

# The Involvement of NO–cGMP–ATP Sensitive K<sup>+</sup> Channels Pathway in Protocatechuic Acid Peripheral Analgesia

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## ABSTRACT

Protocatechuic acid (PCA) is a widely distributed natural bioactive phenolic acid. The various pharmacological activities such as antioxidant, antidiabetic and anti-inflammatory activities have been identified. However, the studies focused on the analgesic effect of protocatechuic acid are limited and the action mechanisms of PCA still remain unclear. The NO-cGMP-ATP-sensitive K<sup>+</sup> channels pathway is one of the mechanism of action for various analgesic drugs. The present study was conducted to investigate the involvement of NO–cGMP–ATP sensitive K<sup>+</sup> channels pathway in analgesic effect of *p.o.* administration of 300 mg/kg protocatechuic acid in acetic acid-induced writhing test in mice. It was shown that pre-treatment with glibenclamide (10 mg/kg, *i.p.*), an ATP-sensitive K<sup>+</sup> channel blocker, and methylene blue (20 mg/kg, *i.p.*), a guanylate cyclase inhibitor, did not notably change antinociception produced by 300 mg/kg protocatechuic acid, however administration of nitro-L-arginine methyl ester (10 mg/kg, *i.p.*), a nitric oxide synthase inhibitor, significantly reversed protocatechuic acid antinociception. The results show that the peripheral mechanism of action of protocatechuic acid-induced antinociception involved another nitric oxide related pain pathway, not NO–cGMP–ATP sensitive K<sup>+</sup> channels pathway.

**Key words:** Protocatechuic Acid, Pain, No–Cgmp–Atp Sensitive K<sup>+</sup> Channels, Writhing Test.

## INTRODUCTION

Although a number of analgesics are available, certain problems such as tolerability, tolerance, abstinence syndrome, insufficiency, possible drug interactions, and side-effects also exist. Hence, the development of analgesics with minimal side effects is still ongoing.<sup>1</sup> In this respect, phenolic compounds have gained attention in pain management. Protocatechuic acid (PCA) is a natural bioactive phenolic compound which is widely distributed and present in tasty plants used traditionally.<sup>2</sup> It has been investigated that PCA has various biological effects. It is well anti-oxidant, antibacterial, anticancer agent and it has antidiabetic, anti-inflammatory, neurological and cardiac activity. However, the studies focused on the analgesic effect of PCA are

limited<sup>3</sup> and the action mechanisms of PCA still remain unclear. It is very important to investigate the action mechanism of drugs for rational drug use. *In vivo* pharmacological studies imply that ATP-sensitive K<sup>+</sup> channels in peripheral sensory neurons may be activated indirectly via the NO/cGMP/PKG pathway and this pathway is one of the mechanism of action for various analgesic drugs such as diclofenac and fentanyl.<sup>4,6</sup> The acetic acid induced writhing test is one of the most commonly used methods for measuring peripheral analgesic activity in mice.<sup>7</sup> Previously, in another study,<sup>8</sup> we found that 150 and 300 mg/kg (*p.o.*) PCA have a statistically significant ( $P \leq 0.05$  and  $P \leq 0.01$ , respectively) peripheral analgesic

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effect in acetic acid induced writhing test. So, for this action mechanism studies, 300 mg/kg PCA was chosen. It was aimed to investigate the involvement of NO–cGMP–ATP sensitive K<sup>+</sup> channels pathway in analgesic effect of *p.a.* administration of 300 mg/kg protocatechuic acid in acetic acid induced writhing test in mice by pre-treatment with glibenclamide (10 mg/kg, *i.p.*), an ATP-sensitive K<sup>+</sup> channel blocker, methylene blue (20 mg/kg, *i.p.*), a guanylate cyclase inhibitor, and nitro-L-arginine methyl ester (L-NAME) (10 mg/kg, *i.p.*), a nitric oxide synthase inhibitor.

## MATERIAL AND METHODS

### Animals

The balb/c male mice were housed in a well-ventilated room with 12-h light/dark cycles at 22 ± 1°C and allowed free access to food and water *ad libitum*. Six hours before the experimental procedures, the animals received only water to avoid possible food interaction with PCA. Animal care and research protocols approved by the Local Ethics Committee of Osmangazi University, Eskisehir, Turkey.

### Experimental groups and treatments

Five experimental groups were formed as follows; **I.**Control group: 0.2 cc vehicle saline (*p.a.*), **II.**Treatment group: 300 mg/kg PCA (*p.a.*), **III.**Pre-treatment group I: 10 mg/kg L-NAME (*i.p.*) + 300 mg/kg (*p.a.*), **IV.**Pre-treatment group II: 20 mg/kg methylene blue (*i.p.*) +

300 mg/kg PCA (*p.a.*), **V.**Pre-treatment group III: 10 mg/kg glibenclamide (*i.p.*) + 300 mg/kg PCA (*p.a.*).

L-NAME was injected 20 minutes before, methylene blue and glibenclamide were injected 15 minutes before the *p.a.* administration of 300 mg/kg PCA. The acetic acid-induced abdominal writhing test was performed 45 minutes after the *p.a.* administration of 300 mg/kg PCA.

### The acetic acid induced writhing test

Mice were injected with 10 ml/kg of 0.6% acetic acid solution (*i.p.*) 45 min after the administration of the PCA or vehicle. 5 min after the administration of acetic acid, the number of writhing was counted for 10 min.

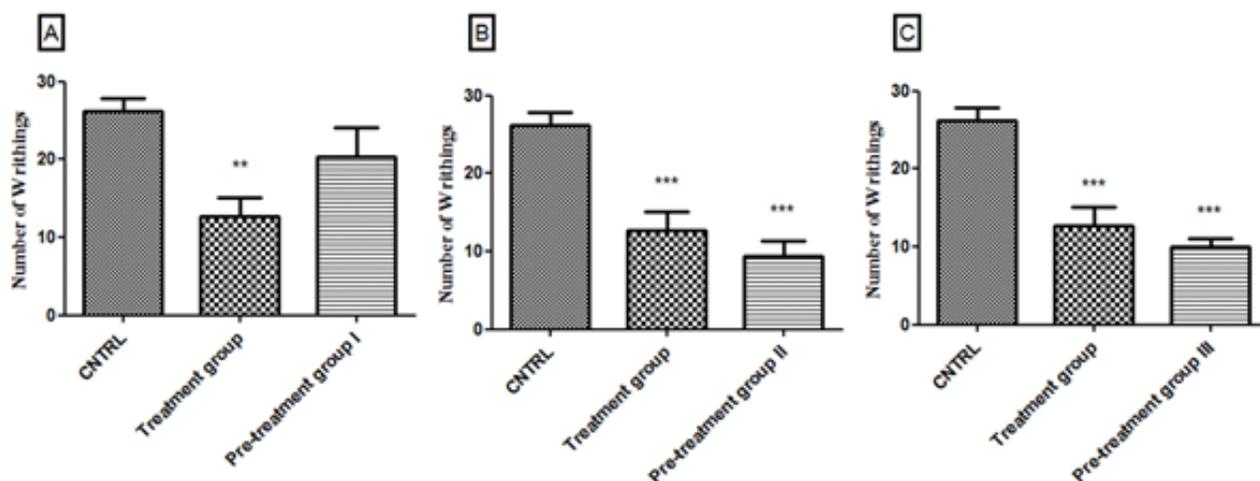
### Data analyses

Statistical differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The results were expressed as the mean ± standard error of the mean to show variation in groups. Differences were considered significant when  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Involvement of L-arginine/NO pathway

The number of abdominal writhing reduced in just 300 mg/kg PCA administered group as expected. L-NAME pre-treatment reversed the reduction of writhing induced by 300 mg/kg PCA administration. This reversible effect did not significant when compared to 300 PCA group, but pre-treatment group also not effective as control group (Figure 1.A).



**Figure 1.** (A) Involvement of L-arginine/NO pathway in acetic acid induced writhing test. (B) Involvement of cGMP pathway in acetic acid induced writhing test. (C) Involvement of ATP-sensitive K<sup>+</sup> channel pathway in acetic acid induced writhing test. Treatment group: 300 mg/kg PCA, Pre-treatment group I: L-NAME+ 300 mg/kg PCA, Pre-treatment group II: methylene blue+ 300 mg/kg PCA, Pre-treatment group III: glibenclamide + 300 mg/kg PCA. \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ : as compared to control group.

### Involvement of cGMP pathway

In experiment which researches the involvement of cGMP pathway; it was observed that the number of abdominal writhing was not significantly altered by pre-treatment with methylene blue as seen in Figure 1.B. PCA was still effective despite the methylene blue injection.

### Involvement of ATP-sensitive K<sup>+</sup> channel pathway

In last step of mechanisms of action studies; it was observed that the number of abdominal writhing was not significantly altered by pre-treatment with glibenclamide as in methylene blue injected group. PCA was still effective as you can see despite the glibenclamide injection

The pre-treatment with an ATP-sensitive K<sup>+</sup> channel blocker glibenclamide (10 mg/kg, *i.p.*), and GC inhibitor methylene blue (20 mg/kg, *i.p.*), did not pronounced change antinociception produced by 300 mg/kg PCA, however administration of NOS inhibitor L-NAME (10 mg/kg, *i.p.*) reversed antinociceptive activity induced by PCA.

The acetic acid-induced writhing test that used in this study, is an inflammatory pain model for acute nociception. Acetic acid damages the tissues and provokes the releasing hyperalgesic substances such as prostaglandins, which stimulate the peripheral nociceptors on the terminals of sensory nerve fibers.<sup>9</sup> It is also known hyperalgesic mediators inhibit the potassium channels which cause the reducing of the nociceptor threshold and inducing neuronal membrane excitability. PCA reduces the number of abdominal writhing significantly therefore it can be said that PCA has peripheral analgesic effect. ATP-sensitive K<sup>+</sup> channels which is found in the central and peripheral nervous system, regulate the neurotransmitter release, neuronal excitability and ligand effects. Upon activation of potassium channels, the potassium channel will open and thus permits the efflux of potassium ions from nerve cell later cause repolarization and/or hyperpolarization that decrease the induction of action potential.<sup>10</sup> Because of that effectiveness, they become the therapeutic target in finding analgesic drugs. Specifically, *in vivo* pharmacological studies imply that ATP-sensitive K<sup>+</sup> channels in peripheral sensory neurons may be activated indirectly by way of the NO/cGMP/PKG pathway. The NO/cGMP/ATP-sensitive K<sup>+</sup> channel pathway is one of the mechanism of action of some analgesics such as diclofenac and fentanyl.<sup>4-6</sup> However, according to our test results, the peripheral antinociceptive action of PCA involved another NO

related pain pathway, not NO–cGMP–ATP sensitive K<sup>+</sup> channels pathway. The other possible NO related pain pathway involvement in PCA antinociception is still under investigation.

### CONCLUSION

It seems that PCA shows peripheral analgesic effect and one of the mechanism of action is NO related pain pathway. It has a potential for using in pain management as a co-adjuvant or monotherapeutic agent

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### CONFLICT OF INTEREST

None

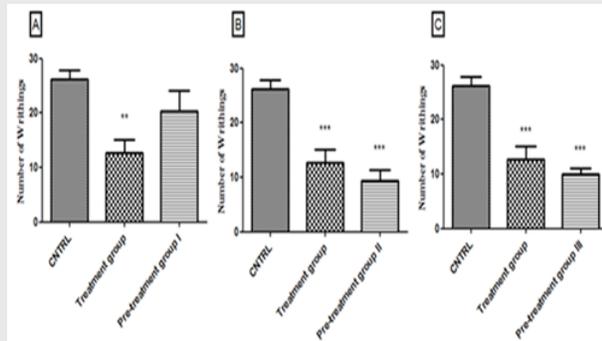
### ABBREVIATION USED

PCA: Protocatechuic acid; NO: Nitric oxide; cGMP: cyclic guanosine monophosphate; PKG: Protein kinase G; L-NAME: L-arginine methyl ester; NOS: Nitric oxide synthase.

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## PICTORIAL ABSTRACT



## SUMMARY

- Protocatechuic acid possesses peripheral analgesic effect in mice.
- Glibenclamide, an ATP sensitive K<sup>+</sup> channel blocker, and methylene blue, a guanylate cyclase inhibitor, did not notably change antinociception produced by 300 mg/kg protocatechuic acid.
- Administration of nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, significantly reversed protocatechuic acid antinociception.
- The peripheral mechanism of action of protocatechuic acid-induced antinociception involved another nitric oxide related pain pathway, not NO-cGMP-ATP sensitive K<sup>+</sup> channels pathway

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