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Aggression behaviour induced by oral administration of the Janus-kinase inhibitor tofacitinib, but not oclacitinib, under stressful conditions

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ABSTRACT

Janus kinase (JAK) inhibitors have recently been developed for allergic diseases. We focused on the 2 different JAK inhibitors, tofacitinib (selective for JAK3) and oclacitinib (selective for JAK1 and 2), to clarify the mechanism of anti-inflammatory and anti-itching potency of these drugs. In the process of detecting anti-itching potency, we observed that tofacitinib treated mice showed aggression behaviour. The objective of the study reported here was to investigate the aggressive behaviour induced by tofacitinib by using a mouse model of allergic dermatitis and the resident–intruder test. For the allergic dermatitis model, female BALB/c mice were sensitised and challenged topically with toluene-2,4-diisocyanate (TDI). Vehicle, tofacitinib or oclacitinib, was administered orally 30 min before TDI challenge. Scratching, aggression and standing behaviours were monitored in the 60 min period immediately following challenge of TDI. Another group of male BALB/c mice treated with vehicle, tofacitinib or oclacitinib was evaluated in the resident–intruder test and brains were obtained to determine blood brain barrier penetration. In the allergic dermatitis model, a significant increase in aggression and standing behaviour was only obvious in the tofacitinib treatment group. There was no effect in non-sensitised mice, but similar aggression was also induced by tofacitinib in male resident–intruder test. Penetration of blood–brain barrier was observed both in tofacitinib and oclacitinib treated mice. These results suggest that aggression was induced by tofacitinib under some kind of stressful environment. This study indicates a possible role of the JAK-STAT pathway in modulation of aggression behaviour.

1. Introduction

The Janus kinases (JAK) family of enzymes, namely JAK1, JAK2, JAK3, and tyrosine kinase (TYK) 2 play an important role in mediating intracellular signalling of cytokines (O'shea et al., 1997), and eventually play a key role in several types of immune responses such as innate and adaptive immunity, inflammatory, and embryonic development (Mascarenhas et al., 2014).

Until now, numerous cytokines are known to activate the JAK family of enzymes when bound to their receptors (Schindler et al., 2007). In particular, pro-inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-6, IL-13, IL-21 and IL-23 have been implicated in allergic and inflammatory skin disease that utilise the JAK pathway. Therefore, several JAK inhibitors have recently been developed for inflammatory skin diseases such as atopic dermatitis and psoriasis (Cosgrove et al., 2013a, 2013b; Fujii and Sengoku, 2013; Gonzales et al., 2014; Ports et al., 2013). However, the exact mechanisms of the anti-inflammatory and anti-itching action of these JAK inhibitors are still not fully understood. The primary objective of our study was to elucidate mechanisms of anti-inflammatory and anti-itching potential of JAK inhibitors in a model of allergic skin disease.

We focused on the 2 different types of JAK inhibitors, tofacitinib and oclacitinib, which differ in their JAK inhibitory profile, with oclacitinib being more selective for JAK1 and JAK2 compared to tofacitinib, which shows higher selectivity against JAK3 (Gonzales et al., 2014; Meyer et al., 2010). Tofacitinib has been approved as an oral JAK inhibitor by the US Food and Drug Administration for the treatment of adults with moderately to severely active rheumatoid arthritis (Meyer et al., 2010). Oclacitinib is also a novel JAK inhibitor that has been approved in the United States and European Union for the control or treatment of pruritus associated with allergic dermatitis in dogs (Gonzales et al., 2014).

We selected a toluene-2,4-diisocyanate (TDI) induced allergic dermatitis model with female BALB/c mice to reveal the

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mechanism of anti-itching potency of the JAK inhibitors. However, in the process of this study, we observed that tofacitinib treated mice displayed aggression behaviour. Although tofacitinib has been already approved in several countries, to the authors' knowledge, studies on altered behaviour have not been reported. Therefore, the objective of the study reported here was to investigate the side effects of tofacitinib by using a mouse model of TDI-induced allergic dermatitis and an aggression specific resident–intruder test.

2. Materials and methods

2.1. Animals

Both sexes of BALB/cAnN mice (6-wk-old) were purchased from Charles River Laboratories (Raleigh, NC) and housed in groups of four mice per cage at under controlled lighting (a 12 h light-dark cycle), temperature (22 ± 3 °C), humidity (55% ± 15%), and ventilation (at least 10 complete fresh-air changes/h). All behaviour testing occurred between 8 a.m. and 13 p.m. Standard rodent chow and water were available ad libitum. All aspects of the current study were conducted in accordance with the Animal Care and Use Program of the North Carolina State University (IACUC Protocol No. 13-111-B).

2.2. Drugs

Tofacitinib (CP-690550) was purchased from Tocris (Minneapolis, MN), and Oclacitinib (PF-03394197) was purchased from Zoetis Inc. (APOQUEL, Kalamazoo, MI). TDI and acetone were obtained from Sigma (St. Louis, MO). Methylcellulose and Tween 20 were ordered from Thermo Fisher Scientific Inc. (Waltham, MA). Tofacitinib and oclacitinib were diluted in 0.5% methylcellulose/0.25% Tween 20 solution to concentrations described below. toluene-2,4-diisocyanate (TDI) was diluted in acetone to concentrations described below.

2.3. TDI-induced allergic contact dermatitis model

TDI-induced allergic contact dermatitis model was performed with female mice as described earlier with minor modifications (Baumer et al., 2004, 2010). Following 2 weeks acclimatisation period, the shaved abdominal skin was stripped 10 times with adhesive tapes and 100 µl of 5% TDI solution in acetone was applied to the stripped epidermis (day 1). On day 2 and 3, 50 µl of 5% TDI in acetone was applied to the same site without tape stripping. The allergic reaction was boosted 21 days later by application of 50 µl of 0.5% TDI in acetone onto the shaved abdomen (day 25). The allergic reaction was challenged 7 days later by application of 30 µl of 0.5% TDI in acetone onto the shaved dorsal region (day 32). JAK-inhibitors tofacitinib or oclacitinib were administered orally 30 min before TDI challenge, because the absorption of tofacitinib and oclacitinib is rapid, with plasma concentrations for both tofacitinib and oclacitinib peaking at around 1 h after oral administration (Collard et al., 2014; Dowty et al., 2014). For each drug, vehicle-only control group (oral administration with 0.5% methylcellulose/0.25% Tween 20 solution), low- and high-dose groups were set. Chemical doses were: Tofacitinib, 10 and 30 mg/kg; Oclacitinib, 30 and 45 mg/kg. During the video monitoring, the mice were observed in pairs at the same cage and both mice were treated at the same dosage. In general, scratching bouts of allergic dermatitis mice are monitored for 60 min period and we could find significant different between vehicle and treatment group. Therefore, the test lasted for 60 min and aggressive (biting, chasing and wrestling) as well as standing bouts were counted immediately following challenge of TDI according to the method described previously (Inagaki et al., 2001). We also monitored social interaction, defensive behaviour and restlessness as important features in the behaviour profile. Restlessness is one of the behaviour which is reflected the central nervous system stimulation. In parallel, the same procedure was performed with normal female mice (non-TDI sensitised and challenged). Aggression and standing bouts were counted in the 60 min period 30 min after tofacitinib (10 and 30 mg/kg) or oclacitinib (10 and 30 mg/kg) oral administration. The doses of tofacitinib and oclacitinib used in this study were selected based on the previously published studies (Kudlacz et al., 2004; Yew-Booth et al., 2012). Apart from aggression behaviour no other abnormalities were noticed during the examination period.

2.4. Resident–intruder test

Aggressive behaviour toward a stranger was tested by the resident–intruder test. The resident–intruder test was performed as described earlier with minor modifications (Kalinine et al., 2014; Rammal et al., 2010). Briefly, 48 h prior to the test (following 2 weeks acclimatisation period), the home cage bedding and enrichment of male resident mice was not changed for the mice to maintain territoriality with in their home cage. Vehicle, tofacitinib (10 and 30 mg/kg) or oclacitinib (10 and 30 mg/kg) were administered orally 30 min prior to introducing the male intruder mouse. This encounter occurred twice with a 48 h interval. In our study, we could find significant different between vehicle and treatment group in the 5 min period. Therefore, each test lasted for 5 min and attack latency, aggressive and standing bouts were counted in the 5 min period. Since our aim of resident–intruder test was just to confirm the aggressive behaviour which observed in female mice model of allergic contact dermatitis, we focused on biting, chasing and wrestling which were shown in allergic model. It was reported that tested male animals should be pre-exposed to female presence (with oviduct ligation to avoid maternal aggressiveness) in order to facilitate the development of territoriality and prevent the social isolation. However, based on our preliminary trial, we judged it was not necessary to be pre-exposed to female presence for our objective.

2.5. Molecular imaging of drug transit through the blood–brain barrier

After finishing the resident–intruder test, mice were anaesthetised and killed by carbon dioxide gas. Whole brains were removed and embedded in Tissue-Tek® O.C.T Compound (Sakura Finetek U.S.A., Inc. Torrance, CA) until used for freeze sectioning. Ten micrometre thick sections of brains were used for matrix assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) to visualise tofacitinib or oclacitinib penetration in brain tissue, similar to a procedure described earlier (Liu et al., 2013; Qi et al., 2015). A Bruker (Bremen, Germany) solariX 7 T Fourier transform ion cyclotron resonance mass spectrometer was used for the measurement. In MS/MS mode, precursor ions were isolated in the quadrupole, externally accumulated in the hexapole for 0.1 s, and 5–30 eV collision energy was applied for CID to find the characteristic product ions for tofacitinib and oclacitinib measurement. In MSI mode, homogeneous MALDI matrix layers were deposited onto the tissue surface by a Bruker ImagePrep system using dihydrobenzoic acid (DHB) at 30 mg/ml (for oclacitinib) and cyano-4-hydroxycinnamic acid solution at 7 mg/ml (for tofacitinib) dissolved in 50% methanol–water (v/v)+0.2% trifluoroacetic acid. The pixel size in imaging experiments was 50 µm, and both precursor and characteristic product ions were monitored in experiments.