Inhibition of leukotriene biosynthesis as a molecular basis for the anti-inflammatory actions of Salvinorin A

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Salvinorin A (SA), a neoclerodane diterpene, is the principal active component of *Salvia divinorum* (usually referred to as salvia) used by Shamans of the Mazatec Indians of Oaxaca for divinatory and religious purposes as well as in traditional healing practice for the treatment of inflammatory disorders, rheumatism and headache. SA is a potent and highly selective k-opioid receptor (KOR) agonist with anti-inflammatory effects (Capasso et al., 2008). In particular SA reduced the production of nitrites, tumor necrosis factor and interleukin 1beta and down-regulated the expression of inducible nitric oxide synthase in LPS-stimulated murine macrophages (Aviello et al., 2011). The role of SA in the production of leukotrienes (LTs), active lipid mediators with key roles in inflammation (Rådmark et al., 2015)], has never been studied in detail so far.

The aim of this study is to evaluate the effects of SA in *in vitro* (rat peritoneal macrophages activated with calcium ionophore A23187) and *in vivo* (mouse zymosan-induced peritonitis and rat carrageenan-induced pleurisy) models of inflammation related to LTs.

SA inhibited in a concentration-dependent manner (0.01-1 μM; 30 min before A23187, 0.5 μg/ml 60 min) LTB_4 production in activated rat peritoneal macrophages. Moreover the compound exerted anti-inflammatory effects in *in vivo* models of acute inflammation. In particular SA (1, 3 and 10 mg/kg i.p. 30 min before zymosan injection, 1 mg/mouse) reduced LTC_4 peritoneal levels 30 min after peritonitis induction (31% at 1 mg/kg and 63% and 81% at 3 and 10 mg/kg, respectively). This effect was associated to inhibition of acute inflammatory reaction evaluated as vascular permeability (30 min after peritonitis induction, 50% at 10 mg/kg) and cell recruitment (4h after peritonitis induction) [cell number (63% at 10 mg/kg) and myeloperoxidase (MPO) activity (71% at 10 mg/kg), a specific marker of polymorphonuclear cells]. We next studied the effects of SA in another LT-related model of inflammation: carrageenan-induced pleurisy in rats. The i.p. treatment (30 min before carrageenan administration, 0.2 ml 1%) of rats with 10 mg/kg of compound significantly reduced, 4 h after pleurisy induction, the inflammatory reaction measured as exudate volume (61%), inflammatory cell numbers (60%), MPO activity (30%) and LTB_4 (58%) levels in the pleural exudates. Moreover SA reduced lung injury (histological examination) as well as extracellular signal-regulated kinase activation.

In conclusion, our results demonstrate that SA exerts anti-inflammatory effects *in vitro* and *in vivo* by the LT inhibition and offer a novel therapeutic approach for the management of acute inflammation**.**

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Rådmark O (2015). Biochim Biophys Acta. 1851, 331-339.