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Application of various antioxidants in the treatment of influenza

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Abstract

We determined the effect of the antioxidants superoxide dismutase, desferrioxamine and allopurinol on the survival of male CBA mice infected intranasally with 2-5 LD_{50} lung influenza virus A/Aichi/2/68. Survival for at least 20 days was observed for 45% of the mice that received 1000 U/day superoxide dismutase prepared from red blood cells on days 5, 6, 7 and 8 after infection, and 75% survival was observed for mice that received the same dose on days 4, 5, 6, 7 and 8. Desferrioxamine, 25 mg/kg per day and 100 mg/kg per day injected subcutaneously, resulted in survival rates of 5 and 0%, respectively, compared to 10% survival observed for saline-injected controls. Allopurinol at doses of 5 to 50 mg/kg per day had no effect on mouse survival. These data demonstrate the efficacy of superoxide dismutase for the protection of mice against hemorrhagic lung edema. The ineffectiveness of allopurinol suggests that the xanthine oxidase system does not play a major role in hemorrhage or lung edema and that caution is necessary when desferrioxamine is administered during an acute inflammatory process accompanied by erythrocyte lysis.

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The question of how to effectively treat influenza infection after the onset of virus resistance to typical chemotherapeutic drugs is still unsolved (1). The effectiveness of most anti-influenza drugs correlates with the suppression of virus reproduction and this is why these medications are effective only during the early phase of infection when intensive reproduction of the virus takes place in the upper respiratory tract epithelium and are ineffective later during infection when virus multiplication no longer plays a major role in the development of the infection. The role of free radical processes in the pathogenesis of influenza-induced hemorrhagic lung edema has been reported and the potential treatment with antioxidant drugs has been discussed recently (2,3).

The objective of the present study was to compare the action of some antioxidants on mouse survival during influenza infection.

It has been proposed that superoxide radical ($O_2^{\bullet -}$) generation plays an important role in the pathogenesis of influenza infection and it is assumed that the xanthine-xanthine oxidase system is responsible for $O_2^{\bullet -}$ generation and for the activation of free radical processes during influenza (4). Since allopurinol is a powerful inhibitor of xanthine oxidase (5) we used this drug in our experiments.

We also studied superoxide dismutase

(SOD), an enzyme that catalyzes the inactivation of the superoxide radical by a dismutation reaction (5):

$$O_2^{\bullet-} + O_2^{\bullet-} + 2\ H^+ \longrightarrow H_2O_2 + O_2$$

Thus, SOD was used in our experiments to trap $O_2^{\bullet-}$ which is generally considered to be the major factor in oxygen toxicity (5).

The morphological substrate of influenza infection is hemorrhagic lung edema and influenza virus can induce erythrocyte hemolysis with the release of hemoglobin (6). The iron released from hemoglobin can catalyze the Haber-Weiss reaction forming hydroxyl radicals (7):

$$H_2O_2 + O_2^{\bullet-} \xrightarrow[Fe]{} O_2 + OH\bullet + OH^-$$

The reactivity of hydroxyl radicals is so great that, when formed in a living system, they will react immediately with whatever biological molecule is in their vicinity, thus producing secondary radical(s). Since the iron chelator desferrioxamine (DEF) is a powerful inhibitor of hydroxyl radical formation dependent on the presence of iron salts (8), DEF was used as the third drug in our experiments.

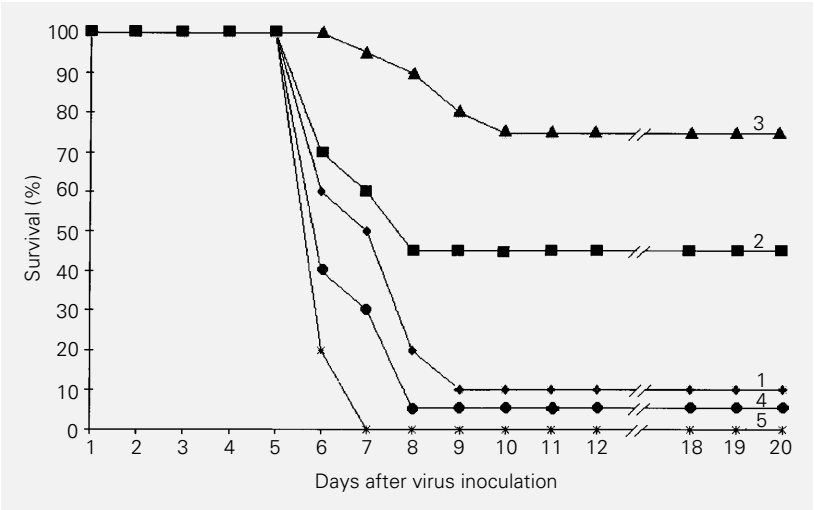

Figure 1 - Effect of antioxidant treatment on the mortality of mice infected with influenza virus. Male CBA mice were infected intranasally with 2-5 LD_{50} lung influenza virus A/Aichi/2/68 (H2N2). Data are reported as the mean of 3 independent experiments for which 20 mice were used for each group. *Curve 1*: Control group. *Curve 2*: SOD, 1000 U/day *iv* on days 5, 6, 7 and 8 after inoculation. *Curve 3*: SOD, 1000 U/day *iv* on days 4, 5, 6, 7 and 8 after inoculation. *Curve 4*: Desferrioxamine, 25 mg/kg per day *sc*, divided into two applications. *Curve 5*: Desferrioxamine, 100 mg/kg per day *sc*, divided into two applications.

Male mice of the CBA line were infected intranasally with lung influenza virus A/Aichi/2/68 (H2N2) adapted to mice at the dose of 2-5 LD_{50}. Desferrioxamine B (Desferal) was obtained from Ciba-Geigy (Basel, Switzerland) and administered subcutaneously at a daily dose of 5 to 150 mg/kg, twice a day. Human erythrocyte superoxide dismutase was obtained from AO Rosbio (St. Petersburg, Russia) and administered *iv* at the dose of 1000 U once a day. Allopurinol was purchased from Sigma (St. Louis, MO) and administered orally at doses of 5 to 50 mg/kg once a day.

All drugs were administered during the late period of infection on the 4th or 5th day after virus inoculation. The control group of infected mice received saline *iv* or heat-inactivated SOD.

Mouse survival curves for different treatments are shown in Figure 1. Mice that received saline or heat-inactivated SOD (curve 1) had similar 10% survival rates. SOD was effective (45% survival) in protecting the mice when given on days 5, 6, 7 and 8 (curve 2) and even more effective (75% survival) when treatment was started earlier, i.e., given on days 4, 5, 6, 7 and 8 (curve 3). Administration of DEF (curves 4 and 5) increased mortality when compared to saline-treated controls. These data demonstrate the protective effect of SOD against influenza virus mortality in CBA mice.

Allopurinol at doses of 5 to 50 mg/kg per day did not show any effect on mouse survival rate, which did not differ from control mice (data not shown). This lack of effect of allopurinol confirms the suggestion that the main source of free radicals during influenza-caused lung edema seems to be due to activated neutrophils (9) which appear in large numbers in the bronchoalveolar fluid and lungs of infected mice and not to the xanthine-xanthine oxidase system as proposed by others (4). It has been shown that

SOD can prevent $O_2^{\bullet -}$-dependent formation of a neutrophil chemotactic factor (10).

We suggest that in the present experiments SOD was effective mainly due to its ability to inhibit neutrophil influx into the lungs by decreasing the concentration of the chemotactic factor and therefore by rupture of the "feedback loop" during the inflammation process.

The results obtained with DEF were unexpected. Doses of 10, 25 and 50 mg/kg per day potentiated the effect of the virus and increased mortality (Figure 1, curve 4) and more than 100 mg/kg led to the rapid death of all the mice, i.e., 5-7 days after inoculation (Figure 1, curve 5). DEF at the dose of 5 mg/kg per day had no effect on the survival of infected mice. Since the LD_{50} for mice is 250 mg/kg per day (11), the death of mice inoculated with doses of 25 to 100 mg/kg per day was not due to the toxicity of DEF. Indeed, doses of 50, 100 and 150 mg/kg per day had no effect on healthy mice. It has been shown that DEF can stimulate the acute inflammatory induction phase of chronic allergic monoarthritis at low doses (12), presumably due to its prooxidant effect under certain conditions. Indeed in our experiments both low (10 mg/kg per day) and high (150 mg/kg per day) doses of DEF aggravated the condition of mice infected with influenza virus. The prooxidant properties of DEF have been reported by others (13-16). *In vitro* studies have shown that in the presence of O_2 and Fe(II), DEF is a prooxidant (17). In our experimental model the pathological process occurs in the lungs which have a high level of oxygen compared to other tissues. When this process is accompanied by erythrocyte lysis and hemoglobin release with an increase in Fe(II) concentration, conditions similar to those reported by Yegorov (17) probably occur, and therefore DEF acts as a prooxidant leading to aggravation of the pathological process and, in the case of infection with the influenza virus, to increased mortality.

The present results suggest that SOD can protect against the hemorrhagic lung edema caused by influenza virus and furthermore indicate that caution is necessary when DEF is administered during the acute inflammatory process accompanied by erythrocyte lysis. The lack of effect of allopurinol indicates that the xanthine-xanthine oxidase system does not play a major role in the hemorrhagic lung edema of mice infected with influenza virus.

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