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The hallucinogenic diterpene salvinorin A inhibits leukotriene synthesis in experimental models of inflammation

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ABSTRACT

Leukotrienes (LTs) are lipid mediators derived from arachidonic acid (AA) involved in a number of autoimmune/inflammatory disorders including asthma, allergic rhinitis and cardiovascular diseases. Salvinorin A (SA), a diterpene isolated from the hallucinogenic plant *Salvia divinorum*, is a well-established analgesic compound, but its anti-inflammatory properties are under-researched and its effects on LT production is unknown to date. Here, we studied the possible effect of SA on LT production and verified its actions on experimental models of inflammation in which LTs play a prominent role.

Peritoneal macrophages (PM) stimulated by calcium ionophore A23187 were chosen as *in vitro* system to evaluate the effect of SA on LT production. Zymosan-induced peritonitis in mice and carrageenan-induced pleurisy in rats were selected as LT-related models to evaluate the effect of SA on inflammation as well as on LT biosynthesis.

SA inhibited, in a concentration-dependent manner, A23187-induced LTB_4 biosynthesis in isolated PM. In zymosan-induced peritonitis, SA inhibited cell infiltration, myeloperoxidase activity, vascular permeability and LTC_4 production in the peritoneal cavity without decreasing the production of prostaglandin E_2. In carrageenan-induced pleurisy in rats, a more sophisticated model of acute inflammation related to LTs, SA significantly inhibited LTB_4 production in the inflammatory exudates, along with reducing the phlogistic process in the lung.

In conclusion, SA inhibited LT production and it was effective in experimental models of inflammation in which LTs play a pivotal role. SA might be considered as a lead compound for the development of drugs useful in LTs-related diseases.

1. Introduction

SalvinorinA (SA), a neoclerodane diterpene, is the major psychoactive component of *Salvia divinorum*, a plant of the mint family, long used for divinatory and religious purposes by the Mazatecs of Oaxaca, Mexico [1]. Traditionally, chewing fresh leaves, drinking the juice, infusion or smoked dry leaves of the plant have been utilized. Beside the recreational scope, *Salvia divinorum* preparations have been utilized also in healing practices for several disorders including rheumatism, pain, inflammatory bowel disease and headache [2].

Detailed studies have revealed that the pharmacological analgesic effects of SA in the central and peripheral system are mainly

Abbreviations: 5-LO, 5-lipoxygenase; AA, arachidonic acid; cys-LTs, cysteinyl-leukotrienes; DMSO, dimethyl sulfoxide; EIA, enzyme immunoassay; H&E, hematoxylin and eosin; HPLC, high-performance liquid chromatography; IL-10, interleukin 10; LPS, lipopolysaccharide; LT, leukotrienes; KOR, k-opioid receptors; MPO, myeloperoxidase; PBS, phosphate-buffered saline; PM, peritoneal macrophage; PMNLs, polymorphonuclear leukocytes; RIA, radioimmunoassay; SA, salvinorin A; TNF-α, tumor necrosis factor-α.

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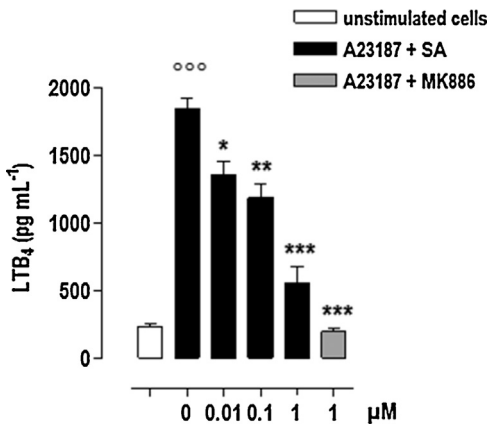

Fig. 1. Capture. Effect of salvinorin A on LTB_4 production in rat activated-peritoneal macrophages. SA (added 30 min prior to A23187) inhibits LTB_4 production in rat peritoneal macrophages (5×10^5 cells mL^{-1}) activated for 60 min with A23187 (0.5 µg mL^{-1}). LTB_4 levels in medium from cell incubations were quantified by EIA. Data are expressed as means ± SEM from $n = 3$ independent experiments performed in triplicates, each. °°° $p < 0.001$ versus unstimulated cells, * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ versus cells activated with A23187 in the absence of SA.

related to interaction of SA with the k-opioid receptors (KOR) [3–5]. Although the analgesic effects of SA are well established [6–9], little is known about its anti-inflammatory actions. It has been demonstrated that SA inhibits lipopolysaccharide (LPS)-stimulated nitrite and tumor necrosis factor (TNF)-α productionin peritoneal macrophages [10], and SA was shown to be effective in experimental inflammatory models *in vivo*, such as the LPS-induced endotoxemia, LPS- and carrageenan-induced paw oedema [10], formalin-induced inflammatory pain [11] and, finally, dinitrobenzene sulfonic acid and dextran sodium sulfate-induced colitis in mice [12].

Among the different models used to study the anti-inflammatory properties of SA there is no knowledge about its possible activity on leukotriene (LT) biosynthesis and on LT-related hallmarks of inflammation.

LTs are bioactive lipid mediators with a key role in the inflammatory process such as the induction of smooth muscle contraction, chemotaxis, vascular permeability and leukocyte extravasation [13]. For LT biosynthesis, arachidonic acid (AA) is released from membrane phospholipids through the action of cytosolic phospholipase A_2 and then rapidly converted by 5-lipoxygenase (5-LO) into the unstable epoxide LTA_4. This intermediate can then be either hydrolyzed into the chemotactic agent LTB_4 by LTA_4 hydrolase or conjugated with reduced glutathione by LTC_4 synthase to form LTC_4. LTC_4 is further metabolized by sequential proteolytic hydrolysis to LTD_4 and LTE_4, which are together known as cysteinyl-LTs (cys-LTs) with prominent actions on smooth muscle cells.

Here, we have investigated the effects of SA on LT biosynthesis and evaluated its effect on experimental models of *in vitro* and *in vivo* acute inflammation, such as activated rat peritoneal macrophages, mouse zymosan-induced peritonitis and rat carrageenan-induced pleurisy, where LTs play pivotal roles. These models are useful for understating the mechanisms of inflammation and testing novel anti-inflammatory molecules [14].

2. Materials and methods

2.1. Materials

SA was isolated from leaves of *S. divinorum*, extracted and purified (purity: 99% by HPLC; Fig. 1S Supplementary data) as described in detail elsewhere [15]. Enzyme immunoassay (EIA) kits were from Cayman Chemical Company (Aurogene, Rome, Italy). [^{3}H-

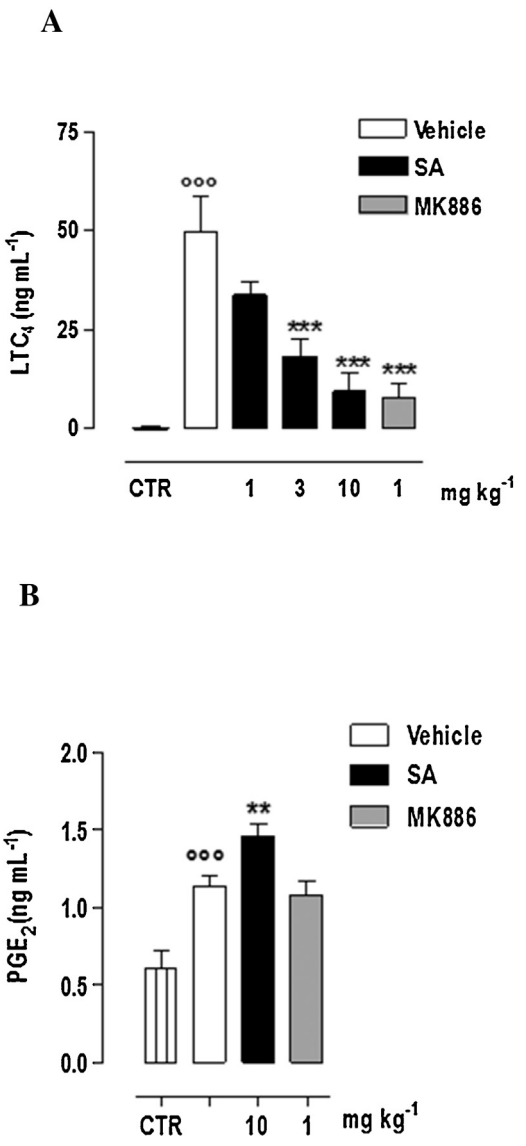

Fig. 2. Capture. Effect of salvinorin A on LTC_4 and PGE_2 production in zymosan-induced peritonitis. (A) SA (1, 3, 10 mg kg^{-1}, i.p. 30 min before zymosan injection) inhibits LTC_4 production in peritoneal exudates 30 min after zymosan injection. LTC_4 levels were quantified by EIA. (B) Effect of SA pre-treatment (10 mg kg^{-1}, applied i.p. 30 min before zymosan injection) on PGE_2 production 4 h after zymosan injection. Control group (CTR) was treated i.p. with saline (0.5 mL) instead of zymosan. Data are expressed as means ± SEM from $n = 6$–7 animals for each group. °°° $p < 0.001$ versus CTR; *** $p < 0.001$ and ** $p < 0.01$ versus vehicle.

PGE_2] was from PerkinElmer Life Sciences (Milan, Italy). All other reagents and fine chemicals were obtained from Sigma–Aldrich (Milan, Italy).

2.2. Animals

CD-1 mice (30–40 g) and Wistar rats (250–300 g) were housed in a controlled environment (21 ± 2 °C) and provided with standard rodent chow and water. All animals were allowed to acclimate for four days prior to experiments and were subjected to 12 h light–12 h dark schedule. Experiments were conducted during the light phase. The experiments described have been carried out in accordance with Italian regulations on protection of animals used for experimental and other scientific purpose (Ministerial Decree 116/92) as well as with the European Economic Community regulations (Official Journal of E.C. L 358/1 12/18/1986).