DOI 10.1007/s11094-019-02056-2
Pharmaceutical Chemistry Journal, Vol. 53, No. 7, October, 2019 (Russian Original Vol. 53, No. 7, August, 2019)

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF VANILLIN DERIVATIVES

Yu. A. Boiko,[1] M. V. Nesterkina,[2,3,*] A. A. Shandra,[1] and I. A. Kravchenko[2,3,]**

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 53, No. 7, pp. 49 – 53, July, 2019.

Original article submitted December 19, 2017.

The analgesic activity of 4-hydroxy-3-methoxybenzaldehyde (vanillin) derivatives with different pharmacophore groups were studied in vivo in models of chemically induced pain induced by subplantar administration of the TRP ion channel agonists capsaicin and allylisothiocyanate. The anti-inflammatory actions of vanillin derivatives were demonstrated in a model of carrageenan-induced edema. The analgesic and anti-inflammatory activities of vanillin and its derivatives were studied; these are linked with the effects of these substances on TRPA1 and TRPV1 receptors.

Keywords: Vanillin derivatives, TRPA1 and TRPV1 receptors, analgesic activity, anti-inflammatory activity.

TRP channels (transient receptor potential channels) were first observed in 1969. Studies using a mutant *Drosophila melanogaster* showed the role of TRP channels in forming electrical responses to exposure to light in the visual analyzer [1]. Despite more than 40 years of work, intense study of TRP channels started only in the 1990s after the cloning and sequencing of the *Drosophila* TRPL channel gene [2]. More recently, studies of TRP channels have paid detailed attention to their undoubted role in the organization of the mechanisms of sensitivity to various phyhsical and chemical stimuli [3, 4]. From the point of view of practical medicine, there is interest in the involvement of TRP channels in generating pain impulses in nociceptive nerve fibers [4]. Substances influencing TRPV channel-associated receptors (the vanilloid family of TRP channels) are currently regarded as a fundamentally novel class of analgesics with great potential [5].

The classical TRPV-1 receptor agonist is capsaicin [6]. Important characteristics of the mechanism of action of this compounds include the ability to induce intense pain sensations on primary activation of capsaicin-sensitive nerve fibers and subsequent analgesia associated with desensitization of TRPV-1 receptors in response to prolonged exposure to capsaicin [7]. In relation to this pain-countering activity, capsaicin has found use as an analgesic in chronic pain [8, 9]. The potential and efficacy of the use of capsaicin in the pain clinic consistently attracts research attention to substances with similar structure. These may include compounds of plant origin, as well as newly synthesized substances [10]. From the pharmacochemical point of view, the capsaicin molecule and similar compounds can be identified as having three functional parts (Fig. 1) [9].

The A segment corresponds to a substituted benzene ring. This segment is the stable part of capsaicinoids and capsinoids, modification of this part in most cases leading to complete loss of biological activity. The B segment also has little variation and consists of an amide or ester bond. Capsaicinoids lacking this segment have lower affinity for TRPV-1 receptors [10]. The C segment is an aliphatic chain, saturated or containing a double bond, and is a variable fragment. From the pharmacological point of view, the most important part of the molecule is the vanilloid segment, which is responsible for affinity to TRPV-1 receptors, the aliphatic "tail" performing a modulatory function.

As indicated, modification of the vanilloid part has extremely significant influences on the biological activity of derivatives. In some cases, substitution of agonist properties in relation to TRPV-1 receptors by antagonist has been

[1] Odessa National Medical University, 65000 Odessa, Ukraine.
[2] Odessa National Polytechnic University, 65044 Odessa, Ukraine.
[3] I. I. Mechnikov Odessa National University, 65082 Odessa, Ukraine.
[*] e-mail: mashaneutron@gmail.com
[**] e-mail: kravchenko.pharm@gmailcom

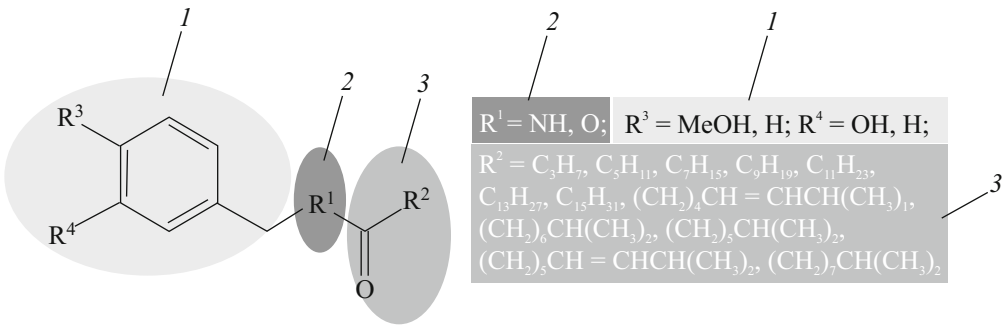

Fig. 1. Chemical structure of the capsaicin molecule: *1*) the slightly varying A segment; *2*) the B segment, corresponding to the amide or ether bond; *3*) the structurally most variable C segment.

achieved by introduction of halogen radicals into the benzene ring [11].

We selected vanillin as the parental molecule; this has significant potential for creating substances with positive biological activity [12] and has affinity for TRP receptors. Modulation of the pharmacological activity was attained using vanillin derivatives with differences in the B segment. Considering the positive influences of these compounds on the structure of biological membranes, it seems appropriate to study these derivatives using transdermal administration, with evaluation of their analgesic and anti-inflammatory activities.

EXPERIMENTAL SECTION

Studies of analgesic and anti-inflammatory activity in the present work used transdermal administration of the following vanillin derivatives: vanillin oxime, vanillyl alcohol, and vanillic acid. These derivatives were synthesized from vanillin (98%, TCI, Belgium) using known methods [13].

The soft base for ointments was prepared by mixing polyethylene glycol 1500, polyethylene oxide 400, and 1,2-propylene glycol at a ratio of 4:2:3. The resulting mixture was warmed with mixing to complete dissolution of polyethylene oxide 1500, after which one of the active substances (vanillin, vanillin oxime, vanillyl alcohol, or vanillic acid) was added. The final concentrations of active substances in ointment was 2% (w/w).

Studies were performed in animals reared and kept in the animal house of Odessa National Medical University. Mice (60 individuals) and rats (30 individuals) were selected randomly for experimental studies; rats were male Wistar rats weighing 180-220 g and mice were male CBA mice weighing 16-20 g. Animals were kept in standard animal-house conditions with free access to food and water. All studies using animals were carried out in compliance with the bioethical norms of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes (Strasbourg, 1986).

Studies of analgesic activity were performed on mice divided into six groups of 10 individuals. The right hindlimb of mice of the four experimental groups was treated with one of the four ointments 10 min before the test started. Approximately 10 mg of ointment was applied to the limb and was lightly rubbed into the skin. Animals of group 5 were controls (treated with ointment base). Animals of group 6 received application of ointment containing reference agent Anaesthesin to the hindlimb. Each group, including the control group, was divided into two equal subgroups (each of five individuals). Animals of subgroup 1 were used in the capsaicin test and animals of the second subgroup were used in the allylisothiocyanate test.

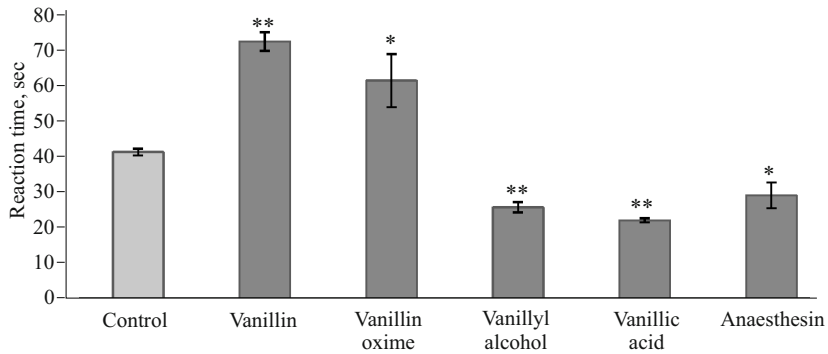

Fig. 2. Analgesic activity of vanillin and its derivatives (capsaicin test): *$p < 0.01$, **$p < 0.001$ compared with animals of the control group.

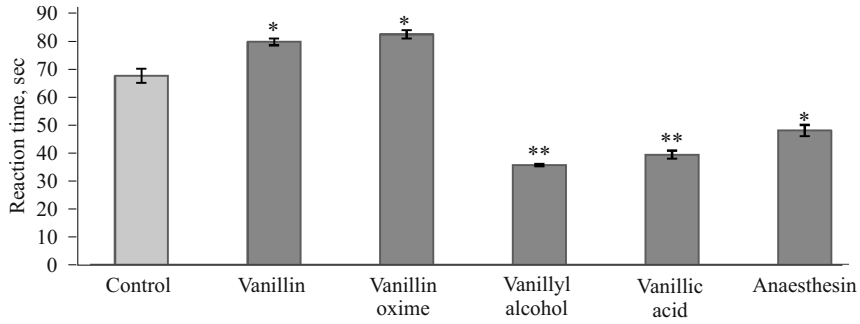

Fig. 3. Analgesic activity of vanillin and its derivatives (allylisothiocyanate test): *$p < 0.01$, **$p < 0.001$ compared with animals of the control group.

Anti-inflammatory activity was studied in rats, which were also divided into six groups, each containing five individuals. Animals of group 1 were treated with ointment containing vanillin, group 2 with ointment containing vanillin oxime, group 3 with ointment containing vanillyl alcohol, and group 4 with ointment containing vanillic acid. Animals of group 5 were used as controls (treated with ointment base). Group 6 were treated with ointment containing reference agent ibuprofen.

Capsaicin and allylisothiocyanate (AITC) tests for analgesic activity. Tests for analgesic activity were performed as described elsewhere [14].

Carrageenan-induced inflammatory edema. Inflammation was induced in experimental animals by subplantar administration of carrageenan as described in [15]. Treatment of animals with carrageenan-induced edema started immediately after administration of phlogogen and 2 h after administration. Ointment was then applied once each day. Ointment was applied at a dose of 50 mg to the area of inflammation, after which it was gently rubbed in for several minutes.

The distribution of the data obtained during the study was checked for normality using the Shapiro-Wilk test. As all data in the experimental groups had normal distributions, unifactorial analysis of variance was performed followed by Student's t test with the Bonferroni correction. Differences between values for which $p < 0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

Studies of analgesic and anti-inflammatory activities were carried out for soft medicinal formulations containing 2% active ingredient (vanillin or its derivatives). Further increases in the content of active component in the ointment did not lead to increases in the pharmacological effect. Reference agents in the ointment base were used at equimolar quantities of Anaesthesin/ibuprofen which, considering the close molecular weights of vanillin derivatives and reference compounds, were also 2%. Administration of capsaicin and allylisothiocyanate led to marked nociceptive reactions. Times spent licking the irritant administration site were taken as a measure of the intensity of the pain response. Prior treat-

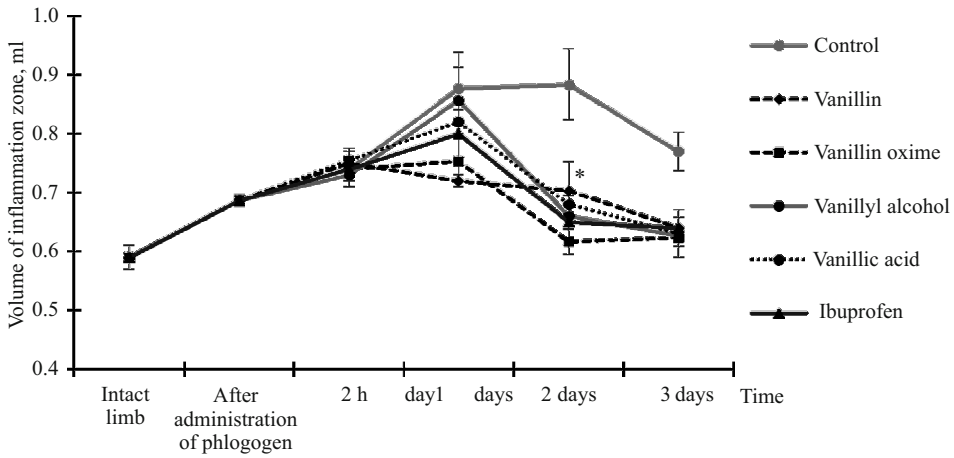

Fig. 4. Anti-inflammatory activity of vanillin and its derivatives in carrageenan-induced edema in rats (limb volume): *$p < 0.05$ compared with animals of the control group. Significance values very applied to all study groups.

ment of the limb in mice with vanillin or its derivatives could either lengthen or shorten the licking period (Figs. 2 and 3). Use of vanillin and vanillin oxime increased the licking period by factors of 1.75 and 1.5 in the capsaicin test and by factors of 1.18 and 1.21 in the allylisothiocyanate test respectively. Vanillyl alcohol and vanillic acid decreased licking time by factors of 1.64 and 1.86 in the capsaicin test and 1.89 and 1.74 in the allylisothiocyanate test respectively.

The anti-inflammatory activity of vanillin and its derivatives was assessed in terms of the ability to decrease carrageenan-induced edema. The dynamics of changes in the volume of edema at the carrageenan administration site in animals of the control and experimental groups are shown in Fig. 4. During the first day after administration of phlogogen, there were no significant differences in the magnitude of edema in animals of the control and experimental groups. The volumes of the inflamed area in groups receiving treatment on days 2 and 3 were 1.44 and 1.24 times lower than in animals of the control group (min $p = 0.04$) on days 2 and 3 respectively. There were no significant differences in the therapeutic efficacies of the ointments used.

Injections of capsaicin into the soft tissues of the footpad led to a pain response associated with activation of TRPV-1 membrane channels in C-type nerve fibers. The study compounds produced different actions on capsaicin-induced pain reactions. Vanillin and vanillin oxime potentiated the action of capsaicin, leading to an increase in the duration of licking, which is evidence of an increase in the pain response. At the same time, vanillyl alcohol and vanillic acid decreased the licking period. According to current views of the structure of the TRPV-1 channel, when this channel is in the closed state, the capsaicin binding site contains a small lipid molecule [16]. To exert its biological effect, capsaicin has to displace this molecule from the binding site. On pretreatment of the limb with ointment containing vanillin or is derivatives, we suggest that these latter displace the lipid molecule from the membrane pocket and take its place. Capsaicin injected into the tissue must then displace the vanillin or vanillin derivative from the receptor binding site. It follows from this proposed mechanism that the affinity of TRPV-1 for vanillin and vanillin oxime is lower than that for the natural "lipid bung" and, conversely, is higher in the cases of vanillyl alcohol and vanillic acid. These factors alter the binding kinetics of capsaicin with TRPV-1 and, ultimately, alter the extent of the pain reaction.

An explanation for these proposed differences in the affinities of the study compounds for the vanilloid binding site from our point of view is provided by the energy of hydrogen bonds formed between the variable fragment of the corresponding substances (the B segment) and hydrophilic groups in amino acid residues. Two groups of hydrogen bonds are critical for holding capsaicin in the membrane pocket. The first group is formed between methoxy and hydroxy radicals in the benzene ring and the corresponding polar fragments of amino acids; the second group of bonds arises in the region of the amide group [17]. Modification of the B segment of vanillin leads to strengthening or weakening of hydrogen bonds between this fragment and the polar groups of amino acids, which is reflected in the process of displacement of capsaicin by the test compounds from the membrane pocket. The allylisothiocyanate pain model is based on binding of allylisothiocyanate with TRPA-1 (TRP ankyrin family) and opening of TRPA-1 channels [18]. TRPA-1 channels, like TRPV-1 channels, are nonspecific cationic channels which can be activated by various chemical and physical stimuli [19, 20]. The activation of the pain response occurring in these conditions is mediated by arousal of nociceptive nerve fibers and subsequent release of neuropeptides [21]. As in the case of TRPV-1 receptors, preliminary treatment of the limb with ointment containing vanillin or its derivatives leads to modulation of the pain response induced by activation of TRPA-1 receptors. It is interesting to note that the influences of vanillin and its derivatives on changes in the pain response are in the same direction in both models.

Studies of anti-inflammatory activity were performed using a carrageenan edema model. Injection of carrageenan into the tissue induces a multiple of extra- and intracellular biochemical cascades, as well as intercellular interactions including release of histamine and serotonin [22] and bradykinin [23], activation of COX and synthesis of eicosanoids [24], and a response on the part of the vascular endothelium [25]. Despite the differences in the processes activated in the case of carrageenan-induced edema, we believe that the therapeutic compounds used in our studies acted at the subsequent points of the inflammatory cascade – delayed desensitization of TRPA-1 and TRPV-1, decreased production of neurogenic peptides, and decreased mast cell and tissue macrophage activity.

The use of vanillin and its derivatives as anti-inflammatory compounds had no significant influences on the dynamics of the development of carrageenan-induced edema in the first hours after administration. The differences seen in the extents of inflammatory edema in animals of the experimental and control groups one day after induction of inflammation and after two treatments were insignificant ($p = 0.27$). These data lead to the conclusion that the study compounds have no marked influence on the first phase of carrageenan edema, which is dependent on biogenic amines, bradykinin, and interleukins. Decreases in edema seen in animals of the experimental groups on day 2, as compared with the controls, were multifactorial in nature. The developing desensitization of TRP (vanilloid and ankyrin) in sensory nerve fibers leads to decreased release of substance P and calcitonin gene-related peptide. These substances stimulate hyperalgesia and increase vascular permeability, and substance P is also an activator of neutrophils, basophils, and mast cells; the monoamines released by them on degranulation also promote growth of capillary porosity [20]. Thus, decreases in the release of these neuropeptides lead to decreases in edema and pain, which was observed in our experimental conditions on the second day of treatment.

Thus, these studies established the analgesic and anti-inflammatory activity of vanillin and its derivatives, which is linked with the influences of these substances on TRPA-1 and TRPV-1. We believe that differences between the effects of study compounds on pain responses in experimental nociception models are explained by the characteristics of the binding of vanillin and its derivatives with the affinity sites of TRPA-1 and TRPV-1. The anti-inflammatory actions are mediated by a number of mechanisms, including suppression of the production of tachykinins, decreased levels of macrophage and mast cell activation, decreases in the production of proinflammatory cytokines, and decreases in COX and NO synthase.

REFERENCES

1. D. J. Cosens and A. Manning, *Nature*, **224**(5216), 285 – 287 (1969).
2. A. M. Phillips, A. Bull, and L. E. Kelly, *Neuron*, **8**(4), 631 – 642 (1992).
3. D. E. Clapham, L. W. Runnels, and C. Strubing, *Nat. Rev. Neurosci.*, **2**, 387 – 396 (2001).
4. M. J. Caterina and D. Julius, *Annu. Rev. Neurosci.*, **24**(1), 487 – 517 (2004).
5. J. Sousa-Valente, A. P. Andreou, L. Urban, and I. Nagy, *Br. J. Pharmacol.*, **171**(10), 2508 – 2527 (2014).
6. M. L. Reyes-Escogido, E. G. Gonzalez-Mondragon, and E. Vazquez-Tzompantzi, *Molecules*, **16**(2), 1253 – 1270 (2011).
7. O. M. E. Abdel-Salam, *Capsaicin as a Therapeutic Molecule*, Springer Science & Business Media, Basel (2014).
8. K. Shimoda, N. Kubota, and M. Akagi, *ACES*, **2**(1), 45 – 49 (2011).
9. B. C. N. Prasad, R. Shrivastava, and G. A. Ravishankar, *Evidence-Based Integrative Medicine*, **2**(3), 147 – 166 (2005).
10. A. Szallasi and P. M. Blumberg, *Pharmacol. Rev.*, **51**(2), 159 – 212 (1999).
11. G. Appendino, N. Daddario, A. Minassi, et al., *J. Med. Chem.*, **48**(14), 4663 – 4669 (2005).
12. L. S. Premkumar, *ACS Chem. Neurosci.*, **5**(11), 1117 – 1130 (2014).
13. L. Tittse, T. Aikher, *Preparative Organic Chemistry* [in Russian], Mir, Moscow (1999).
14. M. Nesterkina and I. Kravchenko, *Pharmaceuticals*, **10**(2), 1 – 10 (2017).
15. I. A. Kravchenko, E. A. Alekseeva, A. I. Aleksandrova, and A. A. Kobernik, *Pharm. Chem. J.*, **49**(3), 163 – 166 (2015).
16. F. Yang and J. Zheng, *Protein Cell*, **8**(3), 169 – 177 (2017).
17. F. Yang, X. Xiao, W. Cheng, et al., *Nat. Chem. Biol.*, **11**(7), 518 – 524 (2015).
18. S. E. Jordt, D. M. Bautista, H. H. Chuang, et al., *Nature*, **427**(6971), 260 – 265 (2004).
19. W. J. Laursen, E. O. Anderson, L. J. Hoffstaetter, et al., *Temperature*, **2**(2), 214 – 226 (2015).
20. C. R. McNamara, J. Mandel-Brehm, D. M. Bautista, et al., *Proc. Natl. Acad. Sci. USA,*, **104**(33), 13525 – 13530 (2007).
21. Y. Kaneko, A. Szallasi, *Br. J. Pharmacol.*, **171**(10), 2474 – 2507 (2014).
22. H. A. Al-Haboubi and I. J. Zeitlin, *Eur. J. Pharmacol.*, **88**(2 – 3), 169 – 176 (1983).
23. M. Di Rosa, J. P. Giroud, and D. A. Willoughby, *J. Pathol.*, **104**(1), 15 – 29 (1971).
24. J. L. Liang, J.-Z. Wu, Y.-H. Liu, Z.-B. Zhang, *Mediators Inflammat.*, 2017, 1 – 14 (2017).
25. M. Bucci, F. Roviezzo, I. Posadas, J. Yu, L. Parente, W. C. Sessa, and G. Cirino, *Proc. Natl. Acad. Sci. USA,*, **102**(3), 904 – 908 (2005).