REVIEW

An Updated Review on the Metabolite (AM404)-Mediated Central Mechanism of Action of Paracetamol (Acetaminophen): Experimental Evidence and Potential Clinical Impact

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Abstract: Paracetamol remains the recommended first-line option for mild-to-moderate acute pain in general population and particularly in vulnerable populations. Despite its wide use, debate exists regarding the analgesic mechanism of action (MoA) of paracetamol. A growing body of evidence challenged the notion that paracetamol exerts its analgesic effect through cyclooxygenase (COX)-dependent inhibitory effect. It is now more evident that paracetamol analgesia has multiple pathways and is mediated by the formation of the bioactive AM404 metabolite in the central nervous system (CNS). AM404 is a potent activator of $TRPV_1$, a major contributor to neuronal response to pain in the brain and dorsal horn. In the periaqueductal grey, the bioactive metabolite AM404 activated the $TRPV_1$ channel-mGlu5 receptor-PLC-DAGL-CB1 receptor signaling cascade. The present article provides a comprehensive literature review of the centrally located, COX-independent, analgesic MoA of paracetamol and relates how the current experimental evidence can be translated into clinical practice. The evidence discussed in this review established paracetamol as a central, COX-independent, antinociceptive medication that has a distinct MoA from non-steroidal anti-inflammatory drugs (NSAIDs) and a more tolerable safety profile. With the establishment of the central MoA of paracetamol, we believe that paracetamol remains the preferred first-line option for mild-to-moderate acute pain for healthy adults, children, and patients with health concerns. However, safety concerns remain with the high dose of paracetamol due to the NAPQI-mediated liver necrosis. Centrally acting paracetamol/*p*-aminophenol derivatives could potentiate the analgesic effect of paracetamol without increasing the risk of hepatoxicity. Moreover, the specific central MoA of paracetamol allows its combination with other analgesics, including NSAIDs, with a different MoA. Future experiments to better explain the central actions of paracetamol could pave the way for discovering new central analgesics with a better benefit-to-risk ratio.

Keywords: paracetamol, acetaminophen, AM404, analgesia, molecular targets

Plain Language Summary

The mechanisms of how paracetamol works are still subject to debate despite its widespread use. Previous scientific research questions the idea that paracetamol relieves pain by inhibiting cyclooxygenases (COX). Now, it is more apparent that paracetamol's pain-relieving effect occurs in the brain and is independent of the COX enzyme. This article reviewed the central paracetamol actions and how the current experimental evidence can be used in clinical practice. Based on the evidence discussed in this review, paracetamol works differently than NSAIDs and has a safer safety profile. With the central MoA for paracetamol in place, we think paracetamol will still be the first choice for acute pain management. When the COX-independent mechanism of action and stable pharmacokinetic properties of paracetamol are considered, it is safe to say that paracetamol should be preferred over NSAIDs for analgesia, especially in special populations. Newer painkillers, based on the central action of paracetamol, are being studied and show promising results in relieving pain without increasing the risk of liver damage.

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Introduction

Pain represents substantial healthcare and financial burden despite the available pain management approaches, high-lighting the urgent need for innovative medications and, possibly, indications of established analgesics.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (also known as acetaminophen or *N*-acetyl-*p*-aminophenol) are commonly prescribed for pain management in a wide range of conditions. Globally, paracetamol is also at the apex of the most commonly utilized over-the-counter (OTC) analgesic and the preferred first-line option for patients with mild-to-moderate acute pain.[2] The good tolerability at the recommended dosage (eg, lack of gastrointestinal adverse effects) is a major contributor to the preference for paracetamol by general practitioners, specialists, and pharmacists.[3] Although it was introduced in clinics more than 100 years ago,[4] paracetamol remains the recommended first-line option for pain management by several scientific societies, and particularly in vulnerable populations such as geriatrics, children, patients with peptic ulcers, and pregnant women.[5,6] Despite its wide use, debate exists regarding the analgesic mechanism of action (MoA) of paracetamol.[7]

Historically, it was believed that the paracetamol-mediated analgesia stems solely from its cyclooxygenase (COX)-dependent inhibitory effect on prostaglandin (PG) synthesis[8]; however, it is now evident that the analgesic MoA of paracetamol is multidimensional and involves several pathways within the central nervous system (CNS), such as the endocannabinoid, serotonergic, and nitric oxide pathways[9–14] or for some authors opioid pathways.[15] The notion that we already know concerning the metabolic pathways for paracetamol has been challenged in the past two decades following the 2005 study by Högestätt et al; in this report, the authors demonstrated that, following hepatic deacetylation to *p*-aminophenol, this paracetamol metabolite crosses the blood–brain barrier (BBB) and is converted to *N*-arachidonoylphenolamine (AM404).[16] Subsequent experimental evidence found that this new metabolite pathway was involved in its analgesic MoA.[17–19] Moreover, several molecular actors targeted by the bioactive AM404, including the Cav3.2 calcium channel, the cannabinoid CB1 receptors and $TRPV_1$ receptors, would participate in the MoA.[10,20,21]

Paracetamol is generally considered safe for many patients, particularly in comparison with NSAIDs and opioids. The growing evidence of the centrally located COX-independent actions of paracetamol can pave the way for optimizing the use of an old drug in a more widened patients' profile. More importantly, the development of paracetamol–based derivatives can maximize the central effects of paracetamol while reducing the risk of toxicity.[22–25] The present article provides a comprehensive literature review of the centrally located, COX-independent, analgesic MoA of paracetamol and how the current experimental evidence can be translated into clinical practice. This review highlighted the advantages of the centrally located MoA and the benefit/risk of paracetamol in mild-to-moderate pain conditions. Consequently, we discussed the potential clinical applications of paracetamol analogs. Besides, we review the safety aspects of paracetamol administration and how it can be preferred over NSAIDs due to its more tolerable safety profile.

Review Development

A bibliographic literature search was performed on Medline via PubMed to collect relevant articles from their inception to May 2022. The following keywords were employed during the literature search: (paracetamol[MeSH Terms]) OR (acetaminophen[MeSH Terms])) AND (AM404). The online bibliographic search was complemented by a manual screening of the relevant references.

The Analgesic MoA is Independent of Central COX Inhibition

Historically, Vane and Flower demonstrated that paracetamol reduced brain PGE2 with a preferred action in the CNS than in the periphery.[8]

PGs are produced by COX enzymes which need to be oxidized by hydroperoxides to be catalytically active. Paracetamol, as reducing agent,[26] reduces the hydroperoxide tone and thus inactivates COX.[27–30] The selectivity of paracetamol for CNS was hypothesized to be based on cellular concentration of peroxide and arachidonic acid. High levels of peroxide (found especially in the periphery, eg, in an inflammatory context) or arachidonic acid, thwart paracetamol COX inhibition.[30–33]

Another proposed COX-dependent MoA of paracetamol is the involvement of another COX isoenzyme, named COX-3. Flower and Vane demonstrated that the COX inhibitory effect of paracetamol was more potent in the brain than in the spleen, suggesting another COX isoenzyme as a sensitive site for paracetamol action.[8] COX-3 is an exon splice variant from COX-1 that was identified in the canine cerebral cortex and exhibited selective sensitivity to paracetamol.[34] However, further genomic and kinetic studies showed that a full-length, catalytically active form of COX-3 does not exist in humans[35,36] and that the COX-3 selective interaction is unlikely to be clinically relevant.[31]

However, the question remained whether the analgesic MoA of paracetamol is primarily mediated by COX inhibition. In mice, administering a weekly dose of paracetamol, which induced an analgesic effect, did not reduce PGE2 content in the brain.[37] Besides, the antinociceptive profile of paracetamol was found to be different from that of the competitive COX inhibitor, ibuprofen, with no observed antinociceptive action of ibuprofen, in the first phase of the formalin test, despite the proposed shared MoA.[37] Altogether, these results indicate that the analgesic action of paracetamol cannot be attributed to the inhibition of central COX, however data from the literature would suggest such an involvement.[38] Furthermore, the inhibitory effect of paracetamol on COX observed by some authors seems more closely related to its hypothermic/antipyretic effects than to its analgesic action.[39–42]

AM404: The Active Metabolite of Paracetamol

Research experiments have shown that the analgesic MoA of paracetamol is primarily COX-independent and mediated by the formation of the bioactive AM404 metabolite in the CNS, highlighting that paracetamol is a pro-drug of a potent analgesic metabolite. The first description of the central conversion of paracetamol was reported in 2005. In this report, Högestätt et al demonstrated that the deacetylated form of paracetamol, the *p*-aminophenol, is converted mainly in the brain to AM404 by the fatty acid amide hydrolase (FAAH) enzyme (Figure 1).[16] These findings were then supported by Muramatsu et al's experiment.[20] The first human evidence of central conversion of paracetamol to AM404 was demonstrated by Sharma et al in 2017; 26 patients received 1g of paracetamol, followed by measurement of the
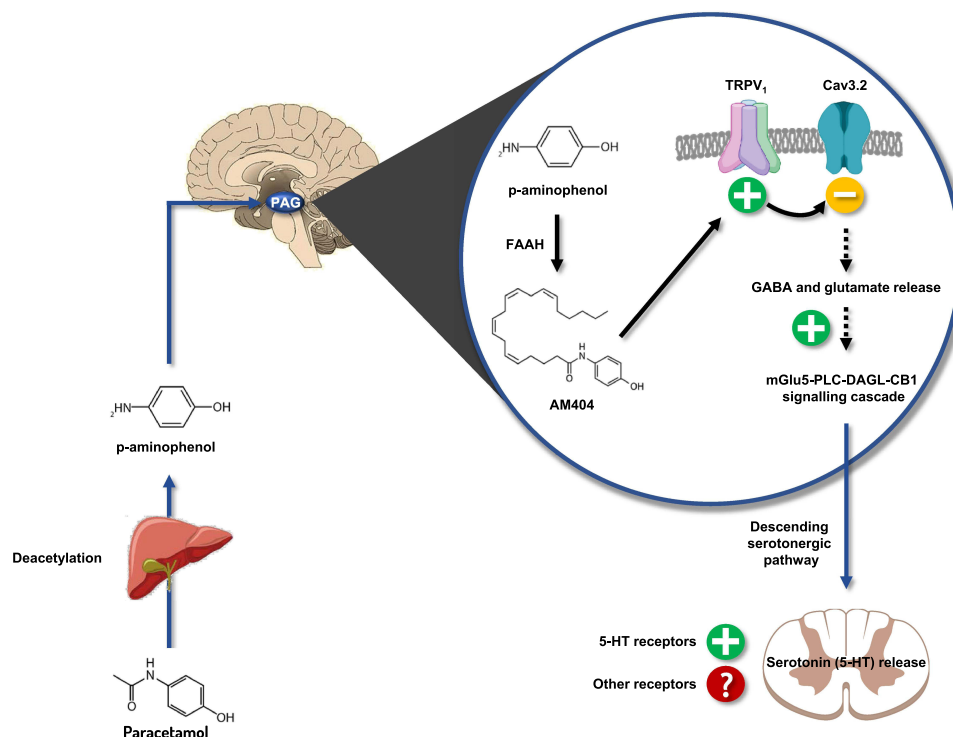

Figure 1 Schematic representation of the central mechanism of action of paracetamol to its antinociceptive activity. Paracetamol is deacetylated in *p*-aminophenol in the liver, then metabolized in the brain by FAAH into AM404. AM404 activates the TRPV1 channel-mGlu5 receptor-PLC-DAGL-CB1 pathway and co-activates the Cav 3.2 T-type calcium channel, which in turn reinforces the activity of the descending serotonergic pathways.

AM404 in the cerebrospinal fluid (CSF) and blood. The study detected AM404 in the CSF of 17 patients, confirming the central conversion of paracetamol to the bioactive AM404 in humans.[43]

Importantly, it has been shown that 1) centrally injected AM404 induced analgesia[37,44,45] and 2) the inhibition of FAAH (genetically and pharmacologically) suppressed the analgesic effect of paracetamol (and *p*-aminophenol), demonstrating the involvement of AM404 as the active metabolite in the analgesic effect of paracetamol.[17,18,37] In the setting of nociceptive pain, Dalmann et al observed the loss of paracetamol analgesic effect after supra-spinal administration of a FAAH inhibitor, which did not occur after peripheral FAAH inhibitor injection. This demonstrated that the FAAH-synthetized AM404 formation occurs mainly at supra-spinal level,[19] even if a study evoked a spinal level.[46]

AM404-Modulated Molecular Targets
TRPV$_1$ Channels
AM404 is a potent activator of TRPV$_1$, a major contributor to neuronal response to pain. Contrary to the periphery, where activated TRPV$_1$ induces nociception, hyperalgesia, and pain transduction modulation,[47] supraspinal TRPV$_1$ activation induces anti-nociception.[37,45,48] In Zygmunt et al's patch-clamp experiment, AM404 activated the vanilloid receptor TRPV$_1$, an effect that was suppressed after administration of the TRPV$_1$ antagonist, capsazepine.[49] Besides, experiments showed diminished analgesic action of paracetamol and *p*-aminophenol in TRPV$_1$ knockout mice and mice pretreated with capsazepine.[18,37,45] Notably, a recent report by Barrière et al[50] demonstrated that paracetamol reduced the connectivity of brain structures in the pain pathway, particularly the periaqueductal grey, where local blockade of TRPV1 suppressed the analgesic action of paracetamol, this confirms a fully TRPV1-dependent mechanism and thus tends to exclude a mechanism involving central COX. As a confirmation of the role of TRPV1 in humans, Pickering et al demonstrated the involvement of a specific TRPV1 rs224534 variant in paracetamol antinociception in healthy volunteers.[51]

Downstream Targets of TRPV$_1$
The T-type calcium Cav3.2 isoform regulates the neuronal response to pain.[52,53] Cav3.2 knockout models and mice pretreated with Cav3.2 blockers showed impaired nociceptive response to pain stimuli.[54] Previous reports indicated that the antinociceptive action of arachidonic compounds involves inhibition of Cav3.2 channels, suggesting a potential interaction between Cav3.2 and AM404.[11] In 2014, Kerckhove et al demonstrated that Cav3.2 knockout mice had impaired response to the analgesic effect of a systemic administration of paracetamol or intracerebroventricular injection of AM404.[55] Interestingly, an experiment aimed to establish the location of Cav3.2 involved in the analgesic effect of paracetamol found that, contrary to supra-spinal channels, spinal Cav3.2 blockade did not affect the analgesic profile of paracetamol, highlighting that the involvement of Cav3.2 in the analgesic activity of paracetamol is mainly supra-spinal.[45]

In Cav3.2 knockout mice, the analgesic effect of the TRPV$_1$ agonist (capsaicin) was diminished, despite the analgesic action of an inhibitor of Cav3 channels (TTA-A2) persisted in TRPV$_1$ knockout mice suggesting that Cav3.2 and TRPV$_1$ support paracetamol's analgesic activity sequentially, Cav3.2 acting downstream of TRPV$_1$.[45]

In the setting of paracetamol injection, CB1 knockout models or mice pretreated with selective CB1 antagonists showed no analgesic response to paracetamol.[17,56] The expected synergism between paracetamol and opiates was also attenuated in mice pretreated with selective CB1 antagonist.[57] Notably, two previous reports showed that paracetamol injection did not alter the brain endocannabinoid content, suggesting that other studies are needed to know how CB1 receptors are involved. Recently, periaqueductal grey-located CB1 receptors were found to be essential for the MoA of paracetamol through a AM404-activated TRPV$_1$ channel-mGlu5 receptor-PLC-DAGL-CB1 receptor signaling.[50]

Serotonin (5-HT) and norepinephrine are engaged in pathways of physiological nociception, both of which can be produced via descending pain pathways to alter nociceptive transmission in the spinal cord. Serotonin appears to have both inhibitory and facilitative effects on pain.[58] The involvement of the serotonergic pathway in the analgesic effect of paracetamol was described even before the 2005 work by Högestätt et al.[59] Earlier reports described the pharmacological blockade of the serotonergic pathway decreased the antinociceptive effect of paracetamol.[60] The involvement of the

serotonergic pathway in paracetamol action was reported to stem from increasing 5-TH levels in the central structures and activating the spinal 5-TH receptors.[60–71] Nonetheless, the evidence was not conclusive regarding the contribution of serotonergic receptors to the paracetamol action. As an example, the involvement of 5-HT3 receptors has been discussed in humans,[62,70,72–74] and experimental work in rodents with 5-HT3 receptor antisense oligonucleotides showed that they were not involved in the analgesic effect of paracetamol, while a tropisetron-sensitive receptor was.[74] However, investigations showed that *p*-aminophenol analgesic action was mediated by the bulbospinal serotonergic system,[18] which may suggest an involvement of this serotonergic system in the AM404 antinociceptive activity, as recently suggested.[63] Finally, if bulbospinal serotoninergic pathways seem to be involved in the analgesic effect of paracetamol or its metabolites, the exact nature of the spinal tropisetron-sensitive receptor involved in this effect remains to be elucidated.

Altogether, the data collected draw the schematic MoA represented in Figure 1.

Potential Clinical Consequences of the Centrally Located Analgesic Action of Paracetamol

Paracetamol, a COX-Independent, Centrally Acted as the First Choice for a Wide Range of Special Populations

Safety is the primary concern in choosing OTC analgesics, particularly for mild-to-moderate pain conditions in vulnerable groups. Patients with health concerns are frequently reluctant to take the appropriate analgesic dose due to safety concerns, leading to inadequate pain management.[6] Most NSAIDs have safety concerns related to the development of gastric ulcers and bleeding, bronchial asthma, acute kidney injury, and long-term adverse cardiovascular outcomes.[75] Initial reports classified paracetamol as a part of the NSAIDs class due to the presumably shared inhibitory effect on PG synthesis. One thing that is already accepted in the scientific community is that paracetamol has almost no anti-inflammatory activity and does not cause the same adverse effects as NSAIDs do.[5] So, it is sensible to assume that paracetamol is different from NSAIDs. The fact that paracetamol has a limited peripheral COX inhibitory effect and potent centrally located actions can help better understand the benefit–risk ratio of this old drug.

The maximum single dose of paracetamol for pain or fever treatment is 1 g every 4–6 hours as needed, up to a recommended maximum daily dose of 4 g. It is well established that paracetamol is clinically effective under the recommended daily dose and has a well-tolerable safety profile.[76] Paracetamol-related adverse events are rare in healthy adults receiving a daily dose of ≤4 g/day.[77] Thus, several guidelines recommend paracetamol as an optimal option for mild-to-moderate pain conditions, such as headache and migraine attacks, postoperative pain and chronic osteoarthritis for limited duration of treatment (however, with some discrepancies here, according to guidelines).[78–81]

Interestingly, the analgesic effect of paracetamol has a distinct dose-response, according to experimental models and consumers' experience.[82] The French National Agency for Medicines and Health Products Safety (ANSM) recommended initiating paracetamol with the minimum dose. In a recent focus review, studies comparing the analgesic response to higher (1 g) and lower (500–650 mg) doses of paracetamol were retrieved and summarized. Both direct and indirect comparisons demonstrated analgesic superiority of higher single dose compared to lower single doses, highlighting that a higher single dose of paracetamol for a short duration (4–5 days) poses more benefit than lower single doses.[80] This observation is consistent with the successful experimental demonstration in healthy volunteers of a lack of acetaminophen ceiling effect on R-III nociceptive flexion reflex.[83] In terms of safety, a Cochrane review concluded that the rate of adverse events was similar between the higher and lower single doses of paracetamol.[84] This was in line with another clinical trial showing similar rates of treatment-related adverse events between the higher and lower single doses of paracetamol.[85]

Still, the safety of paracetamol is surrounded by controversy. Of course, hepatoxicity is well established and remains a major issue. Indeed, paracetamol produces variable amounts of the hepatotoxic compound, NAPQI. Hepatic metabolism of paracetamol via the P450 system (mainly CYP-2E1) leads to the synthesis of NAPQI normally conjugated with glutathione and eliminated in urine. However, when high doses of paracetamol are used or at usual doses, in some patients at risk (malnutrition, alcoholism, some combinations of drugs, etc), glutathione stores can be decreased and/or

CYP activity increased, both leading to a high level of NAPQI and NAPQI-mediated liver necrosis, and thus doses must be adapted in this population. Interestingly, a recent work studying the role of acetaminophen on epitranscriptome suggests that its hepatotoxicity could be reduced by adequate strategy.[86] Otherwise, although some other safety issues associated with paracetamol remain inconclusive, some patients with comorbidities have concerns with the use of paracetamol for common pain conditions. Thus, it is imperative to consolidate the current literature and appropriately weigh the risk–benefit ratio of paracetamol for pain conditions in special populations until good scientific evidence of safety should prevail. Recently, Alchin et al reviewed the current evidence of paracetamol as the first-option analgesic in patients with kidney disease, cardiovascular diseases, gastrointestinal disorders, asthma, and the elderly.[6] In another context, a recent work suggests that acetaminophen would be a potential suppressor of antitumor immunity, suggesting that it should be used with caution in patients treated with immune checkpoint blockers.[87]

Regarding kidney function, NSAIDs, inhibitors of COX, should be avoided in patients with renal impairment.[88] On the contrary, therapeutic doses of paracetamol are generally safe and do not lead to acute renal failure in patients with renal impairment.[89] It was previously hypothesized that paracetamol may cause renal injury due to the reports of nephropathy after phenacetin overuse; however, this hypothesis was then rejected by further evidence.[90] Data show no association between paracetamol and acute renal failure in patients with chronic kidney disease.[91] Thus, paracetamol remains the mainstay analgesic option in patients with chronic kidney disease.[92] It is unnecessary to modify the dose for dialytic patients; however, dose reduction or longer dose interval should be considered in patients with advanced kidney failure.[6]

NSAIDs significantly increase the risk of cardiovascular adverse outcomes.[93] No robust evidence supports an association between paracetamol and adverse cardiovascular outcomes (stroke or myocardial infarction) in patients with atherosclerotic heart diseases.[94–96] On the other hand, the risk of worsening hypertension should be considered, with some observational studies showing an increase in blood pressure among chronic paracetamol users.[97,98] Still, published studies failed to show an association between elevated blood pressure and the risk of serious cardiovascular events in paracetamol users.[96] Paracetamol remains probably safer than NSAIDs which showed a significant increase in adverse cardiovascular outcomes amongst patients receiving non-aspirin NSAIDs.[99] According to an American Heart Association position statement, paracetamol should be preferred over NSAIDs in patients with coronary artery disease.[100]

Aspirin and other NSAIDs significantly increase the risk of gastrointestinal ulcers and bleeding. Unlike NSAIDs, paracetamol, which has a minimal peripheral COX inhibitory effect, does not cause damage to the gastrointestinal tract. The current evidence affirms the more tolerable gastrointestinal safety profile of paracetamol than NSAIDs.[101] Thus, paracetamol is the recommended first-line option for patients at risk of gastrointestinal ulcers or bleeding.[6]

Finally, paracetamol is considered the preferred analgesic option for the elderly, a challenging group to manage due to the high frequency of comorbidities and polypharmacy. Paracetamol represents an effective and safe analgesic option; however, its use in the elderly with chronic pain is still questionable.[102]

Based on the above, it can be concluded that the centrally acted paracetamol is a compelling first-line analgesic option even for special populations if a low dosage is used, and it should be preferred over NSAIDs due to its very limited COX action and tolerable safety profile.

Paracetamol in Combination with Other Analgesics

Multimodal analgesia has become a principal approach for the management of acute postoperative pain conditions that offers additive or synergistic analgesia and lower opioid dose requirements. Theoretically, combining two analgesics with different mechanisms of action potentiates their analgesic actions without increasing their doses and compromising the safety outcomes.[103–106] Paracetamol has linear and consistent pharmacokinetics, and its tolerable profile makes it a good option when a combination is required.[107]

As previously elucidated, paracetamol has a central, COX-independent, analgesic action that can synergize other analgesics with different central or peripheral MoA. Thus, it can potentiate the analgesic effect of opioids without increasing the risk of adverse events (Table 1). The paracetamol plus opioids combination can benefit from the rapid onset of action of paracetamol and the sustained analgesic effect of opioids.[108] Several reports demonstrated that paracetamol plus opioids led to superior pain relief and lower dose requirements than individual components without

Table 1 Selected Clinical Studies Using Paracetamol Plus Opioids for Acute Post-Operative Pain

Study, Year	Type of Surgery	Design	N	Treatment Groups, Doses (mg)	Doses/Timing	Outcome
Fricke et al, 2002[130]	Oral surgery	Double-blind RCT	200	Tramadol/Paracetamol 37.5 mg/325 mg Tramadol/ Paracetamol 75 mg/650 mg Hydrocodone/Paracetamol 10 mg/650 mg Placebo	Single dose, postoperative	Comparable analgesia, but better tolerability for tramadol/Paracetamol than hydrocodone/Paracetamol
MacLeod et al, 2002[131]	Dental	Double-blind RCT	82	Codeine/Paracetamol 30 mg/1000 mg Paracetamol 1000 mg	3 doses over 8 hours	Better analgesia with combination, with a similar rate of adverse events
Smith et al, 2004[132]	Orthopedic and abdominal surgery	Double-blind RCT	305	Tramadol/Paracetamol 75 mg/650 mg Codeine/Paracetamol 60 mg/600 mg Placebo	1 to 2 tablets every 4 to 6 hours as needed for pain for 6 days.	Both active combinations provided significantly greater pain relief than placebo; scores were similar for tramadol/ Paracetamol and codeine/paracetamol.
Daniels et al, 2011[122]	Dental	Double-blind RCT	678	Ibuprofen/Paracetamol 400 mg/1000 mg Ibuprofen/Paracetamol 200 mg/500 mg Ibuprofen/Codeine 400 mg/25.6 mg Codeine/ Paracetamol 30 mg/1000 mg Placebo	Single dose, postoperative	Ibuprofen/Paracetamol groups were superior in pain reduction to ibuprofen/codeine; ibuprofen/Paracetamol, and noninferior to ibuprofen/codeine
Rawal et al 2011[133]	Ambulatory hand surgery	Double-blind RCT	462	Tramadol/Paracetamol 37.5/325 mg Tramadol 50 mg	Before and immediately after surgery and every 6 hours thereafter	Comparable analgesic efficacy, fewer adverse events with tramadol/paracetamol, and reduced tramadol consumption by 24%
Sniezek et al, 2011[123]	Mohs surgery and cutaneous reconstruction	Double-blind RCT	210	Paracetamol 1000 mg Ibuprofen/Paracetamol 400 mg/1000 mg Codeine/Paracetamol 30 mg/ 325 mg	Immediately after surgery and every 4 hours for up to 4 doses	Ibuprofen/paracetamol is superior to other 2 treatments in pain control; Higher rate of AEs under codeine/Paracetamol compared with ibuprofen/Paracetamol and Paracetamol alone
Aweke et al, 2020[134]	Abdominal surgery	Single blind RCT	63	Paracetamol 1000 mg Diclofenac /Paracetamol 75 mg/1000 mg Tramadol/Paracetamol 100 mg/1000 mg	Single dose, postoperative	Paracetamol–tramadol and Paracetamol–diclofenac reduce cumulative postoperative analgesic consumption and prolongs the time to first rescue analgesic
Liu et al, 2021[112]	Arthroscopic knee surgery	Open-label RCT	232	Oxycodone/Paracetamol 10 mg/650 mg Celecoxib	Single dose, postoperative	Oxycodone–Paracetamol increased analgesic efficacy, with comparable safety profiles compared with celecoxib.
Singh et al, 2021[135]	Rotator cuff repair	Open-label RCT	57	Oxycodone/Paracetamol 5 mg/1000 mg Oxycodone 5 mg Paracetamol 1000 mg	Combinations every 6 hours postoperative as needed Paracetamol 1000 mg perioperative	Perioperative acetaminophen significantly decreased opioid consumption and improved overall pain control.

Abbreviation: RCT, randomized controlled trial.

increasing the risk of adverse events in a wide range of pain conditions, such as osteoarthritis and postoperative pain.[109,110] However, we do not have good evidence that paracetamol spares the opioid adverse effects when a high dose of opioid is used.[111] Recently, a comparative trial showed that the oxycodone/paracetamol combination was more effective than celecoxib in terms of reducing postoperative pain and rescue analgesia consumption amongst patients undergoing arthroscopic knee surgery. The oxycodone/paracetamol combination exhibited well tolerable safety profile, with no difference between the two groups.[112]

Paracetamol/NSAIDs combinations have regained much attention following the establishment of the COX-independent MoA of paracetamol. Combining paracetamol with NSAIDs can represent an alternative for opioids-

based analgesics in patients with moderate pain and no contraindication for NSAIDs. Paracetamol can theoretically potentiate the analgesic and morphine-sparing effects of NSAIDs in patients with acute pain without the need to increase NSAIDs doses.[113,114] In a previous systematic review, combining paracetamol and NSAIDs was more effective than either agent alone in acute postoperative pain.[115] The antinociceptive effects derived from paracetamol/NSAIDs combinations appear to be additive rather than synergistic; in 2012, Moore et al demonstrated that paracetamol plus NSAIDs led to additive analgesic rather than supra-additive (ie, synergism) effects.[116]

Previous pharmacokinetic reports demonstrated the lack of significant interactions in the pharmacokinetic profiles of ibuprofen and paracetamol when administered in a fixed-dose combination tablet, highlighting that the effect of this combination is primarily a pharmacodynamic one.[117,118] Thus, the paracetamol/ibuprofen combination has been studied in several trials over the recent two decades. In a 2013 Cochrane review over three trials (n = 1647 patients), paracetamol/ibuprofen combination demonstrated higher reductions in acute postoperative pain and fewer patients needed rescue analgesia than either drug alone in patients undergoing impacted third molars extraction.[119] These findings align with the 2010 systematic review showing that paracetamol/ibuprofen combination achieved superior analgesia than either drug alone.[115] Recently, the PANSAID trial showed paracetamol/ibuprofen combination achieved higher analgesia and morphine-sparing effect than paracetamol alone in the first 24 hours after total hip arthroplasty.[120] The benefit of the paracetamol/ibuprofen combination was not significantly influenced by age, sex, or type of anesthesia.[121] Notably, comparative trials also showed that the non-narcotic paracetamol/ibuprofen combination had a similar or higher analgesic effect compared to the acetaminophen/codeine approach.[122–124]

According to the abovementioned studies performed in acute pain conditions, the analgesic paracetamol/ibuprofen combination appears to be well tolerable without a significant increase in safety concerns compared to either drug alone. In the PANSAID trial, patients on paracetamol/ibuprofen combination for postoperative pain at the first 24 hours after total hip arthroplasty did not experience a significant increase in the risk of serious adverse events compared to patients on either drug alone.[120] However, a 13-week trial showed patients on paracetamol/ibuprofen combination had a higher risk of decreases in hemoglobin level ($\geq$1 g/dl) than monotherapy, suggesting a potential risk of bleeding on long-term use of paracetamol/ibuprofen combination.[122] A population-based retrospective cohort study, using data from the government of Quebec health insurance agency databases, suggested that combining traditional NSAIDs with acetaminophen increases the risk of upper GI events beyond that of either agent alone.[125] Even if one of the limitations of this study pertains to the use of administrative databases in which the indication for which a medication is prescribed and actual drug consumption is not known, the results of these two studies warrant performing further research to establish the beneficial effect of the paracetamol/NSAIDs combinations and to investigate their safety profile for patients in need of chronic therapy.

The combination of paracetamol and gabapentin is another combination that is currently under investigation. Experimental evidence showed that paracetamol had a synergistic effect on gabapentin.[126] In patients who underwent an abdominal hysterectomy, Durmus et al studied the analgesic effect of the preoperative paracetamol and gabapentin combination compared to gabapentin 1200 mg alone. The results showed that the paracetamol and gabapentin combination was superior to gabapentin alone on pain intensity scores.[127] Further investigations are warranted.

Paracetamol Perspective: Future Analogs

As we have elucidated in this article, paracetamol itself can be considered as a pro-drug of the active centrally located metabolite, AM404. Nonetheless, paracetamol must first undergo hepatic deacylation into *p*-aminophenol and also produces variable amounts of the hepatotoxic compound NAPQI. Thus, paracetamol analogs were proposed to improve the safety profile of the drug, while keeping the same or even higher analgesic activity.

The development of paracetamol analogs that target the brain TRPV$_1$ receptors without the production of NAPQI and depletion of glutathione stores is a clinical perspective linked to the central action of paracetamol. It has been recently shown that paracetamol amine analogs undergo FAAH-dependent transformation into TRPV$_1$ activators without producing hepatotoxicity at the same dose that paracetamol produces clinically relevant hepatotoxicity; these analogs exhibited dose-dependent antinociceptive effects without antipyretic action, liver cell necrosis, or elevation in liver enzyme levels.[18,128] Adamantyl, a paracetamol analog that selectively inhibits the TRPA$_1$ receptor, was also proposed.[129]

These findings highlighted that developing centrally acted analgesics is possible; these compounds can exhibit a well-tolerable safety profile without significantly inducing hepatotoxicity.

Conclusion and Expert Opinion

Paracetamol remains among the most commonly prescribed analgesics. Nonetheless, the paracetamol MoA remained complex and not yet fully understood. It is now more evident that paracetamol analgesia has multiple pathways and is mediated by the formation of the bioactive AM404 metabolite in the CNS. The evidence discussed in this review established paracetamol as a central, COX-independent, analgesic medication that has a distinct MoA from NSAIDs and a more tolerable safety profile.

This leads to consider that paracetamol remains the preferred first-line option for mild-to-moderate acute pain management for healthy adults and patients with health concerns, such as the elderly, patients with gastrointestinal, renal impairments and/or cardiovascular disease. Nonetheless, to avoid hepatotoxicity, healthcare practitioners should consider appropriate paracetamol dosing individually in special populations and weigh the potential risk–benefit ratio after considering the patient-specific factors.

The central COX-independent MoA of paracetamol warrants to use it for multimodal analgesic approaches. Paracetamol can potentiate the analgesic effect of opioids without increasing the risk of adverse events; still, the evidence is limited, and further studies are needed on the morphine-sparing effect of combining paracetamol and opioids. Paracetamol/NSAIDs combination resulted in higher analgesia and no increase in the risk of adverse events than NSAIDs alone. Although the interest in paracetamol-based combinations is growing, the evidence is still limited, and further high-quality evidence is warranted.

As our understanding of the AM404-mediated activation of the $TRPV_1$ channel and CB1 receptor signaling evolves, several investigations are being conducted to test the benefit of paracetamol analogs, with promising results. Paracetamol analogs can activate the $TRPV_1$ channel signaling without the hepatic production of the toxic metabolite, NAPQI; hence, they can induce a higher analgesic effect than that of paracetamol without increasing the risk of hepatoxicity. Thus, future experiments to better explain the central actions of paracetamol could pave the way for discovering analgesics with a better benefit-to-risk ratio as genetic studies could help to predict efficacy and tolerability of paracetamol.

Abbreviations

5-HT, serotonin (5-hydroxytryptamine); AM404, *N*-arachidonoylphenolamine; ANSM, Agence Nationale de Sécurité des Médicaments (French national agency for medicines and health products safety); CNS, central nervous system; COX, cyclooxygenase; CSF, cerebrospinal fluid; CYP, cytochrome; DAGL, diacylglycerol lipases; FAAH, fatty acid amide hydrolase; MoA, mechanism of action; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NSAIDs, nonsteroidal anti-inflammatory drugs; OTC, over-the-counter; PG, prostaglandin; PLC, phospholipase C; POX, peroxidase; TRP, transient receptor potential; $TRPV_1$, transient receptor potential vanilloid.

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