

ORIGINAL ARTICLE

JNJ7777120 Ameliorates Inflammatory and Oxidative Manifestations in a Murine Model of Contact Hypersensitivity *via* Modulation of TLR and Nrf2 Signaling

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Abstract— JNJ7777120, a histamine H4 receptor antagonist, was shown to be effective in different experimental settings of allergic inflammation, including contact hypersensitivity. Toll-like receptors (TLRs) are thought to function as a link between innate and adaptive immune responses to various haptens. Here, we studied the suppression of TLR signaling as a possible mechanism by which JNJ7777120 exerts its anti-inflammatory effects against the chemical hapten, fluorescein isothiocyanate (FITC). The potential anti-oxidant effect of JNJ7777120 in this model was also examined. Mice subjected to FITC sensitization and challenge showed significantly elevated plasma immunoglobulin E (IgE) level, ear interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF- α), and thiobarbituric acid reactive substance (TBARS) contents as well as increased myeloid differentiation factor 88 (MyD88) gene expression, nuclear factor-kappa B p65 (NF- κ B p65), and phospho-p38 mitogen-activated protein kinase (p-p38 MAPK) protein expression. This was accompanied by enhanced ear myeloperoxidase (MPO) and eosinophil peroxidase (EPO) activities as well as diminished glutathione (GSH) content and superoxide dismutase (SOD) activity. JNJ7777120 treatment perceivably reversed these effects, denoting profound anti-inflammatory and anti-oxidant character of JNJ7777120 which was confirmed by its mitigation of FITC-induced pathological changes in mouse ear. JNJ7777120 additionally enhanced the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), providing a novel mechanism by which JNJ7777120 functions as an anti-oxidant in this model. To conclude, JNJ7777120 afforded a remarkable amendment of FITC skin insult by virtue of its anti-inflammatory and anti-oxidant effects; the mechanistic basis of these effects may include modulation of TLR and Nrf2 pathways.

KEY WORDS: FITC; H4 receptor; allergic inflammation; MyD88; NF- κ B.

INTRODUCTION

Contact hypersensitivity (CHS) is a T cell-mediated response to epicutaneous sensitization with haptens which results in a marked inflammatory skin response and altered cellular oxidative status at the site of allergen re-exposure [1, 2]. The recognition of haptens by the innate immune system is a crucial step in the elicitation of the skin

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inflammatory response [3]. Fluorescein isothiocyanate (FITC) is a lipophilic hapten that produces an allergic inflammatory response characterized by immunoglobulin E (IgE), T helper 2 (Th2) cytokine production, and accumulation of inflammatory cells including CD4⁺ T cells, mononuclear cells, eosinophils, and neutrophils [4, 5].

Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) expressed by bacteria, parasites, viruses, and fungi [6] as well as damage-associated molecular patterns (DAMPs), which are self-molecules that can be released during cellular necrosis [7]. TLR signaling results in activation of the transcription factor nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPKs) to induce the expression of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) [8]. TLRs are expressed by many types of cells in the skin, including keratinocytes, Langerhans cells (LCs), and other resident and trafficking immune system cells [9]. It was proposed that TLRs are integral for the development of CHS to various haptens including FITC [10], suggesting that TLRs might provide a link between innate and adaptive immune system activation in this model.

Inflammatory cell accumulation in CHS is associated with the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide anion, hydrogen peroxide, and peroxynitrite [11]; thus, alleviation of oxidative stress might prove to be a promising strategy for suppression of atopic inflammation. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor that mainly regulates the cellular defenses against oxidative stress and electrophilic insults. Constitutionally, transcription of Nrf2 is repressed by negative regulator Kelch-like ECH-associated protein 1 (Keap1). Upon encounter with ROS, Nrf2 leaves Keap1 and translocates to the nucleus where it interacts with anti-oxidant response element in the promoter region of genes encoding anti-oxidant enzymes [12].

JNJ777120 (JNJ) is a potent and selective histamine H4 receptor (H4R) antagonist. The H4R has been implicated in various pathological conditions, especially inflammatory processes. H4R antagonists suppressed inflammation in murine models of asthma and dermatitis, and comparable results were observed with H4R-deficient mice [13, 14]. The H4R has been shown to mediate chemotaxis of eosinophils [15], mast cells [16], and dendritic cells (DCs) [17]. In addition, the receptor has been shown to modulate inflammatory mediator production *in vitro* from various immune cells [18, 19]. However, the exact

molecular mechanisms underlying the anti-inflammatory effects of H4R antagonists have not been fully elucidated. A possible cross talk between H4R and TLRs has been suggested in previous studies [20, 21]. Therefore, we hypothesized that modulation of TLR signaling may mediate some of the anti-inflammatory effects of JNJ in the FITC model. The study also aimed to explore the possible involvement of Nrf2 signaling in JNJ-mediated anti-oxidant effects.

MATERIALS AND METHODS

Drugs and Chemicals

FITC isomer I and JNJ were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). FITC was dissolved in acetone/dibutyl phthalate mixture, 1:1, v/v, and JNJ was suspended in 0.5% carboxymethyl cellulose. All other used chemicals were of analytical grade.

Animals

Female BALB/c mice aged 6–8 weeks were obtained from the National Scientific Research Center (Giza, Egypt). Mice were housed under controlled temperature (25 ± 2 °C) and constant light cycle (12 h light/dark) and allowed free access to a standard rodent chow diet and water. The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (PT 808).

Experimental Design

Animals were randomly divided into three experimental groups, each consisting of 10–12 mice. The abdominal skin of mice was shaven. Group I was sensitized and challenged with FITC vehicle and served as the normal control group. Group II was sensitized on days 1 and 2 by application of 400 μ l of 0.5% FITC solution in acetone/dibutyl phthalate mixture, 1:1, v/v, on the shaved ventral skin and challenged 6 days later by application of 20 μ l of the same solution on both ears [22]. Group III was similarly sensitized and challenged and received JNJ (30 mg/kg; p.o.) [23] 1 h before and 7 h after FITC challenge.

Blood samples were collected from the retro-orbital sinus of all mice 24 h after the FITC challenge and animals were sacrificed by cervical dislocation. The ears were

rapidly removed and homogenized in ice-cold saline to prepare 10% homogenate.

Biochemical Parameters

Plasma Immunoglobulin E Level

IgE level was assessed using mouse IgE ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions.

Oxidative Stress Biomarkers

Reduced glutathione (GSH) content was determined according to the method described by Beutler et al. [24]. After precipitating the SH-containing proteins, the yellow color produced by the reduced Ellman's reagent was measured colorimetrically at 412 nm, and GSH content was expressed as micrograms per gram of wet tissue. Lipid peroxide products were determined by the estimation of thiobarbituric acid reactive substances (TBARS) that were measured according to the method described by Uchiyama and Mihara [25] based on the reaction of tissue malondialdehyde with thiobarbituric acid to produce a pink pigment that was extracted by n-butanol and measured colorimetrically at 520 and 535 nm. Ear TBARS contents were then expressed as nanomoles per gram of wet tissue. Superoxide dismutase (SOD) activity was assessed according to the method of Marklund and Marklund [26] by monitoring the SOD-induced inhibition of pyrogallol auto-oxidation. The rate of increase in absorbance at 420 nm was recorded. One unit (U) of enzyme activity was defined as 50% inhibition of pyrogallol auto-oxidation under the assay conditions. The SOD activity was expressed as units per gram of wet tissue.

Ear Total Nitrate/Nitrite

Total nitrate/nitrite (NO_x) was determined spectrophotometrically at 540 nm as previously described [27] based on the Griess reaction and employing vanadium trichloride as a reducing agent. Total NO_x was expressed as micromoles per gram of wet tissue.

Determination of Myeloperoxidase and Eosinophil Peroxidase Activities

Myeloperoxidase (MPO) activity was estimated according to the method of Bradley et al. [28] where homogenates were subjected to three freeze/thaw cycles, 10 s sonication, and 15 min 10,000×g 4 °C centrifugation. o-

Dianisidine hydrochloride (0.167%)/hydrogen peroxide (0.0005%) in phosphate buffer (50 mM, pH 6) were added to the supernatants. The absorbance rate was recorded at 460 nm. Eosinophil peroxidase (EPO) activity was estimated as previously described [5] where homogenates were centrifuged at 1500g for 20 min at 4 °C and substrate solution consisting of 10 mM O-phenylenediamine in 0.05 M Tris-HCl (pH 8.0) and 4 mM hydrogen peroxide was added to the supernatants. The reaction mixture was incubated at room temperature for 60 min before the reaction was stopped by addition of 2 M sulfuric acid and absorbance at 490 nm was recorded.

Ear Tumor Necrosis Factor-Alpha and Interleukin-4 Contents

Tumor necrosis factor-alpha (TNF- α) and interleukin-4 (IL-4) levels were assessed using mouse TNF- α ELISA kit and mouse IL-4 ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions.

Myeloid Differentiation Factor 88 Gene Expression

Total RNA was extracted from ear tissue using RNeasy Kit (Qiagen, CA, USA), and the purity of the obtained RNA was verified spectrophotometrically at 260/280 nm. Equal amounts of RNA (2 mg) were reverse transcribed into complementary DNA (cDNA) using Superscript Choice systems (Life Technologies, USA) according to the manufacturer's guidelines. To assess the expression of myeloid differentiation factor 88 (MyD88) gene, quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, CA, USA) as described by the manufacturer. Briefly, in a 25- μ l reaction volume, 5 μ l of cDNA was added to 12.5 μ l of 2× SYBR Green Master Mix and 200 ng of each primer. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (R&D Systems Inc., USA) was used as a housekeeping gene. The sequences of primers for murine MyD88 were F: 5'- GTCCATTG CCAGCGAGCTAA-3' and R: 5'- GGAGACAG GCTGAGTGCAAA-3' and the sequences for GAPDH were F: 5'- ACCCCAGCAAGGAC ACTGAGCAAG-3' and R: 5'- GGCCCCTC CTGTTATTATGGGGGT-3'. PCR reactions included 10 min at 95 °C for activation of AmpliTaq Gold DNA polymerase, followed by 40 cycles at 95 °C for 15 s (denaturing) and 60 °C for 1 min (annealing/

extension). The mRNA levels were calculated based on the method of $2^{-\Delta\Delta C_t}$ [29].

Western Blot Analysis of Phospho-p38 Mitogen-Activated Protein Kinase, Nuclear Factor-Kappa B p65, and Nuclear Factor Erythroid 2-Related Factor 2

Part of the ear was homogenized with RIPA buffer containing 1.5 ml 5 M NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF) (MW 174.19), 2.5 ml 10% deoxycholic acid (DOC), 0.5 ml 10% SDS, and 2.5 ml 1 M Tris (pH 8.6). The tissue lysate was centrifuged at 12000 rpm for 20 min at 4 °C. The lysate was then collected and protein concentration was determined with a BCA protein assay kit (Thermo Fisher Scientific Inc., USA). An aliquot of 7.5 µg protein of each sample was boiled with Laemmli buffer at 95 °C for 5 min to ensure that proteins were denatured, then each sample was loaded on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Amersham Bioscience, Piscataway, NJ, USA) using a semi-dry transfer apparatus (Bio-Rad, Hercules, CA, USA). The membranes were incubated with 5% milk blocking buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, and Tris-buffered saline with 0.05% Tween-20 (TBST) at 4 °C overnight. The membranes were then washed with TBST and incubated with a 1:1000 dilution of anti-phospho-p38 mitogen-activated protein kinase (p-p38 MAPK) (Thr 180/Tyr 182), anti-p38 MAPK, anti-nuclear factor-kappa B p65 (NF-κB p65), or anti-nuclear factor erythroid 2-related factor 2 (Nrf2) antibodies (ThermoFisher Scientific, MA, USA) for overnight on a roller shaker at 4 °C. The filters were washed and subsequently probed with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Amersham. Life Science Inc., USA).

Chemiluminescence detection was performed with an Amersham detection kit according to the manufacturer's protocols and exposed to X-ray film. The amounts of p-p38 MAPK, p38 MAPK, NF-κB p65, and Nrf2 proteins were quantified by densitometric analysis of the autoradiograms using a scanning laser densitometer (Biomed Instrument Inc., USA).

Histopathological Examination

Ear tissues were embedded in 10% formalin and processed for paraffin sections of 4 µm thickness. After de-waxing and rehydration, sections were stained with hematoxylin and eosin (H&E) for routine histopathological examination using light microscopy.

Statistical Analysis

Data were expressed as means ± S.E.M. Comparisons between means were carried out using the one-way analysis of variance (ANOVA) test followed by the Tukey-Kramer multiple comparison test. For all statistical tests, the level of significance was fixed at $p < 0.05$. GraphPad Prism® software package, version 6 (GraphPad Software Inc., USA), was used to carry out all statistical tests.

RESULTS

JNJ Treatment Suppresses FITC-Associated Th2 Response

Mice sensitized to FITC on days 1 and 2 and challenged 6 days later showed a significant increase in the plasma IgE level by 8.58-fold as compared to the control group. Similarly, a conceivable rise was observed in the ear IL-4 content in the FITC group (3.43-fold) as compared to the control group. Treatment with JNJ reduced the plasma IgE level and ear IL-4 content by 46.04 and 59.84%, respectively, of FITC mice (Fig. 1a, b).

FITC-Driven Enhancement of Downstream TLR Signaling Is Attenuated by JNJ Treatment

FITC exposure activated TLR signaling as manifested by the marked elevation of ear MyD88 gene expression as well as NF-κB p65 protein expression by 7.77-fold and 10.10-fold, respectively, of the control group. JNJ-treated mice showed a substantial suppression of TLR signaling as indicated by the decrease in MyD88 gene expression in addition to NF-κB p65 protein expression by 70.39 and 69.42%, respectively, of the FITC group. FITC application also increased protein expression of phospho-p38 MAPK (10.04-fold), an effect that was significantly mitigated by JNJ treatment. When normalized to total p38 MAPK levels, JNJ decreased phospho-p38 by 70.43% of the FITC group denoting reduced activation of the kinase (Fig. 2a-c).

JNJ Attenuates FITC-Induced Inflammatory Burst

The obtained results illustrated in Fig. 3a revealed that enhanced TLR signaling in the FITC group was accompanied by increased pro-inflammatory cytokine production as indicated by the amplified ear TNF-α content (4.62-fold) of the control mice. Such effect was hampered following JNJ treatment, showing a reduction by 35.88% of the FITC group.

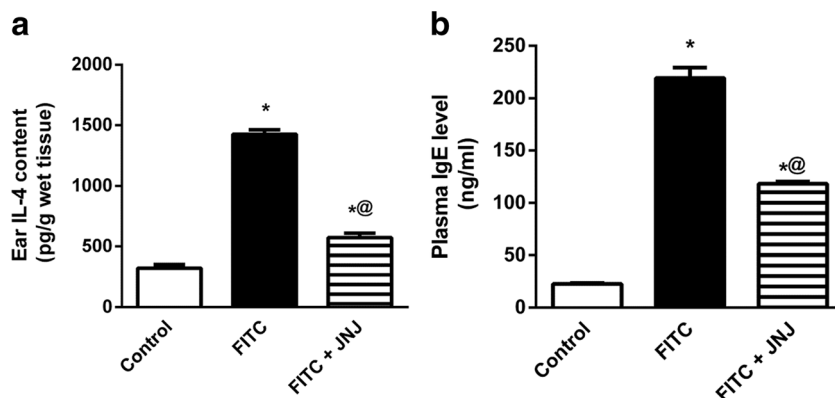


Fig. 1. Effects of JNJ on FITC-induced Th2 response. Twenty-four hours after FITC challenge, mice ears were separated and used for determination of ear IL-4 content (a) and plasma samples were used for determination of plasma IgE level (b). Each bar represents mean $\pm$ S.E.M ($n = 7-10$). *vs normal control; @vs FITC group (one-way ANOVA followed by Tukey-Kramer multiple comparison test, $p < 0.05$).

Furthermore, increased neutrophil and eosinophil infiltration in the ears of FITC mice was reflected in the elevated MPO and EPO activities (593.77 and 199.18%, respectively, of the control group). Treatment with JNJ suppressed both MPO and EPO activities by 27.03 and 41.11%, respectively, of the FITC control group (Fig. 3b, c). As depicted in Fig. 3d, ear NO_x was magnified following FITC challenge by 91.05% relative to the normal control. JNJ treatment halted such a rise, by 20.56% compared to the FITC group.

FITC-Associated Redox Disturbance Is Alleviated by JNJ Treatment

A state of oxidative stress was produced in response to FITC-induced inflammation. The significant boost of ear TBARS content in FITC mice (130.44%) coupled with depleted ear GSH content (84.77%) and diminished SOD activity (78.76%) highlighted the impaired cellular oxidative status. These effects were mitigated following JNJ treatment which was reflected by the reduction of ear TBARS content by 41.04% of the FITC group and replenishment of ear GSH content and SOD activity by 134.53 and 171.87%, respectively, of the FITC group. Besides, JNJ markedly upregulated Nrf2 protein expression by 2-fold as compared to FITC mice (Fig. 4a–d).

JNJ Ameliorates FITC-Associated Histopathological Insults

Figure 5 depicts a photomicrograph of an ear tissue section from normal control mice showing normal histological features of the skin. Sections from the FITC group revealed a diffuse inflammatory cell infiltration and edema

in the dermis. Such alterations were markedly inhibited by JNJ treatment which was reflected in ear sections from the JNJ group that showed only mild inflammatory changes in the dermis.

DISCUSSION

While the propitious effects of JNJ, a selective H4R antagonist, have been demonstrated in various experimental settings of allergic inflammation, the current investigation depicts, for the first time, the involvement of TLR and Nrf2 pathways as possible contributors to the drug's anti-inflammatory and anti-oxidant capacities in the FITC skin model. The development of CHS to chemical haptens involves many events including percutaneous penetration of the sensitizer, haptenization of self-proteins [30], activation of the innate immune system together with release of pro-inflammatory mediators, activation and migration of DCs towards regional lymph nodes, activation of memory and naïve T cells followed by their recruitment to the site of antigen exposure, and initiation of the relevant T cell responses [31].

In the present study, FITC exposure boosted tissue content of the Th2 cytokine, IL-4, which was coupled with the enhanced plasma IgE level, two findings that uphold with former reports [5, 22, 32]. JNJ treatment hampered the level of both parameters which is consistent with the results obtained by Cowden et al. [14]. It has been postulated that H4R activation led to downregulation of a signal transducer and activator of transcription 1 (STAT1) phosphorylation which is associated with Th1 signaling [33]. Additionally, Ohsawa and Hirasawa noticed that JNJ treatment

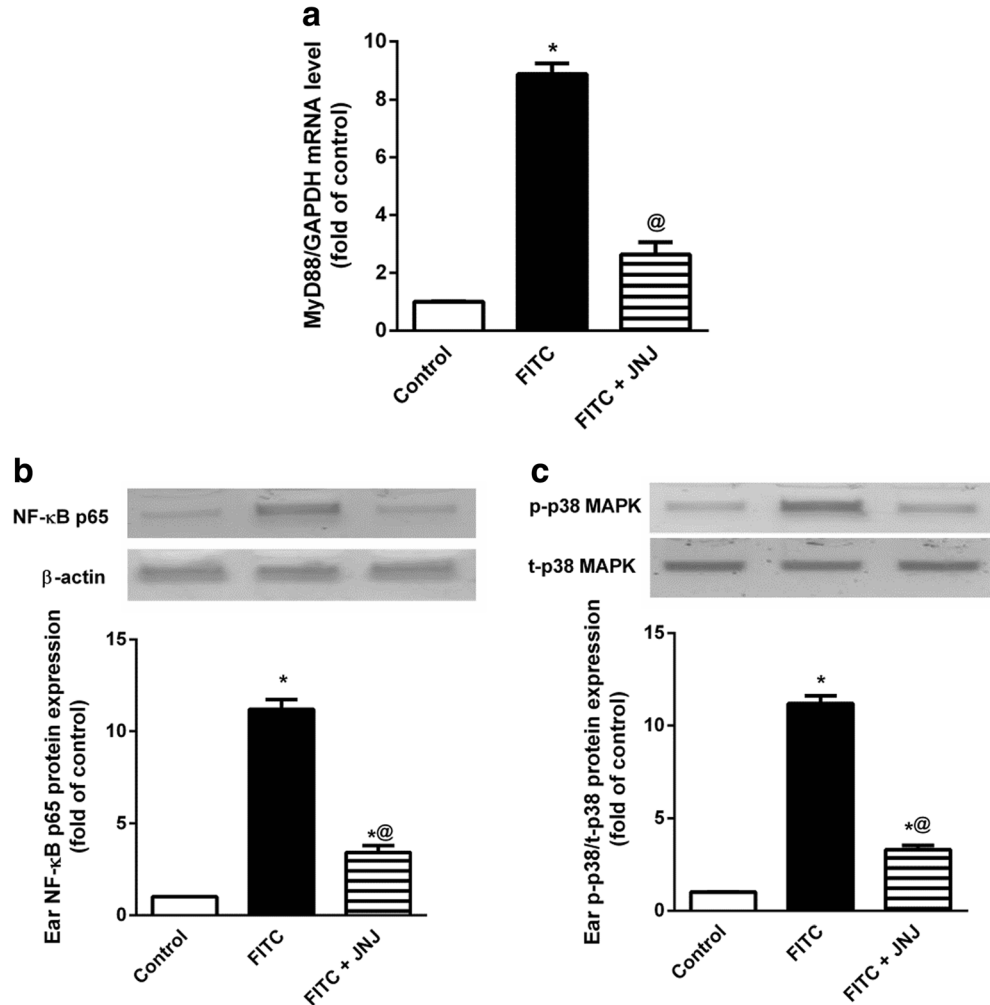


Fig. 2. Effect of JNJ on FITC-driven enhancement of downstream TLR signaling. Twenty-four hours after FITC challenge, mice ears were separated and used for determination of MyD88 gene expression (a) as well as NF-κB p65 (b) and relative p-p38/t-p38 MAPK (c) protein expressions. Each bar represents mean $\pm$ S.E.M (n = 7–10). *vs normal control; @vs FITC group (one-way ANOVA followed by Tukey-Kramer multiple comparison test, $p < 0.05$).

reduced the levels of the Th2 chemoattractants, thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), from antigen-stimulated bone marrow-derived mast cells [34], offering, thus, an explanation of the current findings.

We set out to investigate the involvement of TLR signaling in the development of the allergic inflammatory response to FITC. Ear MyD88 gene expression was markedly elevated in the FITC group. MyD88 is a common adaptor protein for many TLRs that initiates their downstream signaling, ultimately resulting in enhanced NF-κB and MAPK expression [35], a fact that explains the amplified NF-κB p65 and p-p38 MAPK expression in the current study. Activation of the downstream MAPK pathways

leads to the induction of another transcription factor, activator protein-1 (AP-1) [36]. Both NF-κB and AP-1 play a pivotal role in the expression of pro-inflammatory cytokines such as TNF-α, the augmented production of which was similarly delineated by Boehme et al. [22]. Environmental bacterial flora, to which the skin is continuously exposed, might provide the ligands for TLR activation [10]. Besides, it was proposed that hyaluronic acid degradation products are partly associated with this response as they can activate TLR2 and TLR4 on DCs, facilitating their activation and migration. Hyaluronic acid is abundant in the skin and its breakdown might be mediated by ROS that are produced in response to haptens [37, 38]. In context, Martin et al. stated that the absence of both TLR2 and

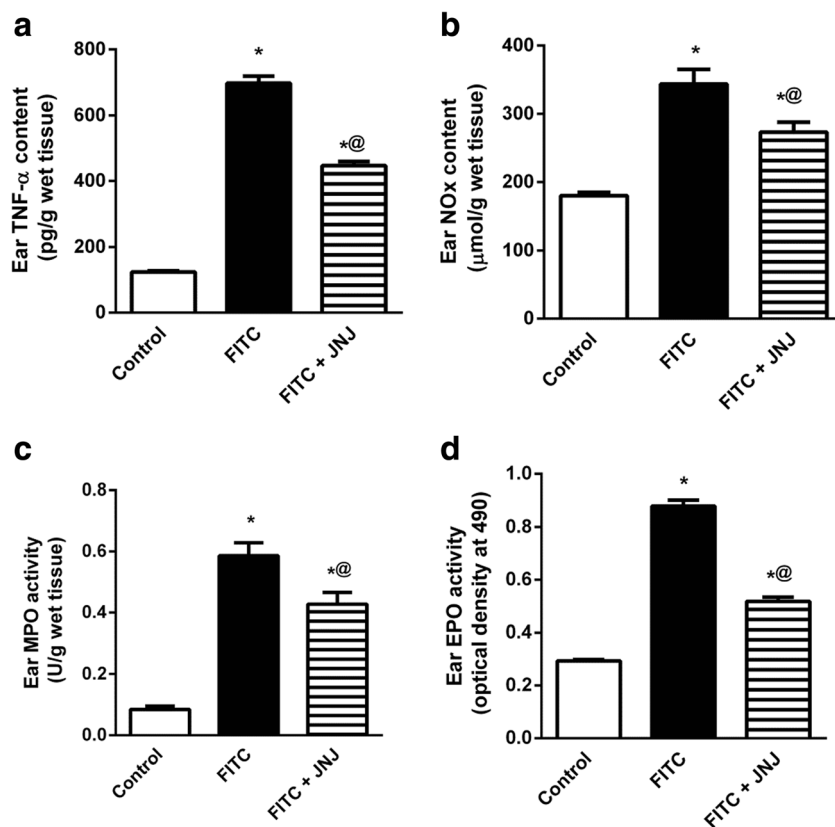


Fig. 3. Effect of JNJ on FITC-induced inflammatory burst. Twenty-four hours after FITC challenge, mice ears were separated and used for determination of ear TNF- α (a) and NO_x (b) contents as well as MPO (c) and EPO (d) activities. Each bar represents mean $\pm$ S.E.M (n = 7–10). *vs normal control; @ vs FITC group (one-way ANOVA followed by Tukey-Kramer multiple comparison test, $p < 0.05$).

TLR4 prevented the induction of CHS to various haptens including oxazolone, trinitrochlorobenzene (TNCB), and FITC [10]. Similarly, Vu et al. reported that small interfering RNA-mediated knockdown of either TLR2 or TLR4 prevented thymic stromal lymphopoietin (TSLP) production by keratinocytes which is essential for elicitation of the Th2 response [39]. It was also demonstrated that MyD88-deficient mice failed to mount CHS response to TNCB and dinitrofluorobenzene (DNFB), thus providing another validation for the pivotal role played by TLRs in the development of CHS response [40].

The current work suggests that JNJ treatment may exert its anti-inflammatory effects in this model *via* modulation of TLR signaling. This was reflected by the mitigated MyD88 gene expression together with the downregulated protein expression of NF- κ B p65 as well as p-p38/t-p38 MAPK ratio, with a resultant reduction of ear TNF- α content. These findings are in close agreement with those of Ahmad et al. [41] who observed that JNJ treatment

decreased mRNA levels of both NF- κ B and TNF- α in the synovial fluid following stimulation with lipopolysaccharide (LPS), a known TLR4 agonist. The H4R was previously shown to potentiate LPS-induced IL-6 production in mouse mast cells [20] and LPS-induced TNF- α production *in vivo* [21]. Furthermore, treatment with H4R antagonists was shown to block neutrophilia induced by zymosan, a TLR2 agonist [42]. It was proposed that H4R activation is required to amplify TLR responses in different cells, including DCs, which is ultimately required for eliciting the T cell response. Alternatively, the reduction of tissue TNF- α content by JNJ could be secondary to the decline in Th2 response which promotes TNF- α production from DCs by TSLP [43].

FITC exposure augmented tissue neutrophil and eosinophil infiltration which was evidenced by the enhanced ear MPO and EPO activities in the FITC group. These observations go in line with previous findings [32, 44]. Neutrophils are extensively found in the inflammatory

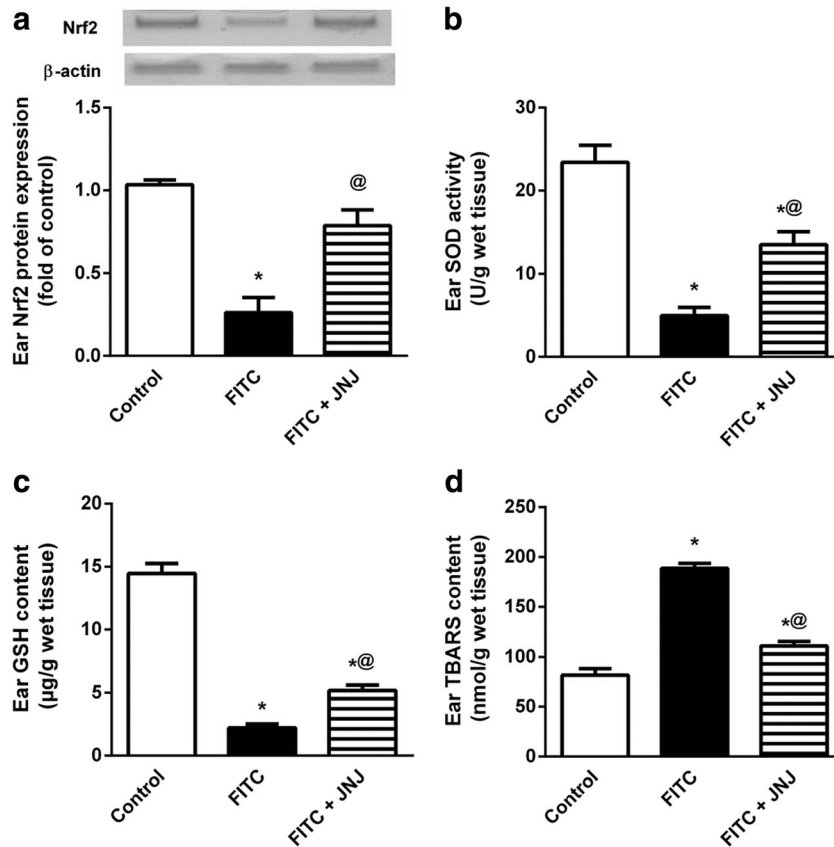


Fig. 4. Effect of JNJ on FITC-induced redox disturbance. Twenty-four hours after FITC challenge, mice ears were separated and used for determination of ear Nrf2 protein expression (**a**) and SOD activity (**b**) as well as GSH (**c**) and TBARS (**d**) contents. Each bar represents mean $\pm$ S.E.M ($n = 7-10$). *vs normal control; @vs FITC group (one-way ANOVA followed by Tukey-Kramer multiple comparison test, $p < 0.05$).

infiltrate induced by FITC challenge [5], and their recruitment is mediated by the CXC chemokines macrophage inflammatory protein-2 (MIP-2) and growth-regulated protein- α (GRO- α) produced by CD4 $^{+}$ T cells, dermis-localized mast cells, and keratinocytes [45]. Neutrophil recruitment to inflammatory lesions might also be indirectly mediated by leukotriene B4 (LTB4) produced from mast cells [46]. Tissue eosinophilia inevitably depends on the presence of the Th2-cytokine interleukin-5 (IL-5) [5, 47] and enhanced expression of eotaxin that attracts CC chemokine receptor 3 (CCR3) + eosinophils [48]. These cells contribute to the release of ROS, proteases, and various cytokines. Moreover, they may provide an additional source of lipid peroxidation, where MPO can convert hydroperoxides into free radicals, triggering, thus, lipid peroxidation [49].

JNJ treatment suppressed both MPO and EPO activities, reflecting the hampered tissue neutrophil and eosinophil accumulation. JNJ was previously reported to suppress

tissue eosinophilia in allergic skin inflammation induced by FITC [14] and croton oil [50]. Similar findings were reported by Somma et al. in a mouse model of OVA-induced airway inflammation [51]. The same study also revealed decreased neutrophil infiltration in the lung and diminished MPO activity by JNJ treatment. The inhibitory effect of JNJ on MPO activity was also reported in a mouse model of zymosan-induced peritonitis [52]. Supporting the present findings, histamine was reported to induce calcium mobilization, actin polymerization, shape change, and migration of eosinophils into inflammatory sites through H4 receptors [53, 54]. In addition, histamine enhances the chemotaxis induced by eotaxin and macrophage chemoattractant protein 3 (MCP-3) and this effect is blocked by H4 receptor antagonists [15]. Moreover, a direct relation between recruitment of neutrophils and TNF- α production has been formerly attested [55], which could explain the reduction in MPO activity by JNJ treatment reported herein. Notably, JNJ's anti-inflammatory

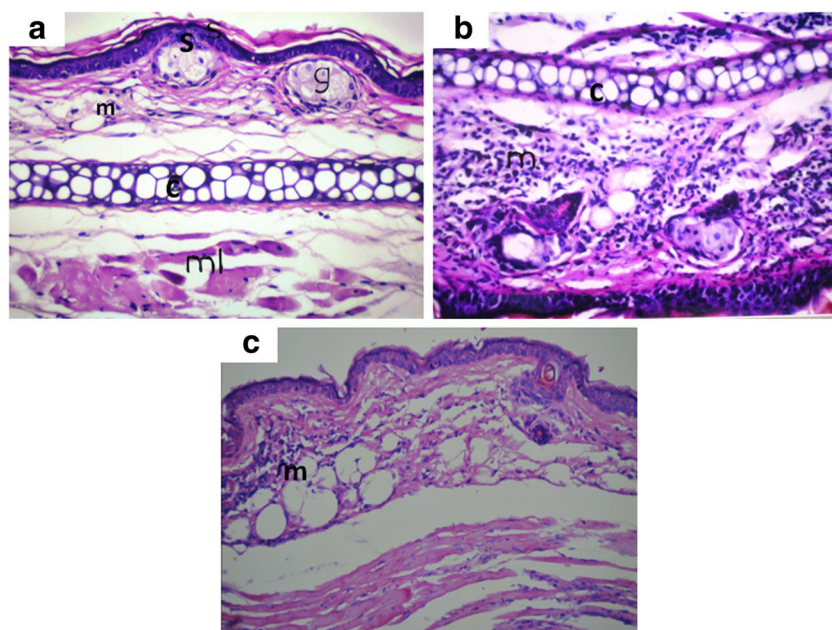


Fig. 5. Effect of JNJ on FITC-induced histopathological alterations. Representative photomicrographs of hematoxylin-eosin stained ear sections from control mice (a) showing normal histological structure of the epidermis (s), dermis (m), subcutaneous glands (g), subcutaneous musculature (ml), and cartilaginous structure (c); mice subjected to FITC (b) showing diffuse inflammatory cell infiltration and edema in the dermis; and mice treated with JNJ (c) revealing mild inflammatory cell infiltration in the dermis (H&E, magnification $\times 40$).

character was confirmed by the suppression of inflammatory cell accumulation in ear tissue afforded by the drug as revealed in histopathological examination.

Accumulating neutrophils and eosinophils are important sources of reactive oxygen intermediates including H_2O_2 produced from superoxide anion and nitric oxide [56] which explains the NO_x surge in the FITC group. This buildup of cellular oxidative burden results in an imbalance of cellular redox status which is reflected by the diminished tissue GSH content and SOD activity. ROS are able to activate various transcription factors including NF- κ B and AP-1, resulting in further expression of iNOS as well as other pro-inflammatory cytokines as well as adhesion molecules [57] and creating, thus, a vicious cycle of inflammatory cell activation and ROS production. ROS and nitric oxide products interact and act as cytotoxic agents by cross-linking lipids, leading to lipid peroxidation and loss of cell membranes' integrity [58]. Both lipid peroxides and their breakdown products, such as malondialdehyde (MDA), can directly or indirectly affect many functions integral to cellular and organ homeostasis.

Considering the extensive role played by ROS in allergic inflammation, a new strategy to counteract ROS-mediated damage could be the activation of constitutive

anti-oxidant enzymes. Nrf2, a curator of redox homeostasis, controls the transcription of many anti-oxidant proteins [59]. Nrf2 activation leads to increased expression of HO-1, an enzyme that profoundly limits lipid peroxidation *via* its product bilirubin [60], nicotinamide adenine dinucleotide phosphate (NADPH): quinone oxidoreductase-1 (NQO1), SOD [61, 62], and γ -glutamylcysteine ligase, an enzyme that catalyzes the rate-limiting step of GSH synthesis [63]. To this end, El Ali et al. demonstrated that ear swelling and inflammatory response to various chemical sensitizers were exaggerated in Nrf2-deficient mice [64], denoting an important role for Nrf2 in controlling CHS response to these sensitizers. Recently, it was shown that melatonin was able to reduce oxidative stress and TSLP production in response to FITC and diisodecyl phthalate exposure in mice partially by virtue of Nrf2 induction [65]. JNJ validated its anti-oxidant capacity by upregulation of Nrf2 expression in the skin of mice treated with the drug. Such surge might explain the boost in tissue SOD activity as well as GSH content along with decreased lipid peroxidation and TBARS production. To the authors' knowledge, this is the first study to correlate the anti-oxidant capacity of JNJ to the enhanced Nrf2 pathway. The improved cellular redox status in response to JNJ

treatment could also be attributed to the reduction in inflammatory cell infiltration exerted by the H4R antagonist.

Taken together, these results verified the anti-inflammatory and anti-oxidant effects of JNJ in the FITC model which could be attributed, at least in part, to modulation of TLR signaling and induction of Nrf2 signaling pathways, hence providing a new insight into the protective effects of H4R antagonists in various inflammatory and allergic conditions.

COMPLIANCE WITH ETHICAL STANDARDS

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (PT 808).

Conflict of Interest. The authors declare that they have no conflicts of interest.

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