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INVITED COMMENTARY

SUPEROXIDE, IRON, VASCULAR ENDOTHELIUM AND REPERFUSION INJURY

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It is proposed that vascular endothelium has an intrinsic capacity to generate O_2^- for regulatory purposes such as inactivation of endothelium-derived relaxing factor. Ischaemia can disrupt the functioning of this oxidant-generating system, resulting in greater O_2^- generation when O_2 is restored. Ischaemia-induced cellular injury can also lead to release of iron ions, that, upon reperfusion, cause conversion of O_2^- and H_2O_2 to powerfully-oxidizing species (such as ·OH) that further injure the endothelium.

KEY WORDS: Ischaemia, reperfusion, endothelium, superoxide, iron, hydroxyl radical, nitric oxide, EDRF.

INTRODUCTION

The superoxide radical, O_2^-, is formed in most, if not all, aerobic cells, which contain the enzyme superoxide dismutase to scavenge it.[1] Superoxide dismutase works in conjunction with H_2O_2-metabolizing enzymes, catalase and especially glutathione peroxidase.[2] Much of the toxicity of O_2^- and H_2O_2 appears to arise by their metal-ion-dependent conversion into highly-reactive species, such as hydroxyl radical, ·OH (reviewed in[3]). Iron seems to be the most likely catalyst of these reactions *in vivo*.[3] Since cells contain an internal low-molecular-mass iron pool, removal of O_2^- and H_2O_2 before they can come into contact with this pool may be an essential part of intracellular defence against oxidant damage.[1,3,4]

Human blood plasma contains very low activities of SOD and glutathione peroxidase and almost no GSH. Thus direct enzymic scavenging of O_2^- and H_2O_2 does not seem to be a major part of extracellular antioxidant defence.[4] Instead, no free catalytic transition metal ions are present in plasma from healthy human subjects, and it has been argued that a major part of extracellular antioxidant defence is the sequestering of transition metal ions in forms incapable of stimulating radical reactions.[4,5,31] For example, iron bound to transferrin does not accelerate ·OH formation or lipid peroxidation.[3–5] Thus, although O_2^- and H_2O_2 can be generated in extracellular fluids (e.g. by activated phagocytic cells and by autoxidizing compounds), they would not normally react to form more dangerous oxidants. Indeed, it has been proposed that O_2^- and H_2O_2 generated at sites of inflammation may perform useful functions.[4,6–9] Thus O_2^- may be involved in neutrophil chemotaxis[6,7] and H_2O_2 may play a role in regulation of lymphocyte function,[8] of platelet aggregation[9] and of prostacyclin synthesis.[34,35] Such roles are possible, provided that O_2^- and H_2O_2 are not allowed to react and form powerful oxidants, such as ·OH.

ISCHAEMIA/REPERFUSION

Depriving a tissue of O_2 produces injury, which increases in extent as the period of ischaemia increases. However, reintroduction of O_2 can exacerbate the tissue damage, and oxygen radicals are involved in this process of reperfusion injury. Their involvement was first demonstrated in studies upon small intestine.[10,11] It was proposed that ischaemia causes rapid conversion of xanthine dehydrogenase to xanthine oxidase within the gut, combined with an accumulation of hypoxanthine resulting from degradation of ATP.[10–12] Thus, on reintroduction of O_2, the hypoxanthine is oxidized by xanthine oxidase with excessive production of O_2^- and H_2O_2.[10–12] Hence some protection against reperfusion injury can be achieved by including superoxide dismutase or catalase in the reperfusion medium.[10–12] Desferrioxamine is also protective in this model.[13] This chelating agent usually suppresses iron-dependent radical reactions[14,15] and its protective effect suggests that O_2^- and H_2O_2 produce damage, at least in part, by iron-dependent formation of more reactive radicals, such as ·OH.

Protective effects of SOD, catalase, desferrioxamine and other antioxidants have also been demonstrated in several reperfusion injury models in heart (reviewed in[12,16,17]). Many studies have used dog or rat hearts, which contain xanthine dehydrogenase activity.[12,16,17] However, the rate of conversion of dehydrogenase to oxidase during the ischaemic phase is very slow, suggesting that the model applicable to gut does not apply to heart.[18,19] Nevertheless, some xanthine oxidase activity appears to be present normally in heart of these species[18,19] and this enzyme might be able to increase radical production upon reperfusion simply as a result of the accumulation of hypoxanthine during ischaemia.

Involvement of oxidants in reperfusion injury can also be demonstrated in pig and rabbit hearts, which have been claimed not to contain xanthine oxidase.[20–22] Human heart has also been reported not to contain xanthine oxidase.[23,24] Debate continues as to whether the enzyme might be concentrated only in the vascular endothelium of the heart, so that assays of tissue homogenates might be insufficiently sensitive to detect the small amount present (reviewed in[23,24]).

THE ENDOTHELIUM

SOD, catalase and desferrioxamine penetrate into intact cells only slowly, yet they have been observed to protect efficiently against reperfusion injury in several experiments.[16,17,25,26] A recent study[27] has shown that the radicals important in producing myocardial "stunning", a form of reperfusion injury, in dog heart are generated within a few minutes of reperfusion and must be scavenged in that time frame for protection to occur.[27] These data, and other experiments,[25,26,28] suggest that the radical generation cannot occur deep within the tissue, but occurs at the endothelium.

The question then arises as to why the endothelium should have such apparently-dangerous potential for oxidant production. Studies of isolated endothelial cells suggest that they may constantly produce small amounts of O_2^-.[25,26,28] Apart from its barrier function, the vascular endothelium synthesises a wide range of products, including prostacyclin, tissue plasminogen activator, platelet activating factor and interleukin 1.[29] It has also been shown to produce a factor that relaxes smooth muscle, the so-called "endothelium-derived relaxing factor" EDRF.[29] EDRF is identical to nitric oxide, NO [reviewed –[29,30,32]]. EDRF can be inactivated by reaction with

O_2^-,[30,32] presumably by forming nitrate ion,

$$NO + O_2^- \rightarrow NO_3^- \quad (1)$$

It thus seems logical to hypothesize that the vascular endothelium has a system to produce O_2^- because the O_2^- interacts with EDRF and inactivates it in a *physiological* control mechanism. Any O_2^- that does not react with EDRF would presumably undergo non-enzymic dismutation to form H_2O_2. As previously,[4] I argue that generation of O_2^- and H_2O_2 is not intrinsically damaging because no metal ions are present to form highly-reactive species such as ·OH.

THE PROBLEM OF ISCHAEMIA/REPERFUSION

It therefore appears that endothelium has an inbuilt physiological mechanism for generating O_2^-. This may be xanthine oxidase, or it may be another system as yet undefined. What goes wrong upon ischaemia/reperfusion? Firstly, ischaemia may lead to an increase of O_2^- generation on subsequent reperfusion, e.g. by hypoxanthine accumulation as discussed earlier in this article. Ischaemia-dependent disruption of cellular electron transport chains, e.g. in mitochondria or plasma membrane, could also contribute to increased O_2^- generation when O_2 is reintroduced.[25] The ischaemia may also do something more significant, however. Cell injury is known to cause release of iron.[3,4,31] Thus the ischaemic period may cause release of iron into the system that, for a brief period upon reperfusion, (presumably until the ions are washed away by the blood or bound by plasma transferrin) is available to convert O_2^- and H_2O_2 into species such as ·OH that should greatly exacerbate the endothelial injury. Evidence for this proposed key role of iron is provided by the observation that, in ischaemia/reperfusion systems in which protective effects of SOD and catalase have been demonstrated, desferrioxamine is also usually protective.[13,16,17] My proposal explains why it appears to be essential to scavenge radicals or bind iron during the first few minutes of reperfusion in order to achieve protection.[27]

EXTRACELLULAR SUPEROXIDE DISMUTASE

Marklund[36,37] has shown that the *very low* SOD activity present in human plasma is largely due to a glycoprotein copper-zinc enzyme called "extracellular SOD" (EC-SOD). The activity of EC-SOD in human body fluids is insufficient to given them any significant ability to scavenge O_2^-.[38] Thus it has been proposed that EC-SOD is normally present attached to the surfaces of endothelial cells, offering localized protection.[39] If SOD is present at the endothelial cell surface, this might be taken to argue against the proposals made in the present paper. However, it does not seem clear from the literature what fraction of the endothelium (if any) would be coated with EC-SOD. The demonstrated ability of isolated endothelium to generate O_2^- (discussed earlier) and the marked protective effects of SOD during reperfusion injury, not only in isolated hearts but also in *in vivo* systems,[16,17] suggests that any EC-SOD present *in vivo* is offering little protection. However, the ability of EC-SOD to bind to cell surfaces does suggest that it might be exceptionally effective as an antioxidant protecting against reperfusion injury.[39] The precise physiological role of EC-SOD thus remains to be determined.

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References

1. Fridovich, I. *Annu. Rev. Pharmacol. Toxicol.*, **23,** 239–257, (1983).
2. Chance, B., Sies, H. and Boveris, A. *Physiol. Rev.*, **59,** 527–605, (1979).
3. Halliwell, B. and Gutteridge, J.M.C. *ISI Atlas Sci. Biochem.*, **1,** 48–52, (1988).
4. Halliwell, B. and Gutteridge, J.M.C. *Arch. Biochem. Biophys.*, **246,** 501–514, (1986).
5. Aruoma, O.I. and Halliwell, B. *Biochem. J.*, **241,** 273–278, (1987).
6. Martin, W., Loschen, G., Gunzler, W.A. and Flohe, L. *Agents Actions,* **16,** 48–49, (1985).
7. McCord, J.M. *Agents Actions,* **10,** 522–527, (1980).
8. Patterson, D.A., Rapoport, R., Patterson, M.A.K., Freed, B.M. and Lampert, N. *Arch. Surg.*, **123,** 300–304, (1988).
9. Del Principe, D., Menichelli, A., De Matteis, W., Di Corpo, M.L., Di Giulio, S. and Finazzi-Agro, A. *FEBS Lett.*, **185,** 142–146, (1985).
10. Parks, D.A., Bulkley, G.B., Granger, D.W., Hamilton, S.R. and McCord, J.M. *Gastroenterol.*, **82,** 9–15, (1982).
11. Granger, D.W., Rutili, G. and McCord, J.M. *Gastroenterol.*, **81,** 22–29, (1981).
12. McCord, J.M. *Fed. Proc.*, **46,** 2402–2406, (1987).
13. Hernandez, L.A., Grisham, M.B. and Granger, D.N. *Am. J. Physiol.*, **253,** G49–G53, (1987).
14. Gutteridge, J.M.C., Richmond, R. and Halliwell, B. *Biochem. J.*, **184,** 469–472, (1979).
15. Halliwell, B. *Biochem. Pharmacol.*, **34,** 229–233, (1985).
16. Bolli, R. *J. Am. Coll. Cardiol.*, **12,** 239–249, (1988).
17. Simpson, P.J. and Lucchesi, B.R. *J. Lab. Clin. Med.*, **110,** 13–30, (1987).
18. Engerson, T.D., McKelvey, T.G., Rhyne, D.B., Boggio, E.B., Snyder, S.J. and Jones, H.P. *J. Clin. Invest.*, **79,** 1564–1570, (1987).
19. Kehrer, J.P., Piper, H.M. and Sies, H. *Free Radical Res. Commun.*, **3,** 69–78, (1987).
20. Naslund, U., Haggmark, S., Johansson, G., Marklund, S.L., Reiz, S. and Oberg, A. *J. Mol. Cell. Cardiol.*, **18,** 1077–1084, (1986).
21. Downey, J.M., Miura, T., Eddy, L.J., Chambers, D.E., Mellert, T., Hearse, D.J. and Yellon, D.M. *J. Mol. Cell. Cardiol.*, **19,** 1053–1060, (1987).
22. Grum, C.M., Ragsdale, R.A., Ketai, L.H. and Shlafer M. *Biochem. Biophys. Res. Commun.*, **141,** 1104–1108, (1986).
23. Maxfeldt, M. and Schaper, W. *Basic Res. Cardiol.*, **82,** 486–492, (1987).
24. Eddy, L.J., Stewart, J.R., Jones, H.P., Engerson, T.D., McCord, J.M. and Downey, J.M. *Am. J. Physiol.*, **253,** H709–H711, (1987).
25. Marklund, S.L. *J. Mol. Cell Cardiol.*, **20,** (suppl. II) 23–30, (1988).
26. Ratych, R.E., Chuknyiska, R.S. and Bulkley, G.B. *Surgery,* **102,** 122–131, (1987).
27. Bolli, R., Jeroudi, M.O., Patel, B.S., Aruoma, O.I., Halliwell, B., Lai, E.K. and McCay, P.B. *Circ. Res.* submitted, (1988).
28. Zweier, J.L., Kuppusamy, P. and Lutty, G.A. *Proc. Natl. Acad. Sci. USA,* **85,** 4046–4050, (1988).
29. Vane, J.R., Gryglewski, R.J. and Botting, R.M. *Trends Pharm. Sci.*, **8,** 491–496, (1987).
30. Moncada, S., Radomski, M.W. and Palmer, R.M.J. *Biochem. Pharmacol.*, **37,** 2495–2501, (1988).
31. Stocks, J., Gutteridge, J.M.C., Sharp, R.J. and Dormandy, T.L. *Clin. Sci.*, **47,** 223–233, (1974).
32. Sneddon, J.M. and Vane, J.R. *Proc. Natl. Acad. Sci. USA,* **85,** 2800–2804, (1988).
33. Palmer, R.M.J., Ashton, D.S. and Moncada, S. *Nature,* **333,** 664–666, (1988).
34. Whorton, A.R., Montgomery, M.E. and Kent, R.S. *J. Clin. Invest.*, **76,** 295–302, (1985).
35. Harlan, J.M. and Callahan, K.S. *J. Clin. Invest.*, **74,** 442–448, (1984).
36. Marklund, S.L. *FEBS Lett.*, **184,** 237–239, (1985).
37. Marklund, S.L. *J. Clin. Invest.*, **74,** 1398–1403, (1984).
38. Blake, D.R., Hall, N.D., Treby, D.A., Halliwell, B. and Gutteridge, J.M.C. *Clin. Sci.*, **61,** 483–486, (1981).
39. Karlsson, K. and Marklund, S.L. *Biochem. J.*, **242,** 55–59, (1987).

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