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Two for one: Cyclic AMP mediates the anti-inflammatory and anti-spasmodic properties of the non-anesthetic lidocaine analog JMF2-1

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ABSTRACT

Inhalation of JMF2-1, an analog of lidocaine with reduced anesthetic activity, prevents airway contraction and lung inflammation in experimental asthma models. We sought to test if the JMF2-1 effects are a consequence of increased intracellular cAMP levels in asthma cell targets, such as smooth muscle cells and T cells. Functional effect of JMF2-1 on carbachol-induced contraction of intact or epithelial-denuded rat trachea was assessed in conventional organ baths. cAMP was quantified by radioimmunoassay in cultured guinea pig tracheal smooth muscle cells, as well as lymph node cells from BALB/c mice, exposed to JMF2-1. We found that JMF2-1 (0.1–1 mM) concentration-dependently inhibited epithelium-intact tracheal ring contraction induced by carbachol challenge. The antispasmodic effect remained unaltered following epithelium removal or pretreatment with NG-nitro-L-arginine methyl ester (100 μM), but it was clearly sensitive to 9-(tetrahydro-2-furyl) adenine (SQ22,536, 100 μM), an adenylate cyclase inhibitor. JMF2-1 (300 and 600 μM) also dose-dependently increased cAMP intracellular levels of both cultured airway smooth muscle cells and T lymphocytes. This effect was consistently abrogated by SQ22,536 and reproduced by forskolin in both systems. JMF2-1 induced apoptosis of anti-CD3 activated T cells in a mechanism sensitive to zIETD, indicating that JMF2-1 mediates caspase-8-dependent apoptosis. Furthermore, forskolin also inhibited anti-CD3 induced T cell proliferation and survival. Our results suggest that JMF2-1 inhibits respiratory smooth muscle contraction as well as T cell proliferation and survival through enhancement of intracellular cAMP levels. These findings may help to explain the anti-inflammatory and antispasmodic effects of JMF2-1 observed in previous studies.

1. Introduction

Asthma is a chronic lung inflammatory disease characterized by airway hyperresponsiveness and lung inflammation, generally marked by eosinophil and lymphocyte infiltration (Barnes, 2004; Moore, 2008). Combination of a bronchodilator with glucocorticoids, can successfully control most of the patients, but a minority of them are resistant to this conventional therapy (Adcock et al., 2008).

The local anesthetic lidocaine has been used as an alternative therapy to control asthma symptoms in patients that respond poorly to classical glucocorticoid treatment (Hunt et al., 2004; Saito et al., 2006). However, the safety and usefulness of lidocaine are debatable since some patients demonstrated irritation of the airways and initial bronchoconstriction, which were associated with its local anesthetic activity (Burches and Warner, 2008; Harrison and Tattersfield, 1998; Hirota et al., 1999).

We have recently reported the pharmacological properties of lidocaine analogs synthesized and screened for reduced local anesthetic activity. Changes in the aromatic ring of lidocaine led to JMF2-1 (2-diethylamine-2′-trifluormethyl-acetoanilide hydrochloride) (Fig. 1), an analog able to combine reduced local anesthetic activity with increased antispasmodic and anti-inflammatory properties (Costa et al., 2008; da Costa et al., 2007). Nebulization with JMF2-1 is a way of achieving the anti-asthma properties of lidocaine without anesthesia, encouraging further research on the mechanism of action and putative therapeutic application of JMF2-1. Our previous study showed that JMF2-1 prevents ovalbumin (OVA) induced lung inflammation and airway hyperresponsiveness by inhibiting activation and survival of T cells (Olsen et al., 2011).

Since the mode of action of JMF2-1 remained poorly understood, the aim of this study was to investigate the molecular mechanisms by which JMF2-1 reduces inflammation and bronchospasm.

2. Material and methods

2.1. Animals

Male guinea pigs (300–400 g) and Wistar rats (250–350 g), as well as BALB/c (18–20 g) and DO11.10 TCR Tg mice were obtained from the Oswaldo Cruz Foundation breeding colonies (Rio de Janeiro,

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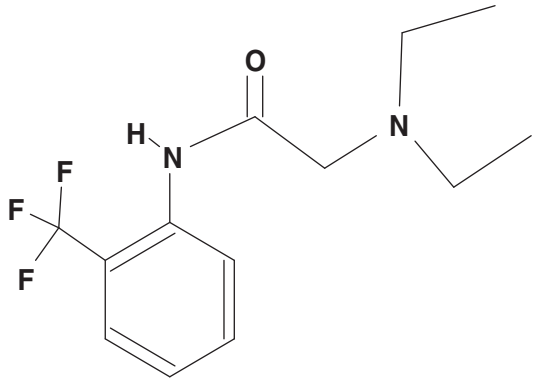

Fig. 1. Chemical structure of JMF2-1.

Brazil). Procedures involving care and use of laboratory animals were examined and approved by the Animal Ethics Committee of Oswaldo Cruz Foundation (CEUAFIOCRUZ, LW-23/10), Rio de Janeiro, Brazil.

2.2. Chemicals

JMF2-1.HCl was synthesized in the Department of Organic Synthesis of Far-Manguinhos (FIOCRUZ, Rio de Janeiro, RJ, Brazil) from 2-trifluormethyl-aniline, with a chromatography purity of >99.8% (Fig. 1). OVA (grade-V), N^{ω}-nitro-l-arginine methyl ester (L-NAME), Propranolol, Forskolin, SQ22,536 and IBMX were purchased from Sigma–Aldrich (St. Louis, MO, USA). JMF2-1 and Propranolol were dissolved in sterile saline solution immediately before use. Forskolin, SQ22,536 and IBMX were freshly prepared in DMSO (final concentration of 0.1%).

2.3. T cell apoptosis and proliferation

Pooled cervical, axial and inguinal lymph node cells (10^6/well) obtained from naïve DO11.10 TCR Tg mice were pre-treated with 50 μM zIETD.fmk (Calbiochem, San Diego, USA) for 1 h before stimulation with OVA (0.5 mg/ml) and exposure to 300 μM JMF2-1. Treatments lasted 72 h at 37 °C in an atmosphere of 5% CO_2. Apoptosis was assessed as previously described (Olsen et al., 2011).

Pooled cervical, axial and inguinal lymph node cells (10^6/well) from BALB/c mice were stimulated with anti-CD3 (10 mg/ml) (BD Biosciences Pharmingen) and treated with either 300 μM JMF2-1 or 100 μM forskolin for 72 h at 37 °C in an atmosphere of 5% CO_2. Proliferating values and DNA fragmentation of retrieved cells were analyzed by permeabilizing and staining cells with propidium iodide (PI), as previously described (Olsen et al., 2011).

2.4. cAMP intracellular levels in T lymphocytes and airway smooth muscle cells

Intracellular cAMP concentrations were assayed in primary cultured guinea-pig tracheal smooth muscle cells as previously described (Wu et al., 2001). Briefly, smooth muscle cells obtained from guinea pig tracheas were cultured in DMEM containing 10% fetal bovine serum, 100 units/ml of penicillin, 100 mg/ml of streptomycin and 2 mM of glutamine for 3 to 7 days. After the third cell splitting, 10^6 cells/well were grown in 24-well plates. At confluence, monolayer cells were washed with PBS and incubated with JMF2-1 (10–1000 μM) or 100 μM forskolin (adenylate cyclase activator), in the presence or absence of 100 μM SQ22,536 (adenylate cyclase inhibitor) for 30 min. All incubations were done in the presence of 100 μM IBMX, a phosphodiesterase inhibitor. Lysed cells were collected and the cAMP was determined by using a radioimmunoassay kit (TRK 432–Cyclic AMP[^{3}H] Biotrak assay system—Amersham Pharmacia Biotech, Buckinghamshire, England).

Intracellular cAMP levels in lymphocytes were also analyzed. Pooled cervical, axial and inguinal lymph node cells (10^6/well) from BALB/c mice were stimulated with 10 μg/ml anti-CD3 (Pharmingen) and treated with JMF2-1 (100, 300 and 600 μM) or forskolin (100 μM), in the presence or absence of 100 μM SQ22,536, for 20 min. All incubations were done in the presence of 100 μM IBMX. Lymphocytes were lysed and cAMP levels were measured as described above.

2.5. Isolated tracheal preparation and measurement of tension

Tracheas from Wistar rats were prepared as described previously (Coelho et al., 2008). Briefly, animals were killed in a CO_2 atmosphere and tracheas were quickly immersed in Krebs' nutritional solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$, and 11 mM glucose). Tracheas were dissected then cut into rings. These tracheal rings were mounted in isolated organ baths filled with 10 ml of Krebs' solution, maintained at 37 °C, and aerated with 95% O_2 and 5% CO_2. To achieve a steady spontaneous tone level, an initial tension of 1 g was applied. Contractions were measured isometrically with a force–displacement transducer (Ugo Basile, Comerio, Italy) and recorded by an Isolated Organs Data Acquisition program (Proto5; Letica Scientific Instruments, Barcelona, Spain). Tissues were allowed to stabilize for 60 min, whereas the bathing solution was exchanged at 10-min intervals. At the end of the equilibration period, the response to 2.5 μM carbachol was recorded. After washout of carbachol and re-establishment of stable baseline tone, tissues were re-exposed to carbachol (10^{-8}–10^{-4} M) in the presence or absence of JMF2-1 (100, 300 and 1000 μM) or lidocaine (300 and 1000 μM). The preparations were preincubated with JMF2-1 or lidocaine 15 min before addition of the spasmodic agent. All responses were expressed as percentages of the response to 2.5 μM carbachol.

In a particular experiment, the epithelial cells were removed mechanically as described previously (Coelho et al., 2008). During the experiment, the contractile response to 2.5 μM carbachol was measured before and after a 15-min exposure to 1000 μM JMF2-1 of intact or denuded epithelium tracheal rings. To further investigate the mechanisms of action of JMF2-1, the tracheal rings were pretreated 10 min before its application with 100 μM L-NAME (nitric-oxide synthase inhibitor), 100 μM SQ22,536 or 1 μM propranolol (β_2-receptor blocker).

2.6. Statistical analyses

Statistical analyses were done with ANOVA followed by the Newman–Keuls–Student test. $P<0.05$ (two-tailed test) was considered significant.

3. Results

3.1. Effect of JMF2-1 on rat tracheal contractions induced by carbachol

Fig. 2 shows the cumulative concentration–response curves of rat tracheal rings triggered by increasing concentrations of carbachol (10^{-8}–10^{-4} M) against either JMF2-1 (100–1000 μM) (part A) or lidocaine (300 and 1000 μM) (part B). Notably, the pre-treatment with JMF2-1 significantly reduced, in a concentration-dependent manner, the maximal contractile response elicited by carbachol, inhibiting the maximal effect of contraction in 67.0% ± 1.9% (n = 8) at the highest concentration, as it shifted the concentration–response curves for carbachol to the right. In contrast, the prototype lidocaine shifted the carbachol response to the right and significantly inhibited its maximal response (15.2% ± 0.9%, n = 8) only at the highest concentration tested.

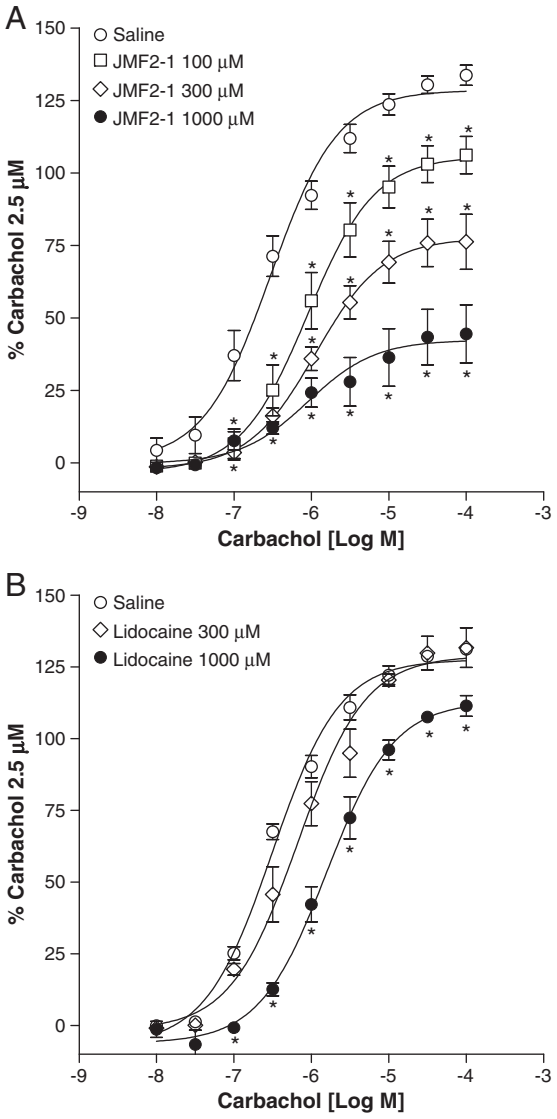

Fig. 2. JMF2-1 induces rat tracheal relaxation. Anti-spasmodic effects of JMF2-1 (100–1000 μM) (Part A) and lidocaine (300 and 1000 μM) (Part B) on the rat tracheal contraction induced by carbachol. Each point represents the mean ± S.E.M. of eight experiments. All results were expressed as a percentage of contractile responses induced by 2.5 μM carbachol. *$P<0.05$ compared with tracheal responses from vehicle-treated preparations. +$P<0.05$ compared with tracheal responses from JMF2-1-treated preparations.

3.2. Role of epithelium, nitric oxide, β_2-adrenoreceptor and adenylate cyclase in the antispasmodic effect of JMF2-1

We studied whether the epithelium would be implicated in the effect of JMF2-1 by mechanically removing the epithelial cells of the internal tracheal surface as reported previously (Coelho et al., 2008). The results showed that JMF2-1 equally inhibited carbachol-induced tracheal contraction in the presence or absence of epithelium as shown in Fig. 3A. This protective response also remained unaltered following pretreatment with 100 μM L-NAME, as added to the medium 10 min before exposure to JMF2-1 (Fig. 3B). In contrast, the protective effect of JMF2-1 upon carbachol-induced contractile responses was clearly sensitive to the pretreatment with the adenylate cyclase inhibitor 100 μM SQ22,536, as shown in Fig. 3C. In addition, propranolol pretreatment did not change the antispasmodic activity of JMF2-1 as shown in Fig. 3D.

3.3. JMF2-1 increases intracellular cAMP levels in airway smooth muscle cells

To check the impact of JMF2-1 treatment on the adenylate cyclase/cAMP pathway, we studied the pattern of the intracellular cAMP levels of primary cultured smooth muscle cells in the absence or presence of increasing concentrations of JMF2-1 (10–1000 μM). As expected, forskolin significantly enhanced cAMP levels in these cells (Fig. 4). Similarly, JMF2-1 (100 and 1000 μM) increased concentration dependently the intracellular cAMP content, an effect which was clearly abrogated by pretreatment with the adenylate cyclase inhibitor SQ22,536 (100 μM). No changes in cAMP levels were noted between untreated and DMSO-treated groups (data not shown).

3.4. T cell apoptosis induced by JMF2-1 is mediated by caspase-8 activation

We have previously shown that JMF2-1 induces caspase-dependent apoptosis of T cells (Olsen et al., 2011). Since increase in intracellular cAMP levels in T cells has led to caspase-8 activation and blockade of cytokine secretion (Naderi and Blomhoff, 2008), we sought to investigate whether JMF2-1 would activate the extrinsic (Fas-mediated) apoptotic pathway. Lymph node cells from naïve DO11.10 mice were pre-treated with the caspase-8 inhibitor 50 μM zIETD for 1 h and then exposed to ovalbumin (0.5 mg/ml) in the presence or absence of 300 μM JMF2-1 for 72 h. As noted in Fig. 5, zIETD prevented the pro-apoptotic effect of JMF2-1.

3.5. Adenylate cyclase activation enhances apoptosis and inhibits proliferation in T cells from mice

As demonstrated before in Jurkat human cells, cAMP elevation induced by an adenylate cyclase activator, forskolin, potentiates Fas-induced apoptosis (Naderi and Blomhoff, 2008). Similar to JMF2-1, forskolin induces both apoptosis (Fig. 6A) and blockade of proliferative response (Fig. 6B) of T cell following anti-CD3 stimulation.

3.6. JMF2-1 increases intracellular cAMP levels in anti-CD3 stimulated T cells

Since elevation of cAMP levels has been implicated in the inhibition of T lymphocyte activity and survival (Bjorgo and Tasken, 2006; Naderi and Blomhoff, 2008; Vendetti et al., 2006), we looked further at the generation of cAMP by anti-CD3 stimulated lymph node T cells following exposure to increasing concentrations of JMF2-1. As indicated in Fig. 7, treatment with JMF2-1 (100–600 μM) for 30 min induced a concentration-dependent increase in the intracellular cAMP levels of anti-CD3-stimulated T cells, in a mechanism clearly sensitive to the adenylate cyclase inhibitor SQ22,536 (100 μM). Forskolin also led to cAMP upregulation which was, as expected, prevented by SQ22,536 (Fig. 7). No changes in cAMP levels were noted between untreated and DMSO-treated groups (data not shown).

4. Discussion

In the current study, we demonstrate that the non-anesthetic lidocaine analog JMF2-1 leads to an adenylate cyclase-dependent increase in the intracellular levels of cAMP in both airway smooth muscle cells and activated T lymphocytes. In line with this data, the adenylate cyclase inhibitor SQ22,536 partially prevents the antispasmodic effect of JMF2-1 on carbachol-induced tracheal ring contraction. Furthermore, we show that JMF2-1 treatment increases cAMP intracellular levels on anti-CD3 stimulated T cells, which might be associated with the induction of apoptosis and the reduction of activity of lymphocytes induced by JMF2-1. These findings support the

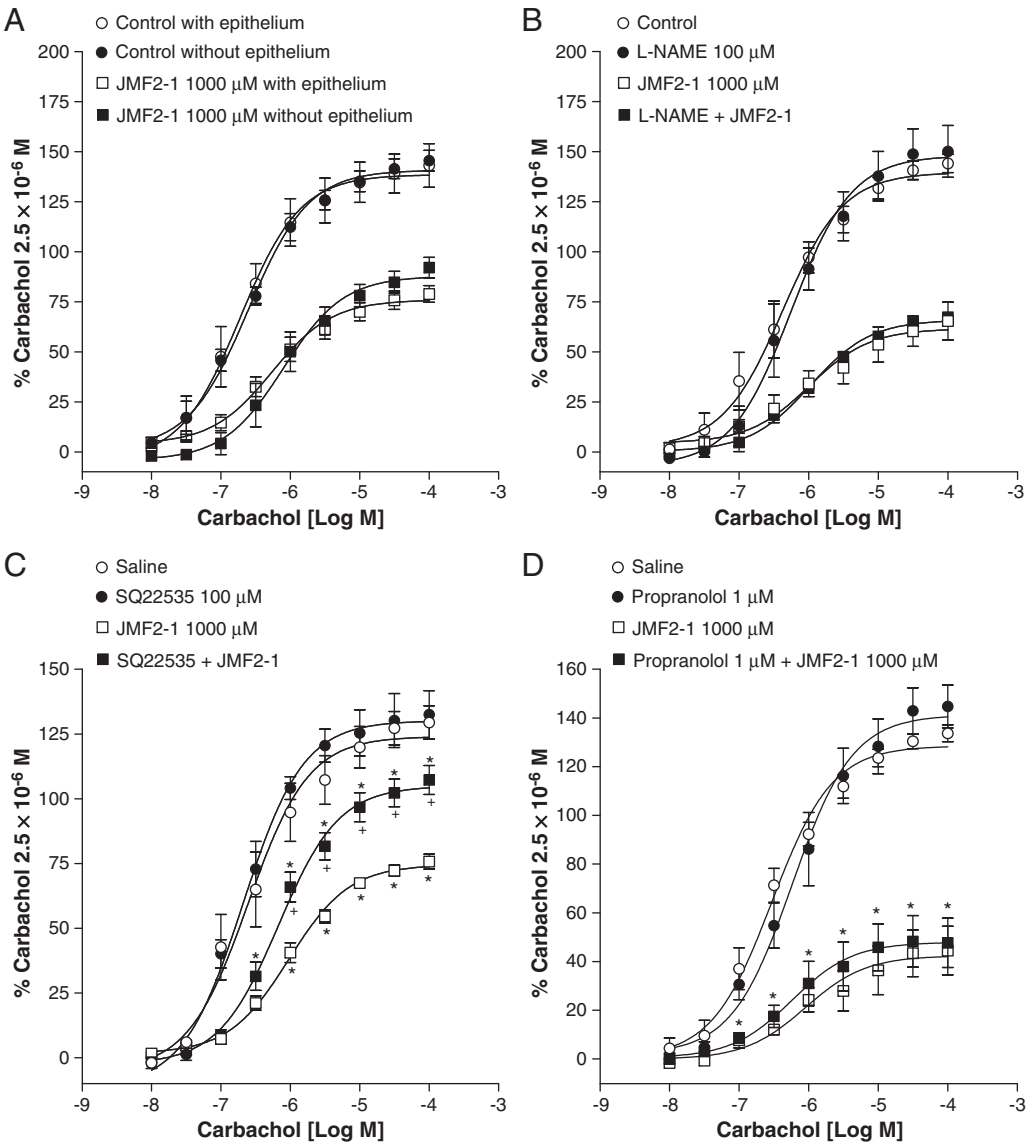

Fig. 3. JMF2-1-induced tracheal relaxation is dependent on adenylate cyclase. Anti-spasmodic effects of JMF2-1 (1000 μM) on the rat tracheal contraction induced by carbachol (10^{-8}–10^{-4} M) in the absence or presence of epithelium (A), L-NAME (100 μM) (B), SQ22,536 (100 μM) (C) or propranolol (1 μM) (D). Each point represents the mean ± S.E.M. of eight experiments. All results were expressed as a percentage of contractile responses induced by 2.5 μM carbachol. *P<0.05 compared with tracheal responses from vehicle-treated preparations. +P<0.05 compared with tracheal responses from JMF2-1-treated preparations.

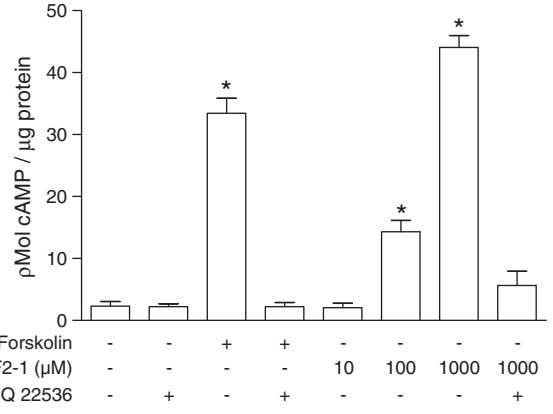

Fig. 4. JMF2-1 increases intracellular cAMP levels in airway smooth muscle cells. Effect of JMF2-1 (10–1000 μM) treatment on guinea pig tracheal smooth muscle cells cAMP intracellular levels. Cells were treated for 20 min with either JMF2-1 or forskolin in the presence or absence of SQ22,536. Values represent the mean ± S.E.M (n = 3) (representative of two experiments). *P<0.05 as compared to untreated cells.

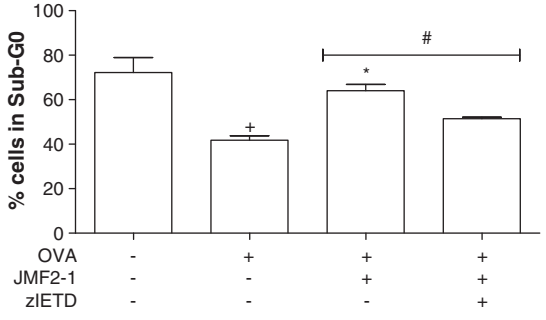

Fig. 5. Apoptosis induced by JMF2-1 is dependent on caspase-8 activation. Effect of JMF2-1 (300 μM) on the sub-diploid DNA content (sub-G_0/G_1) of lymph node cells, obtained from DO11.10 mice, stimulated with OVA (0.5 mg/ml) in the presence or absence of zIETD pre-treatment (50 μM). Values represent mean ± S.E.M from 3 mice (representative of 3 experiments). +P<0.05 as compared to vehicle-stimulated lymph node cells. *P<0.05 as compared to OVA-stimulated untreated cells. #P<0.05 as compared to zIETD-non treated cells.

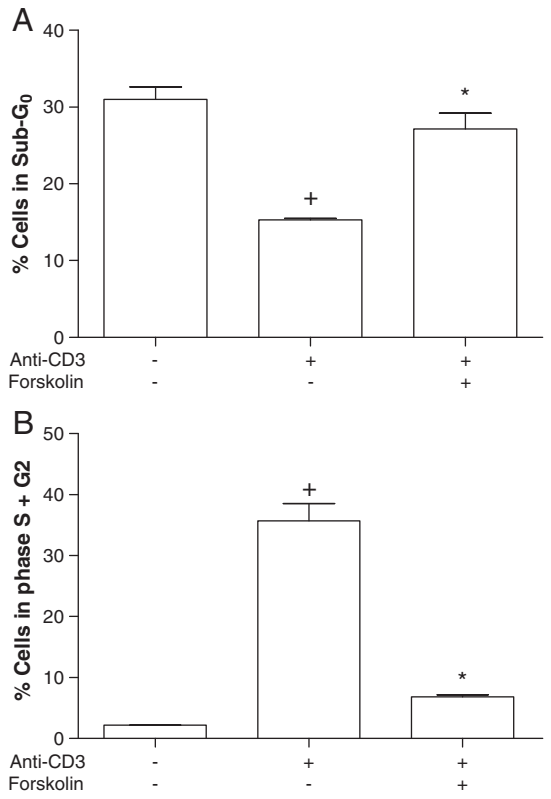

Fig. 6. Forskolin induces apoptosis and inhibition of proliferation of T cells in vitro. Effect of forskolin (100 μM) on the subdiploid DNA (sub-G_0/G_1) content (A) and proliferation (B) of lymph node cells recovered from BALB/c mice subjected to stimulation with anti-CD3. The percentage of cells undergoing DNA fragmentation and cell proliferation was determined by propidium iodide staining (flow cytometry) performed within 72 h after exposure to anti-CD3. Values represent mean ± SEM from three mice (representative of 3 experiments). $^{+}P<0.05$ as compared with saline-stimulated lymph node cells. $^{*}P<0.05$ as compared with anti-CD3-stimulated but untreated cells.

hypothesis that JMF2-1 acts via enhancement of intracellular cAMP levels to promote relaxant and anti-inflammatory responses.

Drugs able to prevent airway obstruction and/or inflammation have potential to benefit asthmatic patients. Remarkably, the local anesthetic lidocaine shows significant effects as an anti-inflammatory and anti-spasmodic agent, and several clinical studies demonstrated that nebulized lidocaine is indeed beneficial in asthma therapy (Decco et al., 1999; Groeben et al., 1999; Hunt et al., 2004; Saito et al., 2006; Siqueira et al., 2005). Since lidocaine is known by its ability

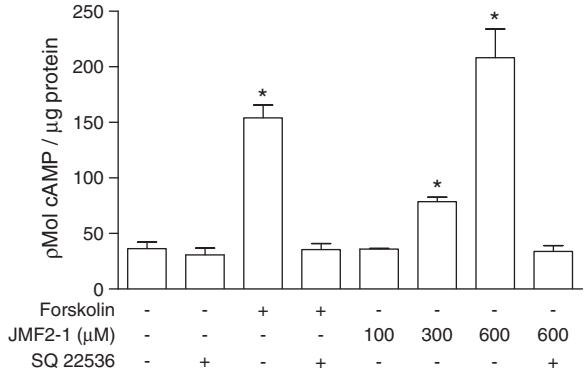

Fig. 7. JMF2-1 increases intracellular cAMP levels in anti-CD3 stimulated T cells. Effect of JMF2-1 (100–600 μM) treatment on cAMP intracellular level of T cells stimulated with anti-CD3 (10 μg/ml). Antigenic stimulated cells were treated for 30 min with either JMF2-1 or forskolin in the presence or absence of SQ22,536. Values represent the mean ± S.E.M (pooled lymph node cells, n = 3) (representative of two experiments). $^{*}P<0.05$ as compared to anti-CD3-stimulated but untreated cells.

to inhibit voltage-dependent sodium channels, it is tempting to assume that lidocaine is working by inhibiting neural regulation of inflammation and respiratory smooth muscle contraction. However, there are mounting evidence that these alternative actions result from lidocaine interacting with targets other than excitable cells and sodium channels (Bankers-Fulbright et al., 1998; Hollmann and Durieux, 2000; Kai et al., 1993; Okada et al., 1998). JMF2-1 is a non-anesthetic lidocaine analog able to inhibit crucial pathological features of asthma, including allergen-induced smooth muscle contraction, airway hyper-reactivity and leucocyte infiltration (eosinophils, CD4 T cells) into the lung (Costa et al., 2008; da Costa et al., 2007; Olsen et al., 2011). But, what are the possible mechanisms by which this occurs? Because the second messenger cAMP is responsible for different signaling pathways in several inflammatory and structural cells, including T lymphocytes and airway smooth muscle cells, we sought to test the hypothesis that JMF2-1 is modulating the function of these cells by increasing the intracellular concentration of cAMP.

Prior studies demonstrated that lidocaine prevents muscarinic receptor-mediated inhibition of adenylate cyclase in the airway smooth muscle, and exert a synergistic effect on the responses to agents that activate this enzyme (such as β_2-agonists and forskolin) under conditions of cholinergic bronchoconstriction (Nakahara et al., 2000; Yunoki et al., 2003). Elevation of intracellular cAMP leads to relaxation of airway smooth muscle via several actions, such as intracellular Ca^{2+} sequestration (Mueller and van Breemen, 1979), activation of Ca^{2+}-dependent K^{+} channels (Kume et al., 1989), inhibition of inositol phospholipid hydrolysis (Hall et al., 1989) and decrease in the sensitivity of contractile proteins to Ca^{2+} (Abe and Karaki, 1992; Bai and Sanderson, 2006; Oguma et al., 2006). Furthermore, it is well established that lidocaine blocks contraction evoked by muscarinic receptors by inhibiting Ca^{2+} influx and decreasing the sensitivity of contractile protein to Ca^{2+} (Kai et al., 1993). In the current study we demonstrated that JMF2-1 presented a higher potency and higher efficacy to inhibit carbachol-induced rat tracheal contraction as compared to lidocaine, and similar effects were noted when mice and guinea pig trachea rings were assessed (data not shown). These findings are consistent with those in which the contractile response caused by allergen provocation or by increasing extracellular Ca^{2+} concentration following high K^{+} depolarization, were more sensitive to JMF2-1 than to lidocaine (da Costa et al., 2007), suggesting that this non-anesthetic lidocaine analog may be indeed a hit for optimized spasmolytic agents.

We have shown, in the current work, that the protective effect of JMF2-1 on the carbachol-induced contraction of rat tracheal rings was neither modified by epithelium removal nor by pretreatment with the nitric-oxide synthase inhibitor L-NAME, discarding the involvement of nitric oxide and other epithelium-derived relaxing factors in this effect. It is noteworthy that similar findings have been reported for lidocaine and bupivacaine (Lautner et al., 2009). In contrast, the antispasmodic effect of JMF2-1 was shown to be inhibited by pretreatment with the adenylate cyclase blocker SQ22,536. This finding led us to investigate whether JMF2-1 could directly upregulate cAMP in cultured airway smooth muscle cells. Yunoki et al. (2003) reported that lidocaine enhanced basal and forskolin-stimulated cAMP accumulation in bovine tracheal tissue, but only under condition of co-stimulation with methacholine. According to these authors, lidocaine acts in this system by attenuating the inhibition of adenylate cyclase induced by the muscarinic receptor agonist (Yunoki et al., 2003). In contrast, our data revealed that JMF2-1, concentration-dependently, enhanced the basal cAMP concentration in primary cultured smooth muscle cells derived from guinea pig tracheas, even in the absence of muscarinic stimulation. Since the phenomenon was abrogated by blockade of adenylate cyclase, JMF2-1 may be acting directly or indirectly through the adenylate cyclase pathway. While trying to clarify whether the cAMP-dependent smooth muscle relaxation elicited by JMF2-1 derives from a β_2

_receptor stimulation, we pretreated rat trachea rings with the β_2-receptor blocker propranolol, but such a treatment failed to alter the JMF2-1 anti-spasmodic effect. Assuming that the β_2-receptor is irrelevant for the JMF2-1 relaxation property, one cannot discard the possibility that JMF2-1 may act, like forskolin, through a direct activation of adenylate cyclase.

A previous study has demonstrated that lidocaine inhibits T cell function and cytokine production without inducing apoptosis of these cells (Tanaka et al., 2002). Although lidocaine does not seem to reduce inflammation by stimulating lymphocyte apoptosis, the lidocaine analog JMF2-1 showed pro-apoptotic effect on T cells both in vitro and in vivo (Olsen et al., 2011). In order to assess if the lymphocyte pro-apoptotic activity of JMF2-1 may account for its ability to reduce the functional activity of these cells, we have used the caspase-8 inhibitor, zIETD, before anti-CD3 stimulation. Under this condition, zIEDT prevented the pro-apoptotic effect of the lidocaine analog, supporting the interpretation that JMF2-1 acts via caspase-8, eliciting the Fas-dependent extrinsic pathway of apoptosis.

Transient increase in cAMP intracellular levels occurs in T cells following TCR antigenic activation, and this event further inhibits cell signaling, unless there is co-stimulation, which induces a reduction in cAMP intracellular levels, allowing the amplification of the signaling induced by the antigenic challenge (Bjorgo and Tasken, 2006; Vendetti et al., 2006). There is evidence in the literature that an increase in cAMP intracellular levels causes a reduction of proliferation and IL-2 production in T cells. In addition, it increases the expression of the inhibitory molecule CTLA-4 and induces anergy in resting T cells (Vendetti et al., 2006). Continuous rise in cAMP intracellular levels in T lymphocytes is also associated with the activation of caspase-8 and inhibition of the cell functional activity (Naderi and Blomhoff, 2008). In line with previous findings (Naderi and Blomhoff, 2008), we demonstrated here that up-regulation of cAMP concentration by forskolin is associated with blockade of anti-CD3-stimulated proliferation and survival of mouse lymph node T cells. It is noteworthy that JMF2-1 was also able to inhibit cell proliferation and enhance apoptosis of T-cells from BALB/c mice stimulated by anti-CD3 similar to forskolin (Olsen et al., 2011). Moreover, we demonstrated that, similar to what was observed in case of airway smooth muscle cells, JMF2-1 increased the cAMP content of T lymphocytes, in a mechanism entirely dependent on adenylate cyclase activity.

In conclusion, these data show that JMF2-1 acts by increasing cAMP concentrations in T lymphocytes and smooth muscle cells leading to two unique effects: a caspase-8 dependent down-regulation of T lymphocytes survival, and relaxation of airway smooth muscle cells. Altogether, these findings help to explain the anti-inflammatory and anti-spasmodic effects of JMF2-1 observed in several experimental asthma systems.

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