

The Effects of the Heat Shock Protein 90 Inhibitor 17-Allylamino-17-Demethoxygeldanamycin, Cannabinoid Agonist WIN 55,212-2, and Nitric Oxide Synthase Inhibitor Nω-Nitro-L-Arginine Methyl Ester Hydrochloride on the Serotonin and Dry Skin-Induced Itch

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Keywords

Pruritus · Heat shock protein 90 · Cannabinoid · Serotonin · Nitric oxide · IgE

Abstract

Introduction: In many types of itch, the interaction between immune system cells, keratinocytes, and sensory nerves involved in the transmission of itch is quite complex. Especially for patients with chronic itching, current treatments are insufficient, and their quality of life deteriorates significantly. **Objective:** In this study, we aimed to investigate the role of the heat shock protein 90 (Hsp90) inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), cannabinoid agonist WIN 55,212-2, and nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) in pruritus. **Methods:** We created a serotonin (5-HT)-induced (50 µg/µL/mouse, i.d.) acute and acetone-ether-water (AEW)-induced chronic itching models. 17-AAG (1, 3, and 5 mg/kg,

intraperitoneally [i.p.]), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) were applied to Balb/c mice. **Results:** We found that 17-AAG suppressed the scratches of mice, depending on the dose. The itch behavior was reduced by WIN 55,212-2, but L-NAME showed no antipruritic effect at the administered dose. The combined application of these agents in both pruritus models showed synergism in terms of the antipruritic effect. Our results showed that NO did not play a role in the antipruritic effect of WIN 55,212-2 and 17-AAG. Increased plasma IgE levels with AEW treatment decreased with the administration of 17-AAG (5 mg/kg, i.p.) and WIN 55,212-2. **Conclusion:** These results demonstrate that Hsp90 may play a role in the peripheral pathway of pruritus, and cannabinoid agonists and Hsp90 inhibitors can be used together in the treatment of pruritus.

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Introduction

Pruritus, commonly known as itching, is an undesirable sensation that produces a desire to scratch and is an important clinical problem that disrupts the quality of life. It is the main symptom of various skin problems such as atopic dermatitis, urticaria, and psoriasis and is also associated with systemic diseases or the undesirable effects of drugs [1, 2]. Effective management of pruritus is essential, but the underlying mechanism of pruritus, especially in chronic itch, is complex, and new treatment strategies are needed.

Nitric oxide (NO) is synthesized from L-arginine via NO synthase (NOS) and is a signaling molecule that has various functions in the nervous system. There are 3 isoforms of NOS: endothelial NOS (eNOS), inducible NOS, and neuronal NOS [3]. NOS is found in many cells in the skin and at sites involved in the transmission and interpretation of itch, such as C fibers, spinal cord, and brain [4]. Latest research studies have shown that NO may also be a neurotransmitter involved in itching [5, 6].

Heat shock protein (Hsp) 90 (Hsp90) is a chaperone protein found in eukaryotic cells that prevents protein aggregation and degradation [7]. Intracellular concentrations of Hsps may increase 2–3 times in conditions such as temperature rise, oxidative stress, hypoxia, inflammation, and ischemia-reperfusion injury [8]. Hsp90 is expressed focally along with the epidermis in cultured human keratinocytes and fibroblasts and in the normal-appearing skin [9]. A study has shown that Hsp90 expression is significantly upregulated in epidermal keratinocytes and mast cells of patients with psoriasis (a chronic inflammatory skin disease) [10]. As a result of Akt binding with Hsp90, eNOS activity increases, and NO production is facilitated [11]. Recent studies have shown that NOS activity is increased by Hsp90 depending on the dose, and Hsp90 inhibitor inhibits NO production and decreases eNOS expression [12].

Cannabinoids act through CB1/CB2 receptors and have an antinociceptive effect [13]. Cannabinoid drugs are now used effectively in many clinical situations such as multiple sclerosis, neuropathic pain, depression, and sleep disorders [14]. However, they cannot be used effectively due to undesirable central effects of cannabinoids such as dependence and tolerance development [15]. Itching and pain sensations are similar in many respects [16]. Recent experimental investigations indicated that cannabinoid agonists have antipruritic effects [17–19].

Our aim in this research was to test whether the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-

AAG), cannabinoid agonist WIN 55,212-2, and NOS inhibitor N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME) have an antipruritic effect in 5-HT- and dry skin-induced itches. We used Balb/c mice because 5-HT, 48/80, or skin dryness causes frequent scratching behavior in this strain [20–22]. We also observed whether 17-AAG, WIN 55,212-2, and L-NAME exhibited a synergistic effect on itch responses and the role of the NO pathway in the expected antipruritic effects of these agents.

Materials and Methods

Animals

A total of 112 male Balb/c mice, 8–10 weeks old and weighing 20–30 g, were used in this study. The subjects were housed with a 12/12-h light-dark cycle at the temperature of $21 \pm 2^\circ\text{C}$ and provided with standard feed and water.

Drugs

Serotonin hydrochloride (H9523-25 mg) and L-NAME (N5751-250 mg) were dissolved in 0.9% saline, while WIN 55,212-2 (W102-25 mg) and 17-AAG (AA17307-50 mg) were dissolved in 20% DMSO, 5% Tween 80, 5% ethanol, and 70% saline. This composition is also used in the vehicle group. Serotonin hydrochloride, WIN 55,212-2, and L-NAME were purchased from Sigma-Aldrich, and 17-AAG was purchased from Biosynth Carbosynth. Drug doses and treatment times were determined from previous research studies [19, 23–25].

5-HT-Induced Model of an Acute Itch

The rostral part of the back hair of mice was shaved 2 days before the experiments. 5-HT-induced scratching behavior was generated by an intradermal (i.d.) injection of 50 μg /50 μL of the pruritogen 5-HT via a 30G needle into the neck and rostral part of the mice as determined from our previous research studies [19, 26]. The control group was injected with 50 μL of saline.

Dry Skin-Induced Model of a Chronic Itch

Dry skin was stimulated with acetone-ether-water (AEW) mixture as indicated in previous studies [27, 28]. The mice were shaved 2 days before starting AEW treatment. AE treatment was provided with a 1:1 acetone/diethylether mixture treatment with a total volume of 1.5 mL. This application was applied to the nape of the mice twice daily (9:00 and 17:00) for 7 days for 10 min by soaked cotton ($2 \times 2 \text{ cm}^2$). After AE treatment, the nape of the mice was cleaned with distilled water for 30 s. Only distilled water was administered to mice in the control group.

Behavioral Analysis

Mice were habituated to the test environment 2 days before starting the experiment. The subjects were placed singly in clear acrylic cages. Then, after i.d. 5-HT injection, the mice were videotaped for 30 min and then counted. Spontaneous itching behavior on the 8th day of the chronic itch experiment was recorded for 1 h and then counted. A scratch count refers to scratching the nape of a mouse with its hind paw and then licking or putting its paw on the ground.

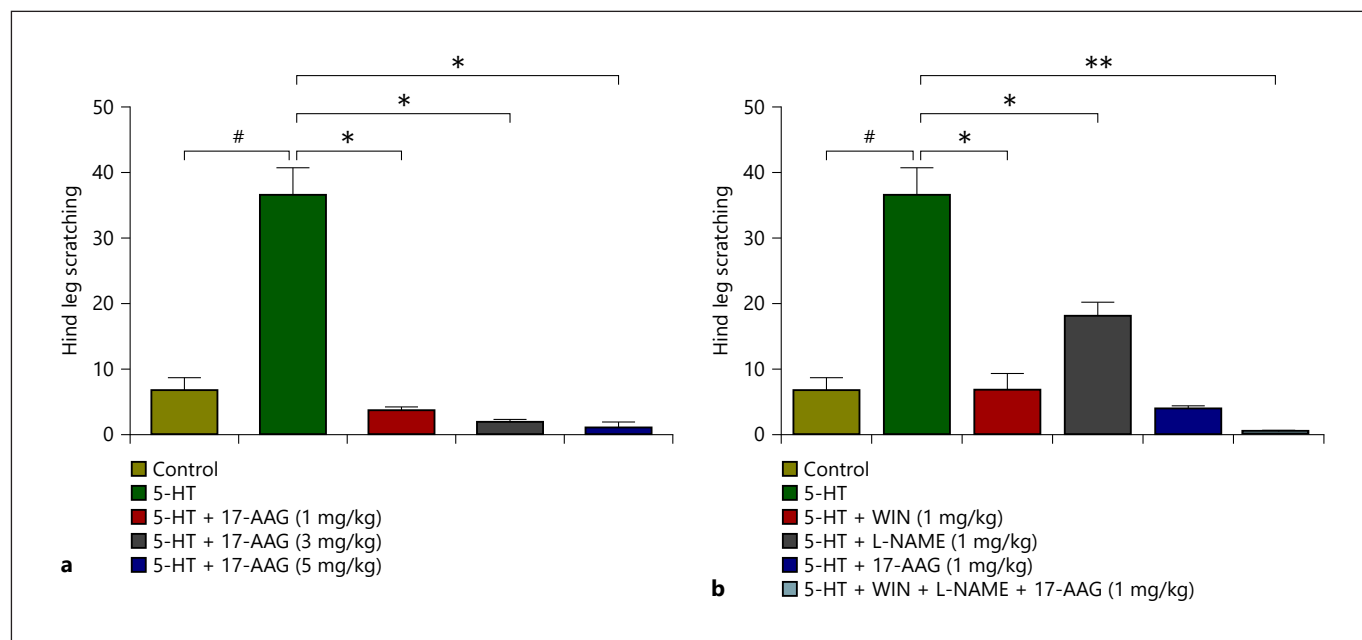


Fig. 1. Effects of 17-AAG, WIN 55,212-2, and L-NAME on 5-HT-induced scratches. **a** Intradermal serotonin (5-HT) injection (50 μ g/50 μ L) stimulated scratches ($\#p < 0.01$). The Hsp90 inhibitor 17-AAG (1, 3, and 5 mg/kg, i.p.) dose-dependently reduced 5-HT-induced scratches ($*p < 0.01$). **b** Cannabinoid agonist WIN 55,212-2 (1 mg/kg, i.p.) reduced 5-HT-induced scratches ($*p < 0.01$). The NOS inhibitor L-NAME (1 mg/kg, i.p.) did not reduce 5-HT-in-

duced scratches. Systemic (1 mg/kg, i.p.) injections of 17-AAG, WIN 55,212-2, and L-NAME synergistically reduced 5-HT-induced scratches ($**p < 0.001$). Data shown are the mean $\pm$ SEM, $n = 7$ for each group. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; Hsp90, heat shock protein 90; NOS, nitric oxide synthase; i.p., intraperitoneally.

Experimental Protocol

To describe the efficacy of the Hsp90 inhibitor on acute and chronic scratches, increasing doses of 17-AAG (1, 3, and 5 mg/kg) were administered intraperitoneally (i.p.) 30 min before 5-HT applications and after 7 days of AEW treatment. In order to define the effects of WIN 55,212-2 (1 mg/kg, i.p.) and L-NAME (1 mg/kg, i.p.), treatment on acute and chronic scratches was administered 30 min before 5-HT administrations and after 7 days of AEW treatment. 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) were given combined to observe the synergistic effects on scratches. Acute itching behavior was counted as described approximately 5 min after 5-HT administration. Chronic itching behavior was counted 30 min after WIN 55,212-2 and L-NAME administrations and 60 min after different doses of 17-AAG administrations.

Enzyme-Linked Immunosorbent Assay

Mice were anesthetized after the behavioral experiments, and the blood samples were collected from animals' hearts. Collected samples were taken into 2 mL EDTA tubes and centrifuged at 3,000 rpm + 4°C for 15 min. Plasma fractions were then collected. The levels of nitrate/nitrite (NO degradation products) and IgE in the plasma samples were measured by commercial enzyme-linked immunosorbent assay kits (CAYMAN-78001 and BT-LAB-E0449Mo, respectively) using an absorbance reader (BioTek ELx800, KC junior).

Statistical Analysis

All data were analyzed with the GraphPad prism 9.0.0. statistical and graphical software. All of the data were expressed as the mean $\pm$ SEM to display variations in groups. $p < 0.05$ was considered statistically significant. The behavioral data and IgE levels were analyzed by one-way ANOVA followed by the Bonferroni test as the post hoc multiple comparison analysis. Unpaired student's t test was used to define the difference between nitrate/nitrite levels.

Results

Effects of 17-AAG, WIN 55,212-2, and L-NAME on 5-HT-Induced Scratches

5-HT (50 μ g/50 μ L, i.d.) administration significantly increased itching behavior compared to the control group ($\#p < 0.01$). Systemic administration of the Hsp90 inhibitor 17-AAG (1, 3, and 5 mg/kg, i.p.) dose-dependently attenuated 5-HT-induced scratches ($*p < 0.01$, Fig. 1a). The cannabinoid agonist WIN 55,212-2 (1 mg/kg, i.p.) decreased 5-HT-induced scratches ($*p < 0.01$, Fig. 1b). Systemic administration of the NO inhibitor L-NAME

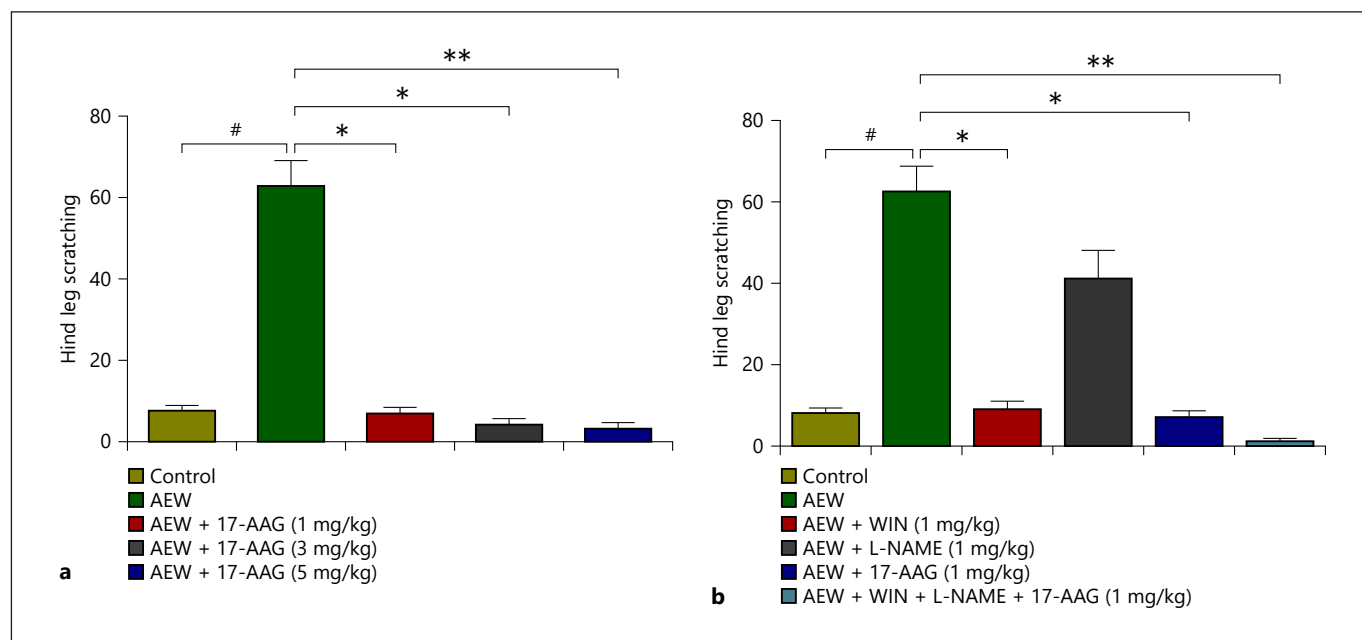


Fig. 2. Effects of 17-AAG, WIN 55,212-2, and L-NAME on AEW-induced scratches. **a** AEW treatment increased scratches ($^{\#}p < 0.01$). The Hsp90 inhibitor 17-AAG (1, 3, and 5 mg/kg, i.p.) dose-dependently reduced AEW-induced scratches ($^*p < 0.01$, $^{**}p < 0.001$). **b** Cannabinoid agonist WIN 55,212-2 (1 mg/kg, i.p.) decreased AEW-induced scratches ($^*p < 0.01$). The NOS inhibitor L-NAME (1 mg/kg, i.p.) did not reduce AEW-induced scratches.

Systemic (1 mg/kg, i.p.) injections of 17-AAG, WIN 55,212-2, and L-NAME synergistically attenuated AEW-induced scratches ($^{**}p < 0.001$). Data shown are the mean $\pm$ SEM, $n = 7$ for each group. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; Hsp90, heat shock protein 90; AEW, acetone-ether-water; NOS, nitric oxide synthase; i.p., intraperitoneally.

(1 mg/kg, i.p.) did not significantly change 5-HT-evoked itching. No scratches were observed with combination of the lowest dose of 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) ($^{**}p < 0.001$, Fig. 1b).

Effects of 17-AAG, WIN 55,212-2, and L-NAME on Dry Skin-Induced Chronic Scratches

AEW (topically, twice daily/week) administration significantly increased itching behavior compared to the control group ($^{\#}p < 0.01$, Fig. 2a). Systemic administration of 17-AAG (1, 3, and 5 mg/kg, i.p.) dose-dependently reduced dry skin-induced chronic scratches ($^*p < 0.01$, $^{**}p < 0.001$, Fig. 2a). Systemic application of WIN 55,212-2 (1 mg/kg, i.p.) relieved dry skin-induced scratches ($^*p < 0.01$, Fig. 2b). Systemic administration of L-NAME (1 mg/kg, i.p.) did not significantly change dry skin-evoked itching. No scratches were observed with combination of the lowest dose of 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) ($^{**}p < 0.001$, Fig. 2b).

Role of NO in the Antipruritic Activity of 17-AAG and WIN 55,212-2

5-HT (50 μ g/50 μ L, i.d.) and AEW administration significantly increased plasma nitrate/nitrite levels compared to the control group ($^{\#}p < 0.01$, Fig. 3a, c). Systemic administration of WIN 55,212-2 (1 mg/kg, i.p.) did not significantly change increased nitrate/nitrite levels in both models of itching (Fig. 3b, d). There was no effect of increasing doses of 17-AAG (1, 3, and 5 mg/kg, i.p.) on the nitrate/nitrite levels (Fig. 3a, c). Similarly, the lowest dose combination of 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) also did not decrease the nitrate/nitrite levels (Fig. 3b, d).

Effects of 17-AAG, WIN 55,212-2, and L-NAME on IgE Levels in Dry Skin-Induced Chronic Scratches

Chronic AEW administration significantly increased the plasma IgE level compared to the control group ($^{\#}p < 0.0001$). Systemic administration of the highest dose of 17-AAG (5 mg/kg, i.p.) decreased the increased IgE level ($^*p < 0.0001$, Fig. 4a). Systemic administration of WIN

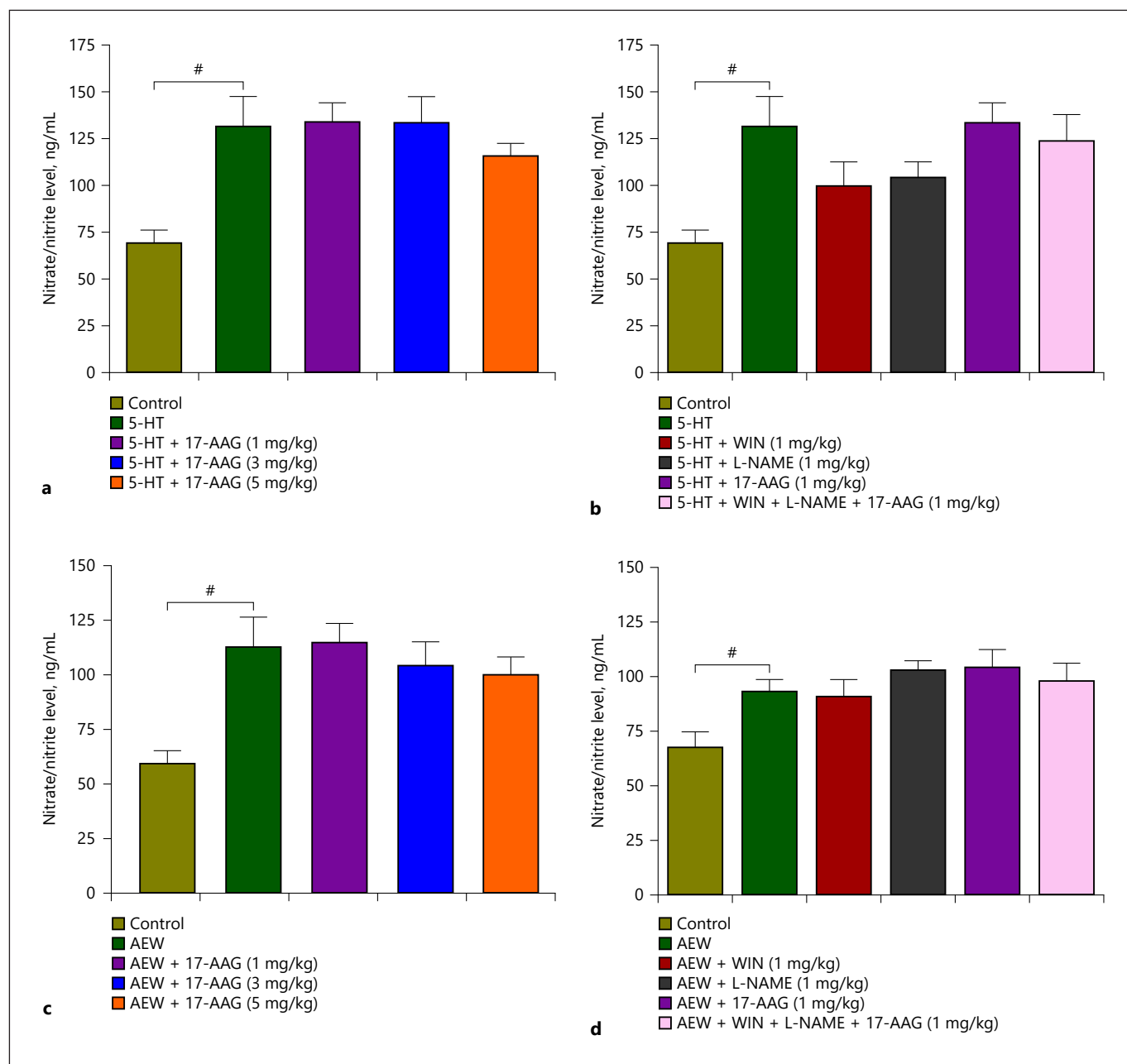


Fig. 3. Effects of 17-AAG, WIN 55,212-2, and L-NAME treatment on plasma nitrate/nitrite level in 5-HT- and AEW-induced itch models. **a** AEW treatment increased the plasma nitrate/nitrite level ($^{\#}p < 0.01$). The Hsp90 inhibitor 17-AAG (1, 3, and 5 mg/kg, i.p.) did not reduce the increasing nitrate/nitrite level by AEW treatment. **b** Cannabinoid agonist WIN 55,212-2 (1 mg/kg, i.p.) and NOS inhibitor L-NAME (1 mg/kg, i.p.) did not reduce the increasing plasma nitrate/nitrite level by AEW treatment. Systemic (1 mg/kg, i.p.) injections of 17-AAG, WIN 55,212-2, and L-NAME did not decrease the increasing plasma nitrate/nitrite level by AEW treatment. **c** Intradermal serotonin (5-HT) injection (50 μ g/50 μ L) increased the plasma nitrate/nitrite level ($^{\#}p < 0.01$). 17-AAG (1, 3,

and 5 mg/kg, i.p.) did not reduce the increasing nitrate/nitrite level. **d** WIN 55,212-2 (1 mg/kg, i.p.) and L-NAME (1 mg/kg, i.p.) did not decrease the increasing nitrate/nitrite level by intradermal 5-HT injection. Systemic (1 mg/kg, i.p.) injections of 17-AAG, WIN 55,212-2, and L-NAME did not decrease the increasing plasma nitrate/nitrite level by intradermal 5-HT injection. Data shown are the mean $\pm$ SEM, $n = 7$ for each group. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; Hsp90, heat shock protein 90; AEW, acetone-ether-water; NOS, nitric oxide synthase; i.p., intraperitoneally.

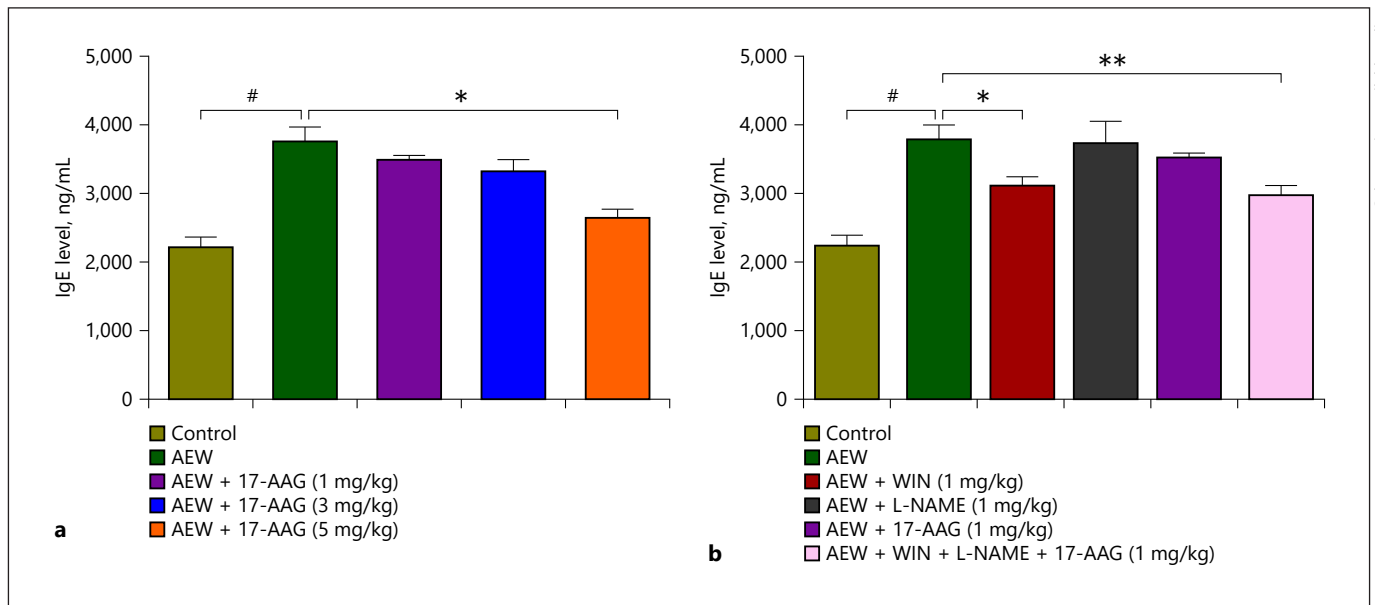


Fig. 4. Effects of 17-AAG, WIN 55,212-2, and L-NAME treatment on the plasma IgE level in AEW-induced itch model. **a** AEW treatment increased the plasma IgE level ($^{\#}p < 0.0001$). The Hsp90 inhibitor 17-AAG (5 mg/kg, i.p.) decreased the increasing IgE level by AEW treatment ($^*p < 0.0001$). **b** Cannabinoid agonist WIN 55,212-2 (1 mg/kg, i.p.) attenuated the increasing IgE level by AEW treatment ($^*p < 0.05$). The NOS inhibitor L-NAME (1 mg/kg, i.p.) did not reduce the increasing IgE level by AEW treatment.

Systemic (1 mg/kg, i.p.) injections of 17-AAG, WIN 55,212-2, and L-NAME decreased the increasing IgE level by AEW treatment ($^{**}p < 0.01$). Data shown are the mean $\pm$ SEM, $n = 7$ for each group. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; Hsp90, heat shock protein 90; AEW, acetone-ether-water; NOS, nitric oxide synthase; i.p., intraperitoneally.

55,212-2 (1 mg/kg, i.p.) decreased the increased IgE level ($^*p < 0.05$, Fig. 4b). Systemic administration of L-NAME (1 mg/kg, i.p.) did not significantly change the increased IgE level. The lowest dose combination of 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) reduced the IgE level ($^{**}p < 0.01$, Fig. 4b).

Discussion

The presence of many mediators and receptors such as histamine, 5-HT, and substance P in the pathological process of itching makes it difficult to characterize [29]. This situation is much more complicated for chronic itching such as atopic dermatitis and psoriasis, and the mechanism underlying such itching has not been fully explained [30]. Therefore, in this study, in which we investigated both acute and chronic pruritus, and we reported the data we obtained by comparing it with the clinical data presented in other studies.

Inhibition of Hsp90 by 17-DMAG (geldanamycin derivative) has been shown to suppress pro-inflammatory

mediators involved in bullous pemphigoid [31]. Kakeda et al. [10] indicated that Hsp90 expression is significantly upregulated in epidermal keratinocyte and mast cells of the lesional psoriatic skin. In another study conducted on 31 healthy volunteers, epidermal mRNA expression levels of Hsp90 and Hsp70 were significantly upregulated associated with cutaneous inflammation induced by tape stripping a pivotal element in enhancing the allergic contact dermatitis response [32]. In addition, in imiquimod-induced pruritus, the antipruritic activity of oxy-matrin has been associated with the inhibition of Hsp90 expression in keratinocytes [33]. These studies suggest that Hsp90 may be a potential drug target for the treatment of pruritus, especially for chronic itchy conditions whose treatment is limited. For this reason, we performed 17-AAG-mediated Hsp90 inhibition in our study. We found that 17-AAG reduced itching in a dose-dependent manner (1, 3, and 5 mg/kg, i.p.) in the 5-HT-induced acute itch model and in the AEW-induced chronic itching model. At the highest dose, we used 5 mg/kg, and 17-AAG eliminated itching in both itching models.

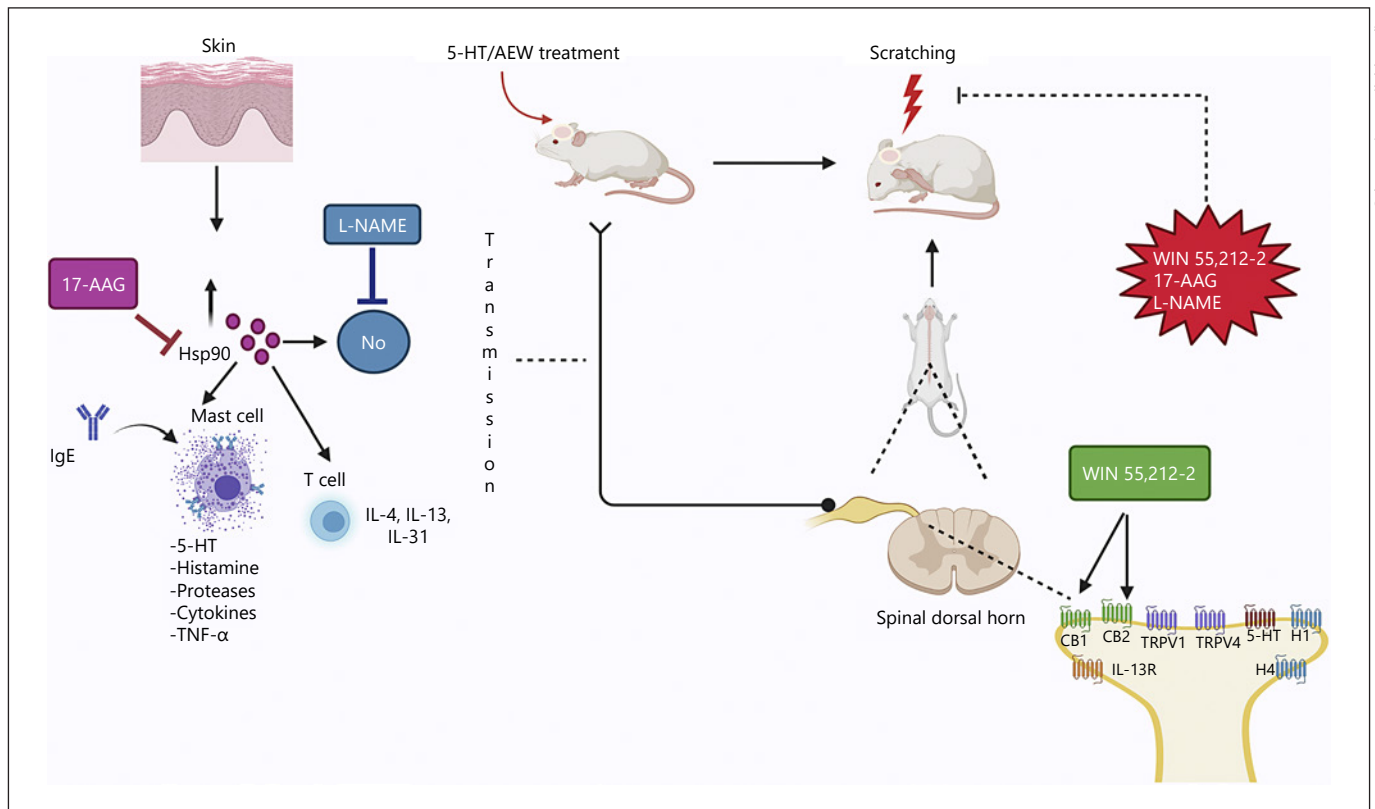


Fig. 5. Possible effects of Hsp90, cannabinoid agonist, and NO on itch transmission. Intradermal application of serotonin (5-HT) and topical application of AEW to the nape of mice produce itching behavior. Induction of itching causes activation of mast cells. Mediators responsible for itch transmission are released from mast cells and T cells [46]. At the same time, the expression of Hsp90 in epidermal keratinocytes and mast cells is increased [10]. Increased Hsp90 expression facilitates NO production [12]. Itching is transmitted to the dorsal horn of the spinal cord through these cellular mechanisms. Cannabinoids, on the other hand, produce antipruritic effects through cannabinoid receptors (CB1/2) and the TRP

ion channels [51]. Hsp90 inhibitor 17-AAG dose-dependently reduced 5-HT- and AEW-induced scratching behavior. These study results show that low-dose cannabinoid agonist WIN 55,212-2, NOS inhibitor L-NAME, and Hsp90 inhibitor 17-AAG synergistically reduce itching (Created with BioRender.com). Hsp90, heat shock protein 90; NO, nitric oxide; TRP, transient receptor potential; 17-AAG, 17-allylamino-17-demethoxygeldanamycin; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; Hsp90, heat shock protein 90; AEW, acetone-ether-water; NOS, nitric oxide synthase.

The data obtained regarding the role of cannabinoid in pruritus show that it is effective in the regulation of itch. In a study conducted in uremic pruritus patients, it was reported that applying a cream containing the endogenous cannabinoid anandamide twice daily for 3 weeks reduced pruritus [34]. Similarly, in humans, peripheral administration of a cannabinoid receptor agonist attenuates histamine-induced itch [35]. It has been speculated that the selective cannabinoid receptor agonist HU210 impedes the signal transmission of histamine and supposes the peripheral expression of cannabinoid receptors on the terminal endings of itch-specific subunits [36]. To-durga et al. [19] found that the nonselective CB1/2 receptor agonist WIN 55,212-2 reduces 5-HT-induced itch in

a dose-dependent manner. In addition, it has been shown that the CB1 receptor antagonist AM-251 partially eliminates the antipruritic effect of WIN 55,212-2 [17]. In these studies, it is concluded that WIN 55,212-2 (1 mg/kg, i.p.) reduces itching at low doses and does not affect motor coordination at this dose. In addition, topical application of cannabidiol, a phytocannabinoid, has been shown to provide relief in patients with atopic dermatitis [37]. In this study, we showed that WIN 55,212-2 (1 mg/kg i.p.) reduced itching behavior due to both 5-HT and dry skin.

In recent years, NO is thought to be a neurotransmitter, mediating itching [6]. In the 5-HT-induced itch model, it has been reported that the application of the nonse-

lective NOS inhibitor L-NAME (3 mg/kg i.p.) 30 min before 5-HT injection reduces itching behavior by approximately 50% [38]. Similarly, in another study, L-NAME (1 mg/kg, i.p.) has been shown to reduce the itching behavior caused by substance P [39]. On the other hand, it has been shown that the same dose of L-NAME (1 mg/kg, i.p.) does not inhibit itching behavior in chloroquine-induced itching [40, 41]. Our results show that L-NAME does not prevent 5-HT-induced and AEW-induced itching behaviors at a dose of 1 mg/kg.

For effective NO generation, Hsp90 and eNOS coupling are required [42]. The binding of Akt with Hsp90 facilitates NO production by increasing eNOS activity [11]. Hsp90 acts as the physiological binding partner and regulator of eNOS [43]. Prangsaengtong et al. [12] demonstrated that stimulation of Hsp90 increased NOS activity, and inhibition of Hsp90 decreased NO production. Recent research shows that there is a close relationship between the cannabinoid system and the NO signaling pathway. Similarly, NO production, considered by THC (tetrahydrocannabinol) and anandamide as an itch mediator, was found to be reduced [44]. The results obtained from these studies show that the NO signaling pathway may be effective in the antipruritic activity created by cannabinoids. Therefore, the cannabinoid agonist, Hsp90 inhibitor, and NOS inhibitor can be used together in the treatment of itching. On the other hand, it has been reported that the antipruritic effect of WIN 55,212-2 is not mediated by the NO pathway [45]. Our findings show that the combined administration of 17-AAG (1 mg/kg, i.p.), WIN 55,212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) eliminates itching in 5-HT- and dry skin-induced itching models. However, we found that these drugs did not cause a decrease in plasma nitrate/nitrite levels. Similarly, there was no decrease in the combined groups. Our findings show that the antipruritic effect of the cannabinoid agonist and Hsp90 inhibitor is not mediated by the NO pathway. On the other hand, the absence of the expected decrease in nitrate/nitrite levels in L-NAME-administered groups may be due to low-dose usage and administration duration.

T helper 2 cell signaling and IgE production play a key role in the pathogenesis of itching. In dermatological diseases such as urticaria, atopic dermatitis, and psoriasis, mast cells are activated by IgE and play a role in allergic responses [46]. Mast cells are affected at different levels by the cannabinoid system. Especially, the CB2 receptor is found intensively on immune system cells [47]. In one study, increased serum IgE levels and expression of the itch mediators IL-13 and IL-6 were shown to be de-

creased by the CB2 receptor agonist in a model of ovalbumin-induced asthma [48]. The Hsp family is closely related to the regulation of inflammatory responses, and the signal transduction cascades associated with these responses [49]. In a model of house dust mite-induced asthma in Balb/c mice, Hsp90 α blockade by 1G-D7 has been shown to significantly reduce elevated serum IgE levels as well as IL-4, IL-5, and IL-13 cytokines [50]. Our results show that increased IgE levels with AEW administration decreased with WIN 55,212-2 (1 mg/kg, i.p.) administration. 17-AAG (5 mg/kg, i.p.) reduced increasing IgE levels only at the highest dose administered. In the groups where these agents were given combined, IgE levels decreased, but there was no difference between the combination application and the single application of WIN 55,212-2. In conclusion, there is a close relationship between the T helper 2-mediated signaling pathway and the cannabinoid system, NO, and Hsp90. Therefore, the antipruritic effect induced by WIN 55,212-2 and 17-AAG may be due to downregulation of inflammatory and pro-inflammatory cytokine expression involved in itching.

In conclusion, pruritus is an orphan disease that cannot be specifically treated effectively as in analgesic drugs. For this reason, itching is still being studied extensively [5]. However, due to insufficient information and the variety of mediators and receptors that play a role in itching, there is no targeted treatment for itching. Thus, our results indicate that Hsp90 inhibitor dose-dependently reduced 5-HT- and AEW-induced scratching behavior. Also, this research study shows that the low-dose cannabinoid agonist, Hsp90 inhibitor, and NOS inhibitor synergistically reduce itching. However, we found that the antipruritic effect of these agents is not mediated by the NO pathway. These results give hope that cannabinoids and the Hsp90 inhibitor can be used in the treatment of pruritus (Fig. 5).

Acknowledgment

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Statement of Ethics

The Istanbul University Animal Experiments Local Ethics Committee confirmed all experimental procedures for this research (Decision No: 2018/17).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Z.G.T.S. and S.O. designed the study. Z.G.T.S. and F.K.T. performed the experiments. Z.G.T.S. and S.O. analyzed and interpreted the data. Z.G.T.S., S.G., and S.O. wrote the original draft and edited the manuscript. All the authors approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. The data obtained in this study are clearly stated in the article, in the figures, and in the figure legends. Further inquiries can be directed to the corresponding author.

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