutase and catalase to alter size of infarction in conscious dogs after 3 hours of occlusion followed by reperfusion. *Circulation*, **73**, 1065–1076.
11. T. Miura, J.G. Downey, D. Hotta and O. Imura (1988) Effects of superoxide dismutase plus catalase on myocardial infarct size in rabbits. *Canadian Journal of Cardiology*, **4**, 407–411.
12. B. Odlind, L-E. Appelgren, A. Bayati and M. Wolgast (1988) Tissue distribution of ^{125}I-labelled bovine superoxide dismutase (SOD) in the rat. *Pharmacology and Toxicology*, **62**, 95–100.
13. S.L. Marklund (1982) Human copper-containing superoxide dismutase of high molecular weight. *Proceedings of the National Academy of Sciences of the United States of America*, **79**, 7634–7638.
14. L. Tibell, K. Hjalmarsson, T. Edlund, G. Skogman, Å. Engström and S.L. Marklund (1987) Expression of human extracellular-superoxide dismutase in Chinese hamster ovary cells and characterization of the product. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 6634, 6638.
15. K. Karlsson and S.L. Marklund (1988) Plasma clearance of human extracellular-superoxide dismutase C in rabbits. *Journal of Clinical Investigation*, **82**, 762–786.
16. S.L. Marklund and K. Karlsson (1989) Binding of human extracellular-superoxide dismutase C to cultured cell lines and to blood cells. *Laboratory Investigation*, **60**, 659–666.
17. N. Aoki, H. Bitterman, M.E. Brezinski and A.M. Lefer (1988) Cardioprotective actions of human superoxide dismutase in two reperfusion models of myocardial ischaemia in the rat. *British Journal of Pharmacology*, **95**, 735–740.
18. B.A. Omar, N.M. Gad, M.C. Jordan, S.P. Striplin, W.J. Russel, J.M. Downey and J.M. McCord (1990) Cardioprotection by CuZn-superoxide dismutase is lost at high doses in the reoxygenated heart. *Free Radical Biology and Medicine*, **9**, 465–471.
19. W. Schaper (1984) Experimental infarcts and the microcirculation. In: *Therapeutic Approaches to Myocardial Infarct Size Limitation* (eds. D.J. Hearse and D.M. Yellon), Raven Press, New York, pp. 79–90.
20. C.E. Hock, L.G.T. Ribeiro and A.M. Lefer (1985) Preservation of ischemic myocardium by a new converting enzyme inhibitor, enalaprilic acid, in acute myocardial infarction. *American Heart Journal*, **109**, 222–228.
21. S.L. Marklund (1985) Direct assay with potassium superoxide. In: *CRC-Handbook of Methods for Oxygen Radical Research* (ed. R.A. Greenwald). CRC Press, Boca Raton, Florida, pp. 249–255.
22. J.M. McCord and I. Fridovich (1969) Superoxide dismutase, an enzymic function for eryhtrocuprein. *Journal of Biological Chemistry*, **244**, 6049–6055.
23. J.H. Kramer, C.M. Arroyo, B.F. Dickens and W.B. Weglicki (1987) Spin trapping evidence that graded myocardial ischemia alters post-ischemic superoxide production. *Free Radical Biology Medicine*, **3**, 153–159.
24. M.H. Johansson, J. Deinum, S.L. Marklund and P-O. Sjöquist (1990) Recombinant human extracellular superoxide dismutase reduces concentration of oxygen free radicals in the reperfused rat heart. *Cardiovascular Research*, **24**, 500–503.
25. R. Bolli, M.O. Jeroudi, B.S. Patel, C.M. DuBose, E.K. Lai, R. Robert and P.B. McCay (1989) Direct evidence that oxygen-derived free radicals contribute to postischemic myocardial dysfunction in the intact dog. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 4695–4699.
26. J.M. Downey, D.J. Hearse and D.M. Yellon (1988) The role of xanthine oxidase during myocardial ischemia in several species including man. *Journal of Molecular and Cellular Cardiology* **20**, suppl 2, 55–63.

27. B.R. Lucchesi, S.W. Werns and J.C. Fantone (1989) The role of the neutrophil and free radicals in ischemic myocardial injury. *Journal of Molecular and Cellular Cardiology* **21**, 1241–1251.
28. M. Suzuki, W. Inauen, P. Kvietys, M.B. Grisham, C. Meninger, M.E. Schelling, H.J. Granger and D.N. Granger (1989) Superoxide mediates reperfusion-induced leukocyte-endothelial cell interactions. *American Journal of Physiology* **257**, H1740–1745.
29. M.B. Grisham, L.A. Hernandez and N. Granger (1986) Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *American Journal of Physiology*, **14**, G567–G574.
30. H. Nakazawa, K. Ban, K. Ichimori, K. Minezaki, H. Okino, T. Masuda, N. Aoki and S. Hori (1988) The link between free radicals and myocardial injury. *Japanese Circulation Journal*, **52**, 645–654.
31. S.W. Werns, M.J. Shea, E.M. Driscoll, C. Cohen, G.D. Abrams, B. Pitt and B.R. Lucchesi (1985) The independent effects of oxygen radical scavengers on canine infarct size: Reduction by superoxide dismutase but not catalase. *Circulation Research*, **56**, 895–898.
32. D.E. Chambers, D.A. Parks, G. Pattersson, R. Roy, J.M. McCord, S. Yoshida, L.F. Parmley and J.M. Downey (1985) Xanthine oxidase as a source of free radical damage in myocardial ischemia. *Journal of Molecular and Cellular Cardiology*, **17**, 145–152.
33. N. Hatori, A. Miyazaki,H. Tadokoro, L. Rydén, J. Moll, R.E. Rajagopalan, M.C. Fishbein, S. Meerbaum, E. Corday and J.K. Drury (1989) Beneficial effects of coronary venous retroinfusion of superoxide dismutase and catalase on reperfusion arrhythmias, myocardial function and infarct size in dogs. *Journal of Cardiovascular Pharmacology*, **14**, 396–404.
34. K. Przyklenk and R.A. Kloner (1989) Reperfusion injury by oxygen-derived free radicals: Effect of superoxide dismutase plus catalase, given at the time of reperfusion, on myocardial infarct size contractile function coronary microvasculature and regional myocardial blood flow. *Circulation Research*, **64**, 86–96.
35. J.M. Downey, T. Miura, J.L. Eddy, D.E. Chambers, T. Mellert, D.J. Hearse and D.M. Yellon (1987) Xanthine oxidase is not a source of free radicals in the ischemic rabbit heart. *Journal of Molecular Cellular Cardiology*, **19**, 1053–1060.
36. B.S. Patel, M.O. Jeroudi, P.G. O'Neill, R. Robert and R. Bolli (1990) Effect of human recombinant superoxide dismutase on canine myocardial infarction. *American Journal of Physiology* **258**, H369–H380.
37. A. Uraizee, K.A. Reimer, C.E. Murry and R.B. Jennings (1987) Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. *Circulation*, **75**, 1237–1248.
38. K.P. Gallagher, A.J. Buda, D. Pace, R.A. Gerren and M. Shlafer (1986) Failure of superoxide dismutase and catalase to alter size of infarction in conscious dogs after 3 hours of occlusion followed by reperfusion. *Circulation*, **73**, 1065–1076.
39. V.J. Richard, C.E. Murry, R.B. Jennings and K.A. Reimer (1988) Therapy to reduce free radicals during early reperfusion does not limit the size of myocardial infarcts caused by 90 minutes of ischemia in dogs. *Circulation*, **78**, 473–480.
40. Y. Tamura, L. Chi, E.M. Driscoll, P.T. Hoff, B.A. Freeman, K.P. Gallagher and B.R. Lucchesi (1988) Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardialischemia/reperfusion injury in canine heart. *Circulation Research*, **63**, 944–959.
41. M. Tanaka, R.C. Stoler, G.P. FitzHarris, R.B. Jennings and K.A. Reimer (1990) Evidence against the "early protection-delayed death" hypothesis of superoxide dismutase therapy in experimental myocardial infarction — Polyethylene glycol-superoxide dismutase plus catalase does not limit myocardial infarct size in dogs. *Circulation Research* **67**, 636–644.
42. P-O. Sjöquist, L. Carlsson, G. Jonasson, S.L. Marklund and T. Abrahamsson (1991) Cardioprotective effects of recombinant human extracellular-superoxide dismutase type C in rat isolated heart subjected to ischemia and reperfusion. *Journal of Cardiovascular Pharmacology*, **17**, 678–683.
43. M. Erlansson, D. Bergqvist, S.L. Marklund, N.H. Persson and E. Svensjö (1990) Superoxide dismutase as an inhibitor of postischemic microvascular permeability increase in the hamster. *Free Radical Biology Medicine*, **9**, 59–65.
44. P-O. Sjöquist and S.L. Marklund (1992) Endothelium bound extracellular superoxide dismutase type C reduces damage in reperfused ischaemic rat hearts. *Cardiovascular Research* **26**, 347–350.
45. S.H. Hale, M.T. Vivaldi and R.A. Kloner (1986) Fluorescent microspheres: a new tool for visualization of ischemic myocardium in rats. *American Journal of Physiology*, **20**, H863–H868.

Accepted by Prof. B. Halliwell