

The contribution of serotonergic receptors and nitric oxide systems in the analgesic effect of acetaminophen: An overview of the last decade

Asetaminofenin analjezik etkisinde serotonerjik reseptörlerin ve nitrik oksit sisteminin katkısı: Son on yıla genel bakış

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Acetaminophen is a widely used analgesic and antipyretic agent. It is also available as over the counter formulations which has increased its wide use. There are many researches accumulated up to now which aimed to evaluate the mechanism of analgesic action of acetaminophen. Additional to the inhibition of cyclooxygenase pathway in the central nervous system, involvement of opioidergic, cannabinoidergic, dopaminergic, cholinergic and nitergic systems as well as contribution of descending pain inhibitory systems like bulbospinal serotonergic pathway have been proposed as the possible mechanisms of analgesic action of acetaminophen. In this review, we aimed to bring the data together from the studies revealing the contribution of the central serotonergic system and the role of central nervous system located serotonergic receptor subtypes in the analgesic effect of acetaminophen. While doing this, we mainly focused on the researches which have been performed in the last ten years and tried to put a link between the previous data and the lately added results. Additional to the serotonergic system involvement, we also reviewed the role of nitric oxide in the analgesic action of acetaminophen, especially with the new findings in the last decade.

Keywords: Acetaminophen, serotonin, pain, nitric oxide

INTRODUCTION

As one of the most commonly used medication, acetaminophen still keeps in its exact analgesic mechanism of action as a mystery. Not only decrease in prostaglandin production via cyclooxygenase (COX) enzyme inhibition (especially COX-2, and a central splice variant of COX-1 which is COX-3) has been proposed as the primary mechanism of analgesic action¹⁻³, but also contribution of cannabinoidergic⁴ and opioidergic⁵ systems have also been shown. In addition to these main contributions, cholinergic⁶ and dopaminergic⁷ systems have also been shown to be involved in the acetaminophen-analgesia. Not only the above neuronal systems, but also the role of calcium channels (T-type voltage-gated calcium channels) has also been proposed to take part in the analgesic effect of acetaminophen.⁸

In this review, it is aimed to discuss the two other proposed mechanisms for the analgesic action of acetaminophen which are serotonergic system with its various receptor subtypes and nitric oxide systems. It is focused on the findings in the last decade regarding the contribution of these two systems in acetaminophen analgesia with the intention of comparing these new findings with the previous results and put these novel findings together.

The role of central serotonergic system in acetaminophen-analgesia:

27 years ago, in 1991, antinociceptive effect of acetaminophen in formalin test was reduced following the chemical impairment of spinal serotonergic pathways (bulbospinal serotonergic pathway) with intrathecal 5,6-dihydroxytryptamine (5,6-DHT) administration in rats. This study indicated the contribution of spinal serotonergic system in the analgesic action of acetaminophen.⁹ This was followed by the finding that, antinociceptive effect of systemic acetaminophen administration to rats was reduced by the administration of *p*-chlorphenylalanine which was known to deplete the brain serotonin levels. Additionally, acetaminophen increased serotonin levels in brain cortex and pons. As a result, this data showed the involvement of supra-spinal serotonergic system in acetaminophen-analgesia.¹⁰

These previous results have been confirmed and also been expanded with some additional researches performed in the last decade. In animal studies, central

serotonergic system impairment with intrathecal and intracerebroventricular 5,7-DHT administration and assessment of brain serotonin levels were the most commonly used methods. These methods served to the evaluation of the serotonergic system involvement in acetaminophen-analgesia. Among these studies, some differences were observed in the effects of chemical destruction of the central serotonergic system on the analgesic effect of acetaminophen between different animal pain models and the doses of acetaminophen. Recent results have been summarized in Table 1.

5,7-DHT	Acetaminophen	Pain model	Effect	Animal	Reference
100 µg; i.t.	3 mg/kg; i.p.	Paw Pressure test	decrease	Rat (Sprague-Dawley)	Mallet C, 2008 ¹¹
50 µg; i.t.	200-600 mg/kg;	Tail Flick test	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
50 µg; i.t.	200-600 mg/kg;	Hot plate test	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
50 µg; i.t.	200-600 mg/kg;	Plantar incision (thermal hyperalgesia)	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Hot plate test	decrease	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Writhing test	decrease*	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Tail immersion	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Paw pressure test	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Formalin test	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³

Table 1: Effect of the deterioration of the bulbospinal serotonergic pathway with 5,7-dihydroxytryptamine (5,7 DHT) in the antinociceptive effect of acetaminophen in different pain models in some studies performed in the last decade. * Decrease in the hot plate test was more obvious than the decrease in the writhing test. i.t.: intrathecal, i.c.v.: intracerebroventricular.

Acetaminophen-induced serotonin increases has also been confirmed in recent studies. 400 mg/kg, intraperitoneal acetaminophen administration induced approximately 40% and 75% increases in the serotonin levels in pons and frontal cortex; respectively. These increases in serotonin levels have been found to be related with central 5-HT₂ receptors as well as opioid receptors (μ_1 and κ).⁵ These data were confirmed in a study by Vijayakaran K. et al.¹⁴ in which acetaminophen

(400 mg/kg; oral) exerted increases in serotonin levels in rat frontal cortex and brain stem. Not only following the acute applications, but also serotonin increases were observed following the chronic acetaminophen applications. 10 and 50 mg/kg doses of subcutaneous acetaminophen were administered to 3 month-old rats and serotonin levels in prefrontal cortex, hippocampus, hypothalamus and striatum were analyzed. Serotonin levels increased in 10 mg/kg acetaminophen dose (not with 50 mg/kg) in prefrontal cortex but not in the other brain regions analyzed in this study. Additionally, 5-HIAA levels decreased in hypothalamus and striatum.¹⁵ All these recent studies have confirmed the idea that systemic acetaminophen administration increases the serotonin levels in brain cortex and brain stem (pons) and meet at a common point which is acute and chronic systemic administration of acetaminophen induces changes in the central serotonergic neurotransmission. It can be concluded that, despite the involvement of 5-HT₂-serotonergic and opioid receptors in acetaminophen-induced serotonin increases in some brain regions⁵, apparently the exact mechanism (alterations in serotonin metabolism, release or uptake) is still not clear and needs to be clarified.

The signs of serotonergic involvement in acetaminophen-induced analgesia in humans were also studied. However, despite to some supportive results, serotonergic contribution seems still doubtful in human studies due to the challenges studying pain in humans. Controversial data have been observed between the results in healthy volunteers and patients with pain.¹⁶ The findings of these studies will be discussed in the following sections.

Some metabolites of acetaminophen and central serotonergic system:

Acetaminophen, following its systemic administration, has been shown to be biotransformed to an amine compound "*p-aminophenol*" which occurs mainly in liver. Enzymatic conversion of *p-aminophenol* to N-arachidonoyl-phenolamine (AM404) which is catalyzed by fatty acid amide hydrolase (FAAH) enzyme with the conjugation of arachidonic acid occurs in brain, spinal cord and dorsal root ganglia.¹⁷ AM404 metabolite of acetaminophen has been shown to activate TRPV1 (transient receptor potential vanilloid-1, capsaicin receptor) channels and act as a CB1 (cannabinoid receptor type-1) ligand.^{17,18} Mallet C. et al.¹¹ showed that, CB1 receptors are vital for the analgesic action of orally administered acetaminophen,

because CB1 receptor antagonism as well as gene deletion totally inhibited the analgesic action of acetaminophen in various pain models; thermal, mechanical and chemical (formalin) painful stimuli in rats. CB1 receptor related activation of descending serotonergic pathway has been suggested as the following step, because the antinociceptive effect of systemic acetaminophen was abolished following the chemical impairment of the spinal serotonergic pathway. As a CB1 receptor ligand, AM404 metabolite of acetaminophen has been claimed to be responsible for this action. Spinal 5HT_{1A} and 5HT_{3/4} receptors have been shown to contribute in the spinal cord level to the analgesic action of acetaminophen eventually. On the other hand, Ruggieri V. et al.⁵ claimed that, AM404 can only partially contribute to the analgesic action of systemically administered acetaminophen, depending on the fact that observed analgesic action of AM404 was approximately the half of the analgesic action of acetaminophen. This AM404 contribution seems to be related with the central 5HT₃ receptors, but not with 5HT_{1A} or 5HT₂ receptor subtypes. Interestingly, central 5HT₂ receptor subtype, but not 5HT₃ or 5HT_{1A}, has been found to be related in the analgesic action of acetaminophen depending on the dose-dependent inhibition of acetaminophen's analgesic action with systemic ketanserin. Another important finding of this study was the increase of serotonin levels in pons and frontal cortex following the administration of acetaminophen, but not with AM404. All these results of this study were pointing out that, acetaminophen and its metabolite AM404 have both analgesic actions but the mechanisms that play role in this analgesic action differs between these two compounds. These differences about the contribution of AM404 in the acetaminophen-analgesia and involvement of different serotonergic receptor subtypes can be related with the use of different type and different administration routes of serotonergic receptor subtype antagonists as well as the dose of acetaminophen which was different in two studies.

Finally, Barrière DA et al.¹⁹, in their study showed the contribution of descending serotonergic antinociceptive pathway in the analgesic effect of 4-aminophenol, another metabolite of acetaminophen as mentioned above. The analgesic effect of intraperitoneally administered 4-aminophenol was reported to depend on the AM404 formation in the brain which is catalyzed by FAAH enzyme and TRPV1 and CB1 receptor stimulation-induced descending antinociceptive

serotonergic system activation which eventually spinal 5-HT₃ and 5-HT_{1A} serotonergic receptor subtypes were claimed to play an important role.

As a result, studies showed that not only acetaminophen itself, but its metabolites like AM404 and 4-aminophenol may play an important role in the analgesic action of acetaminophen. It can be concluded that AM404 metabolite contributes to the analgesic action of systemic acetaminophen to some extent and activates the descending serotonergic antinociceptive pathway via the contribution of central TRPV1 and CB1 receptors. Spinal serotonergic receptor subtypes eventually play role in the antinociceptive action which may act differently to acetaminophen and its metabolites.

Role of 5-HT₁ receptors:

It is known that, 5-HT_{1A} and 5-HT_{1B} serotonergic receptor subtypes are largely located in the supra-spinal level; 5-HT_{1A} on the cell bodies and dendrites of serotonergic neurons and 5-HT_{1B} mainly on the axon terminals. Both 5-HT_{1A} and 5-HT_{1B} serotonergic receptor subtypes have important functions on the extracellular serotonin levels via modulation of the nerve firing mainly for 5-HT_{1A} receptors and by modification of serotonin release for mainly 5-HT_{1B} serotonergic receptor subtypes.²⁰ Blockade of 5-HT_{1A} receptor subtypes have been shown to enhance the extracellular levels of serotonin.²¹ Involvement of 5-HT₁ serotonergic receptor subtypes in the analgesic effect of acetaminophen has been studied in various studies with different animal pain models and with different ligands for 5-HT_{1A} and 5-HT_{1B} receptor subtypes. Incompatible results were obtained in earlier studies regarding the contribution of 5-HT₁ serotonergic receptors in the analgesic effect of acetaminophen. An earlier study showed that, pre-administered WAY-100635 (5HT_{1A} receptor antagonist, 10 µg/rat; intrathecal) did not change the analgesic effect of intravenous acetaminophen (200 mg/kg) in rat paw pressure test.²² However; intrathecal administration of WAY-100635 (40 µg/rat) has been shown to block the acetaminophen-analgesia (3 mg/kg, i.p.) in both phase I and II of rat formalin test.¹¹ Besides, intraperitoneal administration of NAN-190 (5-HT₁ serotonergic receptor antagonist, 1-5 mg/kg) didn't change the acetaminophen-analgesia in hot-plate and paw pressure tests and didn't show any blockage on the acetaminophen-induced serotonin increases in frontal cortex and pons.⁵ However, it should not be

underestimated that NAN-190 could also block the α_2 -adrenergic receptors and this finding can raise some suspicious approaches to NAN-190 when using it as a specific 5-HT_{1A} receptor antagonist.²³ Interestingly, another earlier study showed that systemic administrations of 5-HT_{1A} and 5-HT_{1B} receptor antagonists enhanced the acetaminophen-analgesic action whereas stimulation of the same receptor subtypes blocked the acetaminophen-analgesia in hot plate test.²⁴ This was in good accordance with the findings of Sandrini M et al.²⁵ in which systemic administration of CP 93129 (5-HT_{1B} receptor agonist) prevented the acetaminophen analgesia in hot-plate and paw pressure test. These two findings suggested that increased serotonin release and/or enhanced firing of serotonergic nerves which liberate themselves from the suppressing effects of 5-HT_{1A} and 5-HT_{1B} receptors augment the antinociceptive action of acetaminophen. Findings of a recent study also were in good accordance with those previous results. Oral buspirone as a 5HT₁ serotonergic receptor agonist blocked the antinociceptive action of intraperitoneal acetaminophen (200 mg/kg) in hot plate test and in the early phase of the formalin test in mice.²⁶

As a result, when taken together it can be concluded that despite to some negative results, 5-HT_{1A} and 5-HT_{1B} serotonergic receptor subtypes are likely to contribute to acetaminophen-analgesia. However, characteristics of this contribution seem to depend on the ligands and animal pain models tested as well as the location (spinal/supra-spinal or presynaptic/postsynaptic) of 5-HT₁ serotonergic receptors and still needs to be evaluated.

Role of 5-HT₂ receptors:

The possible involvement of 5-HT₂ serotonergic receptor subtypes in the analgesic effect of acetaminophen has also been studied in recent studies. Ruggieri V. et al.⁵ showed a statistically significant reduction in the antinociceptive action of acetaminophen when ketanserin (5 mg/kg; subcutaneous) was administered systemically before acetaminophen (400 mg/kg; intraperitoneal), whereas it did not change the antinociceptive effect of AM404 in hot-plate and paw pressure tests. In another study, Dogrul A. et al.¹² showed that, intrathecally administered ketanserin (10 μ g) did not change the antinociceptive effect of acetaminophen (200-600 mg/kg; oral) in hot-plate and tail-flick tests as well as in thermal hyperalgesia after the incision of the hind paw. This recent study seems to reveal opposite findings from

the study of Courade JP et al.²² due to the fact that intrathecally administered ketanserin (5-HT_{2A} antagonist) as well as mesulergine (5-HT_{2C} antagonist) decreased vocalization thresholds which have been increased by intravenously administered propacetamol (water soluble prodrug form of acetaminophen). The differences in the study designs, like the animals (mice/rat), animal pain models (tail flick-hot plate/paw pressure test) that have been used and the timing of ketamine administration (before or after acetaminophen) between these two studies should be considered. Even so, when these studies are considered together, although the involvement of spinally located 5-HT₂ receptors in acetaminophen-analgesia needs to be elucidated, it can be speculated that supra-spinal 5-HT₂ serotonergic receptors may contribute to the analgesic effects of acetaminophen. Additionally, 5-HT₂ receptors are likely to be involved in the antinociceptive effect of acetaminophen, not in the antinociceptive effect of its metabolite, AM404. Supporting this assumption, systemic ketanserin has also been shown to block the acetaminophen-induced serotonin increases in frontal cortex and pons.⁵ Acetaminophen administration has also been shown to increase the serotonin levels in supra-spinal structures and led a down-regulation of 5-HT_{2A} receptor subtypes in frontal cortex and brain stem.¹⁴ In this study, authors claimed that increase in serotonin release triggered by acetaminophen caused down-regulation of 5-HT_{2A} receptors related with long-duration of stimulus by serotonin. This assertion was supported with the study of Srikiatkachorn A et al.²⁷ claiming that 5-HT_{2A} receptor down-regulation is important for the analgesic effect of acetaminophen. Thus, it may be speculated that supra-spinal located (most likely post-synaptic) 5-HT₂ receptor stimulation by serotonin which is enhanced following acetaminophen administration contributes to the analgesic action of acetaminophen.

ACETAMINOPHEN	5-HT ₂ ANTAGONIST	ANIMAL	PAIN MODEL	EFFECT ON PARACETAMOL-ANALGESIA	REFERENCE
200 mg/kg, i.v.	Ketanserin & mesulergine (10 µg, i.t.)-5 min before paracetamol	Rat	Paw pressure	Decrease	Courade JP, 2001 ²²
400 mg/kg, i.p.	Ketanserin (5 mg/kg, s.c.)	Rat	Hot-plate	Decrease	Ruggieri V, 2008 ⁵
400 mg/kg, i.p.	Ketanserin (5 mg/kg, s.c.)	Rat	Paw pressure	Decrease	Ruggieri V, 2008 ⁵
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Tail Flick test	No effect	Dogrul A, 2012 ¹²
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Hot-plate	No effect	Dogrul A, 2012 ¹²
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Post-incision	No effect	Dogrul A, 2012 ¹²

Table 2: Some studies on the effect on the role of 5-HT₂ receptors on the analgesic effect of acetaminophen in different pain models. i.v.: intravenous, i.p.: intraperitoneal, s.c.: subcutaneous, i.t.: intrathecal

Role of 5-HT₃ receptors:

Contribution of 5-HT₃ receptors in the analgesic effect of acetaminophen has been tested in various animal pain models as well as in human studies. Different 5-HT₃ receptor antagonists, like granisetron, ondansetron and tropisetron have been used up to now to study the interaction of these receptor subtypes in acetaminophen-analgesia. In 1996, indirect contribution of spinal 5-HT₃ serotonergic receptor subtypes had been pointed out depending on the findings of a research. In this research, it had been shown that spinal tropisetron totally inhibited the antinociceptive action of systemically and spinally administered acetaminophen in rat paw pressure test.²⁸ This finding had also been confirmed in inflammatory pain models.²⁹ In the last decade, a study by Mallet C et al.¹¹ showed that, intrathecal application of 0.5 µg tropisetron pre-treatment blocked the increased vocalization thresholds by systemic administration of acetaminophen which were in good accordance with the previous findings. However, the studies with the other tested 5-HT₃ receptor antagonists revealed mostly opposite results. Ondansetron administration (systemic as well as intrathecal) had been shown not to alter the analgesic effect of acetaminophen significantly.^{25,30} Recent studies also confirmed

this finding. Systemic ondansetron pretreatment (2 mg/kg; subcutaneous) did not alter the effect of acetaminophen in both hot-plate and paw pressure tests in rats⁵ which were in good accordance with the finding that spinally administered ondansetron was without any change in the effect of orally administered acetaminophen-induced analgesia in hot-plate, tail flick test and in thermal hyperalgesia in plantar-incision model.¹² An exception is a study in which acetaminophen-induced analgesia was blocked by ondansetron in mouse formalin test.³¹ Among these studies, differential involvement of 5-HT₃ receptors in acetaminophen and AM404-induced analgesia (similar to ketanserin) has been shown which ondansetron administration was able to block the analgesic effect of AM404.⁵ Another 5-HT₃ receptor antagonist, granisetron was lack of statistically significant changes in the analgesic effect of acetaminophen in paw pressure test.^{22,30} As a result, when the animal studies in the last ten years are considered together with the previous data, we can conclude that ondansetron and granisetron administrations are not likely to alter the effect of acetaminophen-induced analgesia, whereas tropisetron inhibits the analgesic effect of acetaminophen in various animal pain models. These different contributions can be explained by the differences between these antagonists regarding the pharmacokinetical properties (especially primary responsible cytochrome p450 system in the liver for their metabolism), 5-HT₃ receptor binding affinities, selectivity and specificity on 5-HT₃ receptors and their duration of their actions.^{32,33} However, another discussion raised at this point was the interrogation of 5-HT₃ receptor subtype contribution in the interaction between tropisetron and acetaminophen induced analgesia due to the finding that acetaminophen-analgesia was not altered by other 5-HT₃ receptor antagonists like ondansetron and granisetron. Additionally, spinal 5-HT₃ receptor antisense oligodeoxynucleotide pre-treatment, which aimed to decrease the synthesis of 5-HT₃ receptors, didn't inhibit the antinociceptive action of acetaminophen.³⁰ As a result, it has been started to be speculated that not the spinal 5-HT₃ receptor subtypes, but another tropisetron-sensitive receptor may play role in the analgesic action of acetaminophen.³⁰ Additionally, it has been indicated that tropisetron can also show affinity to other receptors like α 7-nicotinic receptor subtypes.^{30,34} When all these are considered together, the role of central 5-HT₃ serotonergic receptors in the analgesic effect of acetaminophen seems to be clarified with further studies.

The contribution of 5-HT₃ receptors in the analgesic effect of acetaminophen has also been studied in humans by using tropisetron, granisetron and ondansetron. These studies had two important goals; to reveal the involvement of 5-HT₃ serotonergic receptors in acetaminophen-analgesia in humans and evaluate the possible drug interaction between 5-HT₃ blockers and acetaminophen which are used in cancer patients together for vomiting and pain management, respectively. The first report showed the blockage of analgesic effect of acetaminophen (1 g, oral) when administered after tropisetron (5 mg, i.v.) or granisetron (3 mg, i.v.) in healthy volunteers tested with electrically-stimulated pain.³⁵ Results of a following study revealed that stimulation of descending serotonergic inhibitory pathway by acetaminophen contributed to the acetaminophen-analgesia in healthy volunteers where central 5-HT₃ receptors were involved.³⁶ These data were confirmed by a randomized, double-blind, and placebo-controlled study conducted in 16 healthy volunteers in which the combination of 1 g intravenous acetaminophen with 5 mg of tropisetron exerted no analgesic action in electrically-stimulated pain. In this study, tropisetron and acetaminophen alone both led analgesic actions.³⁷ The analgesic action of tropisetron administration alone was also confirmed by Tiippana et al.¹⁶ in healthy volunteers. Due to the fact that co-administration of acetaminophen with tropisetron in healthy volunteers did not lead to statistically significant changes in the blood levels of acetaminophen^{35,37}, it has been claimed that interaction between acetaminophen and tropisetron was in a pharmacodynamic fashion. However; studies performed in post-operative patients revealed confusing results which were not totally parallel and clear with the results of healthy volunteers. Ondansetron (4 mg) did not change the analgesic action of acetaminophen in women who went under laparoscopic hysterectomy.³⁸ A study performed in 36 patients who underwent to ear surgery, combination of tropisetron and acetaminophen reported higher pain scores in which the increase was not statistically significant. However patients who received tropisetron and acetaminophen needed more *rescue analgesic* agent.³⁹ A randomized, double-blinded study showed that ondansetron (8 mg) reduced the analgesic effect of acetaminophen (1 g) in patients who had abdominal hysterectomy; however this reduction was in a short period of time.⁴⁰

The results of those human studies indicate that, there is a questionable interaction between acetaminophen-analgesia and 5-HT₃ blockers due to some

conflicting results. Those conflicting results which were lack of obvious interaction were mainly related with the post-operative pain conditions.^{38,39} However, in healthy volunteers, interaction between acetaminophen and 5-HT₃ blockers (tropisetron and granisetron) seems more obvious and is likely to be a pharmacodynamic interaction.³⁵⁻³⁷ Apparently, studies with larger patient populations with different painful conditions are needed to clarify the interaction between 5-HT₃ blockers and acetaminophen in humans.

Role of 5-HT₇ receptors:

5-HT₇ receptors are G protein-coupled receptors linked with adenylyl cyclase and detected in central nervous system regions which are involved in pain transmission, like cerebral cortex, thalamus and the superficial lamina of the dorsal horn.⁴¹ Despite the fact that, 5-HT₇ receptors are one of the serotonergic receptor subtypes which have been studied less compared to the other subtypes (5-HT₁, 5-HT₂ and 5-HT₃ subtypes)⁴¹, some studies pointed out the contribution of these receptors in the antinociceptive action of acetaminophen in the last decade. Dogrul A et al.¹² used SB-269970 as a selective 5-HT₇ receptor antagonist to evaluate the role of these receptors in acetaminophen-analgesia and administered intrathecally (10 µg) after the oral administration of 200-600 mg/kg acetaminophen in mice. Intrathecal administration of SB-269970 blocked the antinociceptive action of acetaminophen in tail flick and hot plate tests. Similarly, intrathecal SB-269970 blocked the antihyperalgesic action of oral acetaminophen in plantar-incision model. This study was the first study which revealed the contribution of spinal 5-HT₇ receptors in the antinociceptive action of acetaminophen. Following study showed that intrathecally administered lower dose of SB-269970 (3 µg) was again successful to reverse the analgesic action of systemic acetaminophen in phase II of formalin test in mice. This data was important to confirm the contribution of spinal 5-HT₇ receptors in acetaminophen-analgesia, but also revealed reduction in the reversing effect of SB-269970 administration on acetaminophen-analgesia in mice lacking adenosine type-1 receptors which additionally indicated a strong interaction between adenosinergic system and 5-HT₇ receptors in the analgesic action of acetaminophen.⁴²

The role of nitric oxide in acetaminophen-analgesia:

Nitric oxide (NO) is widely accepted as an important messenger molecule and neurotransmitter in central nervous system which is involved in various physiological functions.^{43,44} NO plays important roles in pain transmission, either induce hyperexcitability leading to hyperalgesia or exert antinociceptive actions.⁴⁵⁻⁴⁷

Björkman R et al.⁴⁸ in 1994 showed that suppression of N-methyl-D-aspartate and Substance P-induced pain related behaviors with acetaminophen administration was reversed by L-arginine administration to rats. This study pointed out the involvement of neuronal NO systems in the analgesic action of acetaminophen. Additionally and in good accordance with this study, neuronal nitric oxide synthase has been found to be involved in the analgesic effect of acetaminophen when acetaminophen was used in lower doses (especially with 100 mg/kg, oral) in Randall-Sellitto pain model, whereas both neuronal and inducible nitric oxide synthases were found to be involved in the analgesic action of acetaminophen in lower doses (especially 50 and 100 mg/kg, oral) in writhing test. However, the involvement of NO systems was weak or not available with the maximal doses of acetaminophen.⁴⁹ It has also been shown that, acetaminophen inhibited the induced NO synthesis in spinal cord tissue.⁵⁰ As a result, it can be concluded out that NO systems are involved in acetaminophen-analgesia and it is more likely that suppression of the central NO systems contributes to the central analgesic mechanisms of acetaminophen.

When focusing on the findings related with the interaction between acetaminophen and NO in the last decade, it might be appropriate not to underestimate the recent studies related with NO-acetaminophen (NCX-701). NO-acetaminophen is a novel compound with a combination of NO releasing moiety with acetaminophen.⁵¹ This novel compound has been shown to exert enhanced analgesic activity compared to parent compound in non-inflamed, acetic-acid induced and inflammatory pain models⁵¹⁻⁵³ and was also analgesic in arthritis-related pain.⁵⁴ Additionally, NO-acetaminophen owned considerable anti-inflammatory activity and less hepatotoxic potential compared to acetaminophen.^{51,52} Mechanism of action of NO-acetaminophen has been suggested to be different from the acetaminophen itself. It has been proposed that although NO-acetaminophen and

acetaminophen may share some common mechanisms like COX inhibition, the sustained release of low amounts of NO when combined with specific pharmacological actions of acetaminophen may add different but not clearly understood pharmacological properties. Inhibition of the *wind-up phenomenon* that is pointing out a mechanism of action in the central nervous system level, more probably in the spinal cord, and reduction in the amounts of some cytokines in the peripheral tissues has been proposed.^{53,55}

Additional to the above accumulated data related with the promising effects of NO-acetaminophen, the antinociceptive effect of intravenously as well as intrathecally administered NO-acetaminophen has also been shown in neuropathic pain model (partial ligation of sciatic nerve) in rats, where acetaminophen alone was ineffective. In good accordance with the previous speculations, spinal cord was claimed to be the anatomic region involved in this antihyperalgesic action of NO-acetaminophen. Gabapentin addition to NO-acetaminophen showed synergistic effect.⁵⁶ Similar to gabapentin, lowered doses of NO-acetaminophen also have been shown to enhance the analgesic effect of α_2 -adrenergic receptor agonist, medetomidine, when combined with the sub-effective doses of NO-acetaminophen in carrageenan-induced inflammatory model in rats.⁵⁷ These two recent studies with NO-acetaminophen pointed out the beneficial effects of this novel acetaminophen compound in neuropathic and inflammatory pain conditions. Additionally, it is important to note that, NO-acetaminophen was effective in the conditions where acetaminophen alone did not show analgesic action or NO-acetaminophen enhanced the analgesic potency of α_2 -adrenergic receptor agonist where acetaminophen alone did not. As a result, these studies showed that NO-acetaminophen can be an effective analgesic in neuropathic and inflammatory painful conditions and also can lead synergistic actions when used in combination with gabapentin or α_2 -adrenergic receptor agonists in related painful conditions.

CONCLUSION

Findings in the last decade related with the contribution of serotonergic system and nitric oxide in the analgesic effect of acetaminophen confirmed and expanded the involvement of these systems in acetaminophen-analgesia. Due to the finding that direct binding of acetaminophen has not been shown with 5-HT₁, 5-HT₂

and 5-HT₃ serotonergic receptor subtypes⁵⁸, interaction between these serotonergic receptors and acetaminophen are likely to be in indirect fashion. Recent studies confirmed the involvement of bulbospinal serotonergic pathway in acetaminophen-analgesia and the acetaminophen-induced serotonin increases in the central nervous system. The metabolite of acetaminophen, AM404 contributes to the analgesic effect of acetaminophen; however the serotonergic receptor subtypes which contribute to the antinociceptive actions of acetaminophen and AM404 may be different. The involvement of 5-HT₁ receptors in acetaminophen-analgesia is still not clear due to the conflicting results and requires to be evaluated with further studies. Despite to conflicting data, contribution of 5-HT₂ receptors have been shown in acetaminophen-analgesia (but not in AM404), and the localization is most likely to be the supra-spinal centers of the central nervous system. In animal studies, the blockage of acetaminophen-analgesia with tropisetron is more obvious compared to ondansetron and granisetron. It seems that the speculation of the involvement of tropisetron-sensitive receptors instead of 5-HT₃ receptors in the analgesic action of acetaminophen is still valid and waiting to be confirmed and clarified with further studies. Recent studies showed the contribution of 5-HT₇ serotonergic receptor subtypes as well. Despite the fact that there are some conflicting results between the studies in volunteers and post-operative patients, important number of human studies expanded the data regarding the contribution of serotonergic receptors. Although there were not much additional findings related with the contribution of nitric oxide systems in the antinociceptive action of acetaminophen, latest findings expanded the beneficial analgesic effects of nitric oxide releasing derivative of acetaminophen, NO-acetaminophen.

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5,7-DHT	Acetaminophen	Pain model	Effect	Animal	Reference
100 µg; i.t.	3 mg/kg; i.p.	Paw Pressure test	decrease	Rat (Sprague-Dawley)	Mallet C, 2008 ¹¹
50 µg; i.t.	200-600 mg/kg;	Tail Flick test	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
50 µg; i.t.	200-600 mg/kg;	Hot plate test	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
50 µg; i.t.	200-600 mg/kg;	Plantar incision (thermal hyperalgesia)	decrease	Mouse (BALB/c)	Dogrul A.,2012 ¹²
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Hot plate test	decrease	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Writhing test	decrease*	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Tail immersion	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Paw pressure test	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³
70 µg; i.c.v. (neonatal age)	100 mg/kg; oral	Formalin test	no change	Adult rat (Wistar)	Muchacki R.,2015 ¹³

Table 1: Effect of the deterioration of the bulbospinal serotonergic pathway with 5,7-dihydroxytryptamine (5,7 DHT) in the antinociceptive effect of acetaminophen in different pain models in some studies performed in the last decade. * Decrease in the hot plate test was more obvious than the decrease in the writhing test. i.t.: intrathecal, i.c.v.: intracerebroventricular.

ACETAMINOPHEN	5-HT ₂ ANTAGONIST	ANIMAL	PAIN MODEL	EFFECT ON PARACETAMOL-ANALGESIA	REFERENCE
200 mg/kg, i.v.	Ketanserin & mesulergine (10 µg, i.t.)-5 min before paracetamol	Rat	Paw pressure	Decrease	Courade JP, 2001 ²²
400 mg/kg, i.p.	Ketanserin (5 mg/kg, s.c.)	Rat	Hot-plate	Decrease	Ruggieri V, 2008 ⁵
400 mg/kg, i.p.	Ketanserin (5 mg/kg, s.c.)	Rat	Paw pressure	Decrease	Ruggieri V, 2008 ⁵
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Tail Flick test	No effect	Dogrul A, 2012 ¹²
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Hot-plate	No effect	Dogrul A, 2012 ¹²
200 - 600 mg/kg, oral	Ketanserin (10 µg, i.t.)- 60 min after paracetamol	Mice	Post-incision	No effect	Dogrul A, 2012 ¹²

Table 2: Some studies on the effect on the role of 5-HT₂ receptors on the analgesic effect of acetaminophen in different pain models. i.v.: intravenous, i.p.: intraperitoneal, s.c.: subcutaneous, i.t.: intrathecal.