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The effect of cannabinoids on dinitrofluorobenzene-induced experimental asthma in mice

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ABSTRACT

Cannabinoids have anti-inflammatory effects and can produce bronchodilation in the airways. We have investigated the effects of cannabinoids on tracheal hyperreactivity and airway inflammation in dinitrofluorobenzene (DNFB)-induced experimental non-atopic asthma in mice. 5-hydroxytryptamine (5-HT)-induced contraction response was enhanced while carbachol- and electrical field stimulation-induced contractions, and isoprenaline-induced relaxation responses were remained unchanged in DNFB group. The increased 5-HT-induced contractions were inhibited by incubation with either atropine or tetrodotoxin. DNFB application resulted in increased macrophage number in the bronchoalveolar lavage fluid (BALF). *In vivo* ACEA (CB_1 agonist) treatment prevented the increase in 5-HT contractions, while JWH133 (CB_2 agonist) had no effect. However, neither ACEA nor JWH133 prevented the increase in macrophage number in BALF. *In vitro* ACEA incubation also inhibited the increase in 5-HT contraction in DNFB group. These results show that cannabinoid CB_1 receptor agonist can prevent tracheal hyperreactivity to 5-HT in DNFB-induced non-atopic asthma in mice.

1. Introduction

Asthma is a chronic airway disease associated with bronchospasm and airway hyperreactivity, which mostly occurs due to inflammation (Bousquet et al., 2000; Groot Kormelink et al., 2009). Asthma has multiple phenotypes such as atopic and non-atopic asthma with different pathophysiologic and clinical characteristics. In the respiratory research, the interest is mainly focused in atopic asthma although a significant population of asthmatic patients are non-atopic. Non-atopic asthmatic patients give negative results in skin prick test for common allergens and they do not have allergen-specific IgE in their serums (Humbert et al., 1999; Walker, 1993). Non-atopic asthma generally starts following a lower airway infection, and its triggers such as exercise, cold air, air pollution and inhaled irritants are the same as those in atopic asthma despite allergens (Barnes, 2009). The clinical symptoms are also similar to allergic asthma and are alleviated with bronchodilators, but higher doses of corticosteroids are needed in the treatment (Barnes, 2009). Eosinophil, mucosal mast cell and T cell numbers (Barnes, 2008; Humbert et al., 1999) and also T-helper-2 (Th2) type cytokines IL-4, IL-5, IL-9 and IL-13 are increased in both atopic and non-atopic asthma (Ying et al., 1999). On the other hand, it has been shown that the macrophage or neutrophil numbers are increased only in non-atopic asthma (Bentley et al., 1992; Groot Kormelink et al., 2009; Humbert et al., 1996).

The experimental models used in non-atopic asthma are very limited. In some studies, it has been shown that low molecular weight substances like picril chloride, toluen diisocyanide and dinitrofluorobenzene (DNFB) can be used to develop experimental non-atopic asthma in mice (Buckley and Nijkamp, 1994; Garssen et al., 1991, 1989; Scheerens et al., 1996). Following skin sensitization, local application of these substances into the airways produces a hapten-induced pulmonary hypersensitivity reaction which is also a type IV delayed hypersensitivity reaction. In DNFB-induced experimental model, acute bronchoconstriction, pulmonary oedema, mononuclear cell infiltration, *in vitro* tracheal hyperreactivity and *in vivo* airway hyperresponsiveness were demonstrated in mice. Furthermore these reactions were not associated with an increase in serum hapten-specific IgE levels. Most of these findings reflect the characteristics of human non-

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atopic asthma (Dearman and Kimber, 1991; Garssen et al., 1991; Kraneveld et al., 2002; Scheerens et al., 1996).

Cannabinoids are biologically active substances, which are found in the plant *cannabis sativa*. There are also endogeneous cannabinoids in mammalian tissues that contribute to various physiological functions. The effects of cannabinoids are mediated by two G protein-coupled seven trans-membrane receptors namely CB_1 and CB_2 (Pertwee et al., 2010). Although both receptors are expressed in the central nervous system and peripheral tissues, CB_1 receptors are mainly located in the neurons where CB_2 receptors are mostly found in the immune related organs like tonsils, spleen, thymus and bone marrow (Braun et al., 2011; Matsuda et al., 1990; Onaivi et al., 2008). The majority of cannabinoid research has been focused on their effects in the central nervous system and immune system. However, cannabinoids have also significant effects in the airways. Earlier studies have shown that smoking marihuana or ingestion of 9-tetrahidrocannabinol (THC) leads to bronchodilation in human airways (Tashkin et al., 1974, 1975). The bronchodilator effect of THC was observed in both healthy volunteers and asthmatic patients at doses that did not affect central nervous system (Hartley et al., 1978; Tashkin et al., 1973; Vachon et al., 1973). In ovalbumin-induced atopic mice models of experimental allergic asthma, cannabinoids have also been shown to exert an inhibitory effect in airway inflammation along with reduced cell count in the bronchoalveolar lavage fluid (BALF) (Braun et al., 2011; Jan et al., 2003).

Cannabinoids have been shown to possess inhibitory effects on inflammatory cell expression in atopic asthma however no data is available for their effects in non-atopic asthma models. Therefore, in the present study, the effect of cannabinoids on airway inflammation was investigated in an experimental non-atopic asthma model. For this purpose tracheal hyperreactivity and BALF cell count were evaluated in DNFB-treated mice.

2. Methods

20–25 g female CD1 mice were used in the present study. All procedures performed in animals were approved by Hacettepe University Animal Research Ethics Committee.

2.1. DNFB-induced experimental asthma model

DNFB was used to induce experimental non-atopic asthma model in mice (Houtman et al., 2003; Kraneveld et al., 2005, 2002; van der Kleij et al., 2003, 2004). For this purpose, at the beginning (day 0) 0.5% DNFB dissolved in acetone:olive oil (4:1) was applied to the shaved thorax of mice and to each paw epicutaneously in a volume of 50 μl under light ketamine/xylazine anesthesia. At day 1, a second epicutaneous application was made only to thorax, and at day 5, 0.6% dinitrobenzene sulphonic acid (DNS), the water soluble hapten of DNFB which was dissolved in PBS (pH:7.2), was applied intranasally in a volume of 50 μl to mice. The control group received vehicle (acetone:olive oil (4:1)) on days 0 and 1, and challenged with intranasal DNS at day 5. 48 h after DNS application mice were killed by cervical dislocation, and their tracheas and lungs were removed in order to evaluate tracheal reactivity and histopathological changes.

2.2. In vivo *cannabinoid agonist treatment*

In order to evaluate the effects of systemic cannabinoid treatment, control and DNFB groups of mice were treated intraperitoneally with either CB_1 receptor agonist ACEA (7.5 mg/kg/day) (n = 7), CB_2 agonist JWH133 (5 mg/kg/day) (n = 6) or their vehicle (n = 6). The treatments were started one hour before DNS application and repeated after 24 h.

2.3. In vitro *tracheal reactivity*

In vitro tracheal reactivity was evaluated in four groups of mice which were control (n = 6), DNFB (n = 7), ACEA-treated DNFB (n = 7) and JWH-treated DNFB (n = 6) groups.

After isolation of the tracheas, the connective tissues were removed and tracheal rings were prepared. The preparations were mounted in organ baths containing Krebs-Heinseleit solution at 37 °C and gassed with 95% O_2-5% CO_2. The change in tension was measured with an isometric force transducer.

The tissues were equilibrated for 1 h under a resting tension of 1 g by washing every 15 min, and cumulative carbachol (10^{-8} to 10^{-4} M) and 5-HT (10^{-8} to 10^{-4} M)-induced contraction responses, and isoprenaline-induced relaxation in precontracted (10^{-7} M carbachol) tracheas were elicited, respectively. One hour of washout period was applied between each response.

In order to evaluate the contribution of cholinergic nerve stimulation and the release of endogeneous acetylcholine in 5-HT-induced contractions, 5-HT response was examined in the presence of muscarinic receptor antagonist atropine (10^{-6} M) (n = 7 for control; n = 8 for DNFB group) and neuronal sodium channel blocker tetrodotoxin (10^{-6} M) (n = 6 for control; n = 7 for DNFB group). 5-HT-induced contraction responses in the presence of antagonists were elicited in separate tracheal ring preparations due to the tachyphylaxis.

In vitro effects of cannabinoid agonists on 5-HT-induced contractions were also tested by incubation the tracheas with either CB_1 agonist ACEA (10^{-5} M) (n = 7) or CB_2 agonist JWH133 (10^{-5} M) (n = 6) in isolated organ baths. After 30 min of incubation with the agonists, the concentration-dependent contraction response to 5-HT was elicited. 5-HT responses in the presence of cannabinoid agonists were obtained in separate tracheal ring preparations due to tachyphylaxis.

Electrical field stimulation (EFS)-induced contraction responses were also elicited in isolated tracheas in order to assess the cholinergic nerve function in DNFB-induced non atopic asthma model in mice. For this purpose EFS (10 mV, 1 ms, 60 s)-induced contractions were evaluated in a frequency-dependent (0.25, 0.5, 1, 5, 10, 20 Hz) manner in control (n = 6) and DNFB (n = 6) group of mice.

2.4. Histopathology

Isolated tracheas and lungs were fixed in 10% formaldehyte for at least 48 h and stained with hematoxylene/eosin. The histopathological changes were evaluated thereafter.

2.5. Leukocyte accumulation in BALF

Bronchoalveolar lavage fluid (BALF) was collected from all four groups (control (n = 5), DNFB (n = 6), ACEA-treated DNFB (n = 6) and JWH-treated DNFB (n = 5) group of mice) in order to examine the inflammatory cell count. 48 h after DNS application mice were anesthetized with ketamin/xylazine (100/10 mg/kg). Then, the trachea was cannulated and lungs were washed gently three times with 1 ml PBS (37 °C). The samples were kept in 4 °C until cytospin preparations were made and leukocyte (neutrophil, eosinophil, mononuclear cell) cell count was performed by randomly counting 300 cells from each sample.

2.6. Statistical analysis

Carbachol-induced contraction response was represented as absolute value and 5-HT contraction was as the percentage of