Free Rad. Res. Comms., Vol. 1, No. 3, pp. 185–188
8755-0199/86/0103-0185 $25.00/0

Printed in Great Britain

PRO-OXIDANT ACTION OF SUPEROXIDE DISMUTASE IN THE AUTOXIDATION OF RIFAMYCIN SV

A. M. MICHELSON

Institut de Biologie Physico-Chimique
13, rue P. et M. Curie
75005 Paris, France.

(Received June 1st 1985)

Key words: Rifamycin, superoxide dismutase, autoxidation

INTRODUCTION

The antibiotic rifamycin SV has been extensively used as an anti-inflammatory drug in the treatment or rheumatoid knee synovitis, juvenile rhumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, intermittent hydrarthrosis and chondromatosis[1,2]. Since autoxidation of rifamycin SV to the quinone form rifamycin S occurs readily in aqueous solution, reducing agents such as ascorbic acid are often added. Bovine superoxide dismutase (Cu-SOD) is also used in the treatment of such diseases and as for rifamycin SV, by intra-articular injections. In many patients, intra-articular injection of rifamycin SV is followed by mild local pain[1]. It was therefore of interest to study the possible effects of Cu-SOD on rifamycin SV autoxidation with the objective of increasing the stability of the drug, and perhaps to reduce local irritation as well as providing a complementary anti-inflammatory action. However, addition of Cu-SOD to aqueous solutions of rifamycin SV increased the rate of oxidation to the quinone (and more toxic) form and clinical use of a combination of both drugs was not explored.

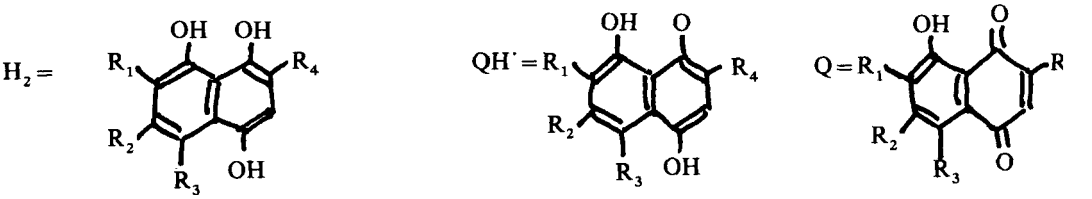
RESULTS AND DISCUSSION

Since rifamycin SV has λ_{max} = 445 nm (absorption of an aqueous solution at 0.05 mg/ml 1.02) and rifamycin S has λ_{max} = 525 nm (absorption 0.31 for 0.05 mg/ml) it is simple to follow oxidation to the quinone form spectroscopically. Solutions of rifamycin SV at 0.05 to 0.1 mg/ml were prepared in 0.1 M phosphate buffers at pH 7.0 and pH 7.8 (i.e. 0.7×10^{-4} M to 1.4×10^{-4} M) at 25°C and the absorption at 445 nm followed as a function of time, compared with solutions con-

taining 20 μg/ml of pure bovine Cu-SOD. The results are shown in Fig. 1a. It can be seen that oxidation of rifamycin SV is exponential and the rate is increased with increase in pH. In presence of SOD, a marked acceleration of oxidation is observed and the kinetics are no longer pseudo 1st order. In the presence of 10% ethanol a similar increased rate of autoxidation was observed when Cu-SOD (20 μg/ml) was present (Fig. 1b). However a lag phase of some 24 hours is observed perhaps due to the scavenging effect of ethanol for hydroxyl radicals produced by the action of H_2O_2 on the semiquinone. In addition, the effect of pH is abolished and kinetics at pH 7.8 are identical with those at pH 7.0 which are unchanged compared with non ethanolic buffer. Indeed, in presence of SOD the effect of pH is inversed and more rapid oxidation occurs at pH 7.0 than at pH 7.8.

Winterbourn has provided convincing explanations for this apparent pro-oxidising activity of SOD[3,4,5], in studies on menadione. The initial reaction is probably rifamycin SV plus O_2 to give O_2^- and the semiquinone in a reversible reaction.

$$QH_2 + O_2 \rightleftharpoons QH^{\cdot} + O_2^- + H^+ \quad (1)$$



$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (2)$$

The semiquinone can then react either with oxygen or with superoxide radicals (the latter acting either as a reductant or an oxidant).

$$QH^{\cdot} + O_2 \rightleftharpoons Q + O_2^- + H^+ \quad (3)$$

$$H^+ + QH^{\cdot} + O_2^- \longrightarrow QH_2 + O_2 \quad (4)$$

$$H^+ + QH^{\cdot} + O_2^- \longrightarrow Q + H_2O_2 \quad (5)$$

Finally, reaction of the semiquinone with H_2O_2 derived from O_2^- can give rise to hydroxyl radicals

$$QH^{\cdot} + H_2O_2 \longrightarrow Q + H_2O + HO^{\cdot} \quad (6)$$

which can then initiate oxidation of QH_2.

It is evident from reaction 4 that presence of SOD will increase the concentration of semiquinone, and that removal of O_2^- in reaction 3 will accelerate oxidation of $QH^{\cdot}$ to the quinone by allowing O_2 to compete more efficiently. Similarly, removal of O_2^- in reaction 5 by preferential dismutation to H_2O_2 can favorise reaction 6, which is an autocatalytic step in the general mechanism.

$$HO^{\cdot} + QH_2 \longrightarrow QH^{\cdot} + H_2O \quad (7)$$

This could explain the biphasic kinetics shown in Fig. 1a, the second phase being considerably delayed in presence of ethanol (trap for $HO^{\cdot}$) as indicated in Fig. 1b.

The action of SOD on the autoxidation of rifamycin SV to rifamycin S thus represents another example of an **apparent** pro-oxidant effect of the enzyme and suggests

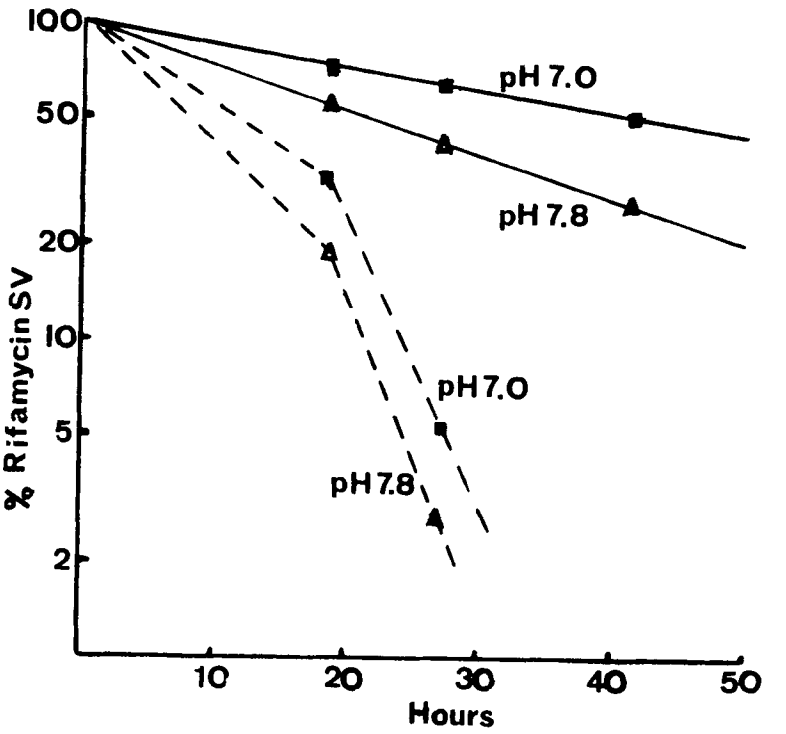


FIGURE 1a Autoxidation at 25°C of rifamycin SV to the quinone as a function of time in 0.1 M phosphate at pH 7.0 (■) and pH 7.8 (△) in absence (——) and in presence (---) of bovine Cu-SOD (20 μg/ml).

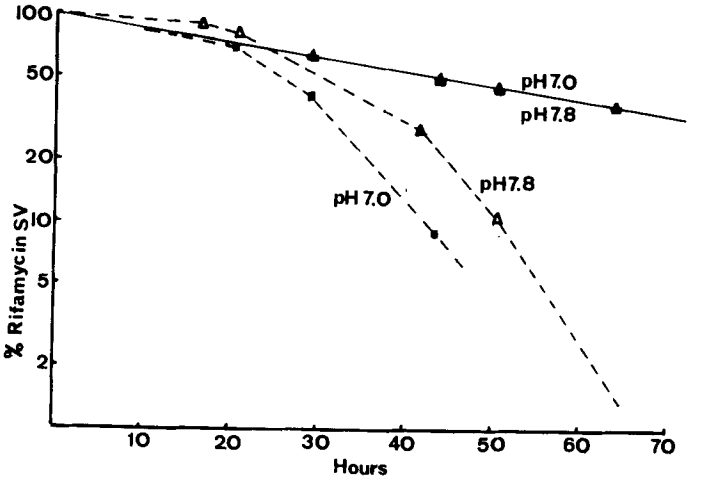


FIGURE 1b As for Fig. 1a but with 10% ethanol in the buffer.

that some reflection is advisable before indiscriminate or promiscuous application of superoxide dismutase to human pathological conditions.

References

1. I. Caruso, F. Montrone, M. Fumagalli, C. Patrono, P. Boselli and F. Scrollini, *Orthopedics*, **5**, 1336–1343, (1982).
2. I. Caruso, F. Montrone, M. Fumagalli, C. Patrono, S. Santandrea and M.C. Gandini, *Ann. Rheum. Dis.*, **41**, 232–236, (1982).
3. C.C. Winterbourn, *FEBS Letters*, **128**, 339–342, (1981).
4. C.C. Winterbourn, *Arch. Biochem. Biophys.*, **209**, 159–167, (1981).
5. C.C. Winterbourn, J.K. French and R.F.C. Claridge, *FEBS Letters*, **94**, 269–272, (1978).

Accepted by Dr. J.V. Bannister