

Activation of cannabinoid receptors reduces allergen-induced oxidative stress damage during asthma-like reaction in sensitised guinea-pigs

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Introduction

A large number of studies indicate that increased levels of oxygen-derived free radicals produced by inflammatory and epithelial cells in the airway are important mediators in the pathogenesis of asthma [1, 2]. Cannabinoids, reported to have antioxidant properties [3], are known to induce their biological effects by binding to specific cannabinoid receptors, designated CB₁ (preferentially expressed in brain) and CB₂ (highly expressed in immune cells) [4]. In this study we used the synthetic compound CP55,940 (CP), a non-selective agonist at CB₁ and CB₂ receptors, the selective antagonist at CB₂ receptors SR144528 (SR), and the selective agonist at CB₁ receptors AM251 (AM). We tested the anti-oxidative properties of CP in a well established in vivo model of allergic asthma-like reactions in sensitized guinea pigs.

Materials and methods

Forty eight male adult albino guinea pigs were used (350–400 g b. wt.; Harlan, Milan, Italy). The experimental protocol was the same used previously for similar purposes [5]. It complied with the recommendations of the European Economic Community (86/609/CEE) for the care and use of laboratory animals and was approved by the animal care committee of the University of Florence (Florence, Italy).

Group 1. Eight guinea pigs were injected with saline (5 ml/kg i. p., plus 5 ml/kg s. c.). They are referred to as naïve controls.

Other guinea pigs were sensitized with ovalbumin (OV, Fluka, Buchs, Switzerland; 100 mg/kg i. p., plus 100 mg/kg s. c.), dissolved in water to a concentration of 20 mg/ml. After 4–8 days, the sensitized animals were randomly divided in 4 further groups, 8 animals each, and treated as indicated below.

Guinea pigs were treated with an i. p. injection of 1 ml PBS (group 2) or with an i. p. injection of CP (Tocris Cookson, Bristol, UK; 0.4 mg/kg b. wt.; group 3), dissolved in 1 ml PBS. Three hours later, the animals underwent challenge with OV aerosol.

Other groups were treated with an i. p. injection of the CB₂ antagonist SR (kindly provided by Sanofi Recherche, Montpellier, France;

10 mg/kg b. wt.; group 4), dissolved in 1 ml PBS; with the CB₁ antagonist AM (Tocris Cookson, Bristol, UK; 10 mg/kg b. wt.; group 5), dissolved in 1 ml PBS; or with an i. p. injection of SR (10 mg/kg b. wt.) and of AM (10 mg/kg b. wt.), dissolved in 1 ml PBS (group 6). One hour later the animals of these groups were treated with CP, and after further three hours, underwent challenge with OV aerosol.

All guinea pigs were placed in a whole body respiratory chamber and challenged with an aerosol of OV (5 mg/ml in water) for 10 s, as previously described [5].

Once extracted from the respiratory chamber, guinea pigs were killed by lethal i. p. injections of sodium thiopental (Abbott, Latina, Italy). The thorax was then opened and tissue specimens from the middle and the lower lobes of the right lung were excised and processed for further analyses.

Malonyldialdehyde (MDA) was determined by measurement of the chromogen generated from the reaction of MDA with 2-thiobarbituric acid [6]. Manganese superoxide dismutase (MnSOD) activity was evaluated as described [5].

Experiments were performed at least in duplicate and the results were expressed as mean ± SEM. Statistical analysis was conducted using a paired Student's t-test.

Results and discussion

It is well known that MDA production is a suitable index of membrane lipid peroxidation and cell damage [7, 8] and that consumption of endogenous antioxidant enzymes, like SOD, is a typical effect of oxidative stress [5]. In our experiments, antigen challenge significantly increased MDA production (Fig. 1A) and decreased MnSOD activity (Fig. 1B) in sensitized guinea pigs of group 2 (indicated as OV) in comparison to naïve animals of group 1. In lungs from animals treated with CP, MDA production was significantly reduced and MnSOD activity was significantly higher. These protective effects were abrogated by pre-treatment with SR (but not with AM).

The results of this study indicate that treatment with the cannabinoid receptor agonist CP is accompanied by a marked reduction in signs of lung injury in response to antigen challenge. We can hypothesise the involvement of CB₂ receptor, since the antioxidative effects of CP were reversed by pre-treatment with SR but not with AM.

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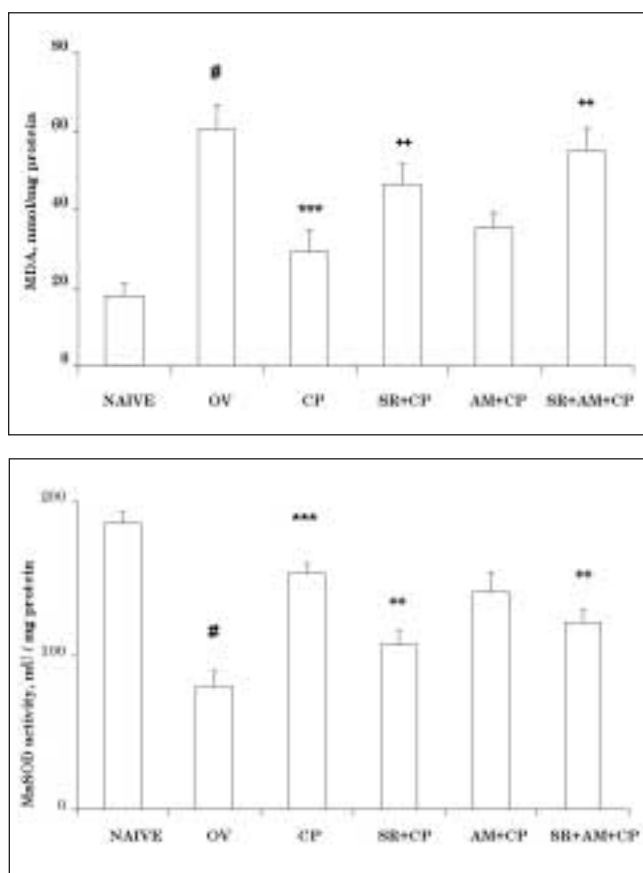


Fig. 1. Measurement of malonyldialdehyde (MDA) production (1A) and of manganese-SOD (MnSOD) activity (1B) in the experimental groups. # $p < 0.001$ vs NAIVE, *** $p < 0.001$ vs OV, ** $p < 0.005$ vs CP. NAIVE: untreated unsensitized animals; OV: untreated sensitized animals; CP: sensitized animals treated with CP55,940; SR+CP: sensitized animals treated with SR144528 and CP55,940; AM+CP: sensitized animals treated with AM251 and CP55,940; SR+AM+CP: sensitized animals treated with SR144528, AM251 and CP55,940.

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