

Research Article**The Antinociceptive Effect of GW5074 in C57BL/6 Mice**Elif Karabacak Tasdemir¹, Mehmet Kilic¹ and Salim Yalcin Inan^{1*}¹Department of Medical Pharmacology, Meram Faculty of Medicine, University of Konya-NE, Konya, Turkey**Abstract**

Visceral pain is one of the most common type of pain in mammals, however its mechanisms of action has not clearly been understood. In common sense, prostaglandins are released depending on tissue damage to reduce the threshold of pain receptors. Thus, pharmacological inhibition of c-Raf kinase signaling which controls the expression of cytokines and other proinflammatory factors may increase the threshold of these receptors. In the present study, we aimed to investigate the possible antinociceptive effect of GW5074, a selective c-Raf inhibitor, in chemically induced pain model. Pretreatment with GW5074 (0.5 and 2 mg/kg) significantly reduced the number of abdominal contractions induced by acetic acid. Therefore, our results suggest the idea that c-Raf kinase signaling could be responsible in peripheral pain mechanisms.

Keywords: c-Raf kinase; GW5074; Pain; C57BL/6 mice.**Introduction**

In recent years, Raf kinases have been shown to be one of the most important parts of signal transduction mechanisms of the mammalian cells. The activation of these kinases promote cellular growth and development [1]. Moreover, Raf kinases (a-Raf, b-Raf and c-Raf) are responsible for proliferation, survival and transformation of the cells [2]. While b-Raf and c-Raf are expressed in the brain, a-Raf is expressed in the peripheral, in which the major kinase is the c-Raf [3,4]. Transient activation of Raf kinases in the brain seem to be neuroprotective, however abnormal activation increase the expression of proinflammatory factors which lead to neuroinflammation, neurodegeneration and cell death [5]. In addition, it has been reported that GW5074, a c-Raf inhibitor, decreased hyperalgesia and allodynia in rats [6,7]. Therefore, in the present study, we aimed to investigate the possible antinociceptive effect of GW5074 in chemically induced pain model in C57BL/6 mice.

Materials and methods**Animals**

Young (9 weeks old) male C57BL/6 mice obtained from Bilkent University Genetics and Biotechnology Research Center (BilGen) - Animal House Unit were used in the experiments. They were housed (5 mice per cage) in an environmentally controlled vivarium under 12 h light-dark cycle, and provided with commercially available food and water ad libitum. Mice were allowed 1 week to adapt to the laboratory before use. Experiments were conducted between

10:00 to 16:00 h. All procedures conformed to NIH guidelines and were approved by the University of Konya-NE Animal Care and Use Committee (Protocol number: 2015-010).

Drugs

GW5074 (Tocris), DMSO (Sigma), acetic acid (Sigma) and metamizole (Novalgine, Sanofi) were used in the experiments. GW5074 (0.1, 0.5 and 2 mg/kg) and metamizole (250 mg/kg) were dissolved in vehicle (1 ml vehicle contains 4 µl DMSO + 996 µl saline) and saline, respectively and injected intraperitoneally (i.p) in a volume of 0.1 ml / 10 g body weight. Acetic acid solution (0.6%) was prepared with saline and injected i.p in a volume of 0.1 ml / 10g body weight 30 minutes after GW5074 or metamizole administrations. Control animals received vehicle injections. Doses were chosen according to our preliminary studies and literature [8,9].

Antinociceptive activity

The antinociceptive activity was evaluated using the acetic acid-induced abdominal contraction test [10,11], namely the writhing test. Briefly, this test is a chemical method used to induce pain of peripheral origin by injection of irritant chemicals, such as acetic acid in which the analgesic activity of the test compound is interpreted from decrease in the frequency of writhings. The typical behaviors of abdominal writhings in mice are arching of back, extension of hind limbs and contraction of abdominal musculature [12]. In the present study mice were injected with 0.6% acetic acid 30 min after GW5074, metamizole or vehicle administrations and they were observed for 15 min for abdominal contractions. The mean number of contractions obtained for each group is calculated

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as % antinociceptive activity (% AA) according to the following formula:

$\% AA = [(N - N^*) / N] \times 100$, where N is the mean number of contractions of vehicle group, and N* is the mean number of contractions of experimental group.

Statistical analysis

Results are presented as means \pm S.E.M. Statistical analyses were performed with SPSS statistical software (Version 20, IBM). Data were analyzed by One-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test. The minimum level of statistical significance was set at $P < 0.05$.

Results

The effects of vehicle (N=6), metamizole (N=5) and GW5074 (0.1 mg/kg N=6, 0.5 mg/kg N=6 and 2 mg/kg N=6) on the number of abdominal contractions and % antinociceptive activity are shown in Figure 1 and Figure 2 respectively. While pretreatment with metamizole protected contractions induced by acetic acid

($P < 0.05$), GW5074 reduced the number of abdominal contractions in a dose dependent manner, in which 0.5 and 2 mg/kg were found statistically significant ($P < 0.05$) when compared to vehicle group.

Discussion

Pain is one of the most common symptoms of various diseases that patients usually feel unpleasant experiences [13], however its mechanisms of action has not clearly been understood. In the present study, we investigated the possible contribution of c-Raf signalization in chemically induced pain model. Our data clearly showed that GW5074, a potent c-Raf kinase inhibitor, significantly reduced the number of abdominal contractions in a dose dependent manner.

Acetic acid induced abdominal contraction test, namely writhing test, is considered to be a clinical relevant model for intestinal pain in humans, and yet it is accepted as one of the best animal models for visceral pain that can be used to evaluate and compare the efficacy of new drugs [14]. Therefore, we used this test in the present study.

In common sense, prostaglandins are released depending on tissue damage to reduce the threshold of pain receptors in which pharmacological inhibition of c-Raf kinase signaling that controls the expression of cytokines and other proinflammatory factors may increase the threshold of these receptors. Moreover, it has been shown that, another Raf inhibitor sorafenib reduced the expressions of cyclooxygenase-2, inducible nitric oxide synthase, amyloid precursor protein and restored memory impairment in transgenic mice brain [5]. It is possible that GW5074 might reduce nitric oxide release which in turns causes antinociception in our study. Another possible mechanism could be the inhibition of proinflammatory factors, such as cytokines, by GW5074, since Lei et al [4] previously reported that GW5074 protected tissue inflammation in sidestream smoke mice model.

In conclusion, c-Raf signalization may be involved in visceral pain and GW5074 could be potentially novel analgesic agents. However, further studies are needed to explain the molecular and histopathological mechanisms of c-Raf signalization in pain.

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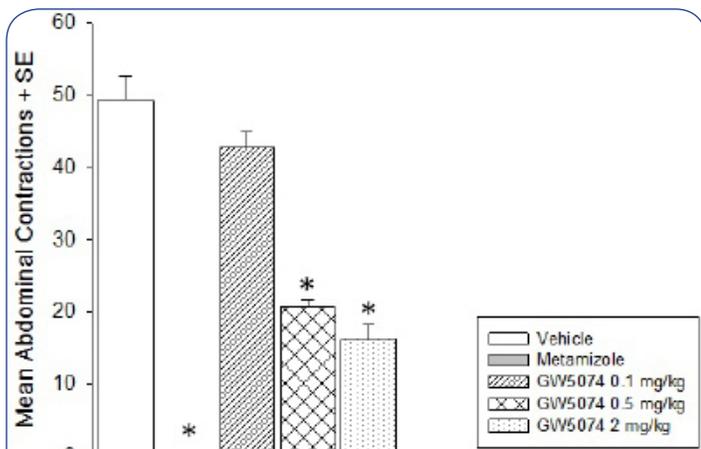


Figure 1: The effects of metamizole and GW5074 on the mean number of abdominal contractions. * $P < 0.05$: significantly different from vehicle group.

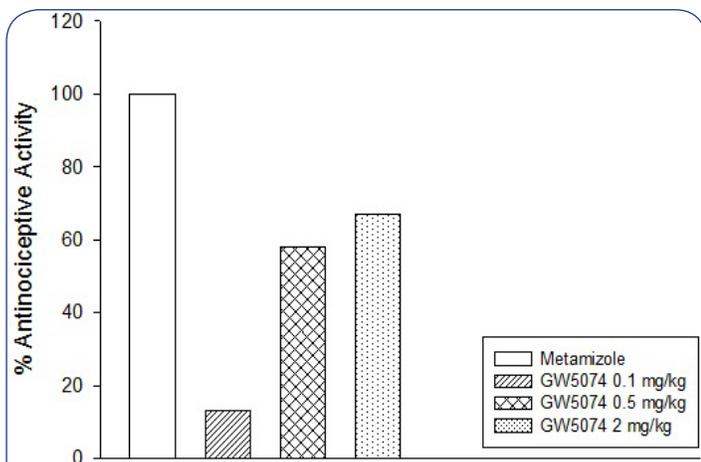


Figure 2: The effects of metamizole and GW5074 on the percentage of antinociceptive activity.

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