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REVIEW ARTICLE

What do we (not) know about how paracetamol (acetaminophen) works?

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SUMMARY

What is known and background: **Although paracetamol (acetaminophen), *N*-(4-Hydroxyphenyl)acetamide, is one of the world's most widely used analgesics, the mechanism by which it produces its analgesic effect is largely unknown. This lack is relevant because: (i) optimal pain treatment matches the analgesic mechanism to the (patho)physiology of the pain and (ii) modern drug discovery relies on an appropriate screening assay.**
Objective: **To review the clinical profile and preclinical studies of paracetamol as means of gaining insight into its mechanism of analgesic action.**
Methods: **A literature search was conducted of clinical and preclinical literature and the information obtained was organized and reviewed from the perspective of its contribution to an understanding of the mechanism of analgesic action of paracetamol.**
Results: **Paracetamol's broad spectrum of analgesic and other pharmacological actions is presented, along with its multiple postulated mechanism(s) of action. No one mechanism has been definitively shown to account for its analgesic activity.**
What is new and conclusion: **Further research is needed to uncover the mechanism of analgesic action of paracetamol. The lack of this knowledge affects optimal clinical use and impedes drug discovery efforts.**

***Keywords*: acetaminophen, mechanism of action, paracetamol**

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BACKGROUND

The recent (June, 2009) convening of an FDA joint meeting of the drug safety and risk management advisory committee with the anaesthetic and life support drugs advisory committee and the non-prescription drugs advisory committee, which had as its primary topic area for discussion the safe use of acetaminophen (acetaminophen: para-acetylaminophenol; paracetamol: para-acetylaminophenol; Tylenol: para-acetylaminophenol; APAP: *N*-acetyl-para-aminophenol) and a recent article (1) and accompanying commentary (2) in this journal about the relative risk of paracetamol vs. aspirin, resurrect the following interesting fact: the mechanism of action of paracetamol, one of the world's most widely used analgesics, is not fully known.

Modern pain therapy

In contrast to the older view in which pain was categorized according to its subjective 'degree' (using terms such as mild, moderate, severe, etc.), the more modern view categorizes pain according to its mechanistic 'type', i.e. according to the underlying (patho) physiology (e.g. nociceptive, inflammatory, neuropathic, etc.) and the biochemical mediators (e.g. prostaglandins, substance P, glutamate, etc.) (3). In many painful conditions, the underlying injury is actually multi-faceted and the pain is transmitted by multiple primary and secondary afferent pathways and by a variety of neurotransmitters and modulators ('mixed' pains). The pain can result from increased activity in excitatory pathways involving, for example substance P, glutamate, etc. decreased activity in inhibitory pathways involving, for example noradrenaline or serotonin (5-HT) or both mechanisms (4). In addition, the underlying pain

(patho)physiology may be time-variant, i.e. the type can change from one to another because of the development of central or peripheral sensitization (or both) or other such phenomena. The modern view of pain is better able to explain the otherwise seemingly contradictory clinical observation that addition of a so-called 'weak' analgesic [such as an non-steroidal anti-inflammatory drug (NSAID) or paracetamol] to a so-called 'strong' analgesic (such as an opioid) can sometimes achieve superior pain relief. According to the new view of pain, optimal pain treatment results from matching the type of pain with the drug that has the appropriate mechanism of analgesic action. Recent advances in the understanding of pain transmission pathways, genetic polymorphisms (5) and analgesic pathways suggest that future pharmacotherapy might be able to target a patient's unique pain with the fewest adverse effects. Until that time, clinicians are faced with the 'analgesic challenge' – namely, trying to treat pain with the currently available drugs. As novel, more targeted analgesics are awaited, the clinician can optimize treatment of pain by using individual drugs, or combinations, which incorporate the appropriate mechanism(s) of action.

Implications of not knowing drug mechanism

The search for a new drug that represents an improvement of an existing drug such as paracetamol requires a mechanistic assay for screening compounds (derived from combinatorial chemistry, molecular modelling or other source) or for testing the activity of such compounds. Without knowledge of the molecular target of the drug, it is impossible to set up such an assay. Likewise, without knowledge of the drug's mechanism, it is impossible to determine if the new drug works the same way as the existing one. Therefore, until the mechanism of action of paracetamol is known, drug-discovery efforts are stalled and a great deal of effort and money will be expended studying and trying to address paracetamol's adverse effects rather than finding a replacement.

METHODS

Identification of studies

Computerized literature searches were conducted using keywords related to clinical use, attributes and toxicity of paracetamol and preclinical investigations of its mechanism of analgesic action. Several comprehensive reviews of the early literature were obtained, as well as an extensive collection of primary literature. In addition, valuable recent reviews of mechanism of action were available, as well as data from one of the author's (RBR) laboratory.

Analysis

Each of the sources was reviewed for its relevance to the mechanism of analgesic action of paracetamol. In many cases, the information was obtained from studies that did not have investigation of the mechanism as the primary outcome, but the results were determined to either reflect on the mechanism or to suggest further avenues of investigation.

Assessment

Each item was evaluated for its relevance and strength of the evidence. In at least one case, an author (authority) was contacted with a series of questions that shed additional light on a particular mechanistic hypothesis that originally seemed to be only weakly supported.

RESULTS

Paracetamol is an aniline (*a.k.a.* phenylamine, aminobenzene, C_6H_7N aromatic amine) derivative. It is an active metabolite of two other anilines [Greek for 'black'], acetanilide and phenacetin, and this played a role in the history of its use. Acetanilide was first synthesized in 1852 and serendipitously found to have antipyretic effects by Cahn and Hepp (6). While studying the effect of naphthalene on intestinal parasites, Cahn and Hepp requested naphthalene from the local pharmacy, but were inadvertently sent the incorrect material. They noted that the delivered substance (acetanilide) behaved differently than expected and had antipyretic properties. The mistake was soon realized and taken advantage of. Acetanilide was marketed as an antipyretic under the clever trade name ANTIFEBRIN. Unfortunately, acetanilide was soon discovered to be quite toxic (causing cyanosis due to methemglobinenia), prompting a search for a safer substitute.

Phenacetin and paracetamol, both derivatives of acetanilide, were studied during the 1880s and 1890s (7, 8). During this period, Hinsberg and Treupel (9) showed that paracetamol was as effective as phenacetin as an antipyretic, but von Mering (7, 9) concluded that paracetamol was more toxic, so phenacetin began to be used. By the early 1900s, phenacetin's analgesic effects were recognized and it began to be used as an analgesic for mild to moderate pain in addition to its use as an antipyretic (7).

In the 1940s, Brodie and Axelrod at the National Institutes of Health (NIH) (10, 11) and Smith and Williams (9) at St Mary's Hospital in London, studied the metabolism of phenacitin and acetanilide. Both groups found that acetanilide and phenacetin are metabolized to paracetamol (see Fig. 1). Paracetamol turned out to be mainly responsible for the antipyretic and analgesic effects of phenacitin and acetanilide, whereas another metabolite, *p*-phenetidine, turned out to be responsible for much of the toxiciy. Paracetamol was first marketed in the US in 1950 as a combination product as TRIOGESIC, which also contained aspirin (acetylsalicylic acid) and caffeine (9). TRIOGESIC was removed from the market 1 year later when it was erroneously associated with agranulocytosis.

Paracetamol was reintroduced into the US market in 1955 as prescription only TYLENOL (9, 12) and it was introduced to the UK in 1956 as prescription only PANADOL (7). Found to be adequately safe at therapeutic doses and free of the gastrointestinal bleeding side effects associated with acetylsalicylic acid, paracetamol gained over-the-counter status in the US in 1960 (12) and was added to the British Pharmacopoeia in 1963 (7).

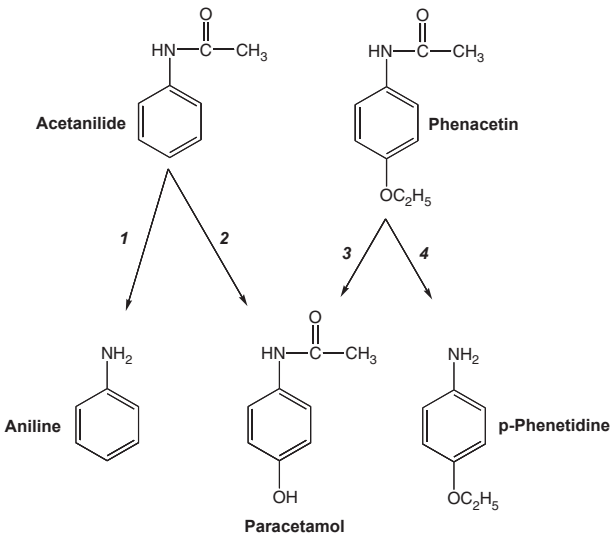

Fig. 1. Chemical structures and pathways involved in the discovery of paracetamol.

By the mid-1960s, paracetamol use increased dramatically overtaking the use of acetylsalicylic acid (7). Combination products containing paracetamol were also introduced at this time. In 1978, paracetamol sales surpassed those of acetylsalicylic acid in the UK (8). Paracetamol gained further popularity in the 1980s when acetylsalicylic acid was associated with Reye's syndrome in children with viral illness, making it the antipyretic and analgesic of choice for children (9).

Currently, paracetamol is one of the most widely used analgesics in both children and adults. It is included in clinical guidelines for multiple pain conditions [e.g. (13, 14)].

APAP Pharmacokinetics

The pharmacokinetics of paracetamol have been well described in humans (15). Paracetamol exhibits an oral bioavailability of 88%, a total body clearance of 5 mL/min/kg and a volume of distribution 0·8 L/kg. About 3% of the drug is excreted unchanged in the urine. Paracetamol is not highly bound to plasma proteins. The mean peak plasma concentration upon a 20 mg/kg oral dose is 20 μg/mL, and time to achieve this peak is on average 0·33 h. Paracetamol crosses the blood–brain barrier. A 0·6 mg i.v. dose in rats achieves quantifiable brain concentrations that are 10–20% of blood levels (16). Highest brain concentrations are observed in the frontal cortex at 15 min post-dose, and in the cerebellum at 120 min post-dose. A steady-state brain concentration of 5·8 μg/mL was reported in mice upon an i.v. infusion of 12·1 mg/kg (with a loading dose of 46·3 mg/kg) (17). Human studies have evaluated paracetamol concentrations in the cerebrospinal fluid (CSF), and successful PK-PD models have been generated with the assumption that CSF concentrations are closest obtainable data to the effect-site concentrations. Thus, a study with 40 mg/kg paracetamol nasogastric administration in paediatric patients reported a C_{max} in the CSF of 15 μg/mL; an EC_{50}

for antipyretic effect was estimated with PK-PD (pharmacokinetic–pharmacodynamic) modelling at 9·6 μg/mL (18). Similar CSF concentrations have been reported in independent studies (19). A meta-analysis of human studies reported an APAP EC_{50} of 4·63 mg/L for antipyresis and 9·98 mg/L for analgesia (20). From these and numerous other studies on paracetamol PK-PD, one can conclude that adequate paracetamol levels are achieved to support a central site of action. In addition, postulated mechanisms of action must be consistent with the levels of paracetamol actually achieved in the central nervous system.

ANALGESIC SPECTRUM

According to a meta-analysis from the American Academy of Family Physicians, <1000 mg of oral paracetamol can reduce over 50% of mild to moderate acute non-specific type pain (21). This includes orthopaedic pain and tension headaches (21), ankle sprain pain (22), acute low back pain (13) and multiple others.

Paracetamol can be effective even against severe pain if it is administered intravenously. For example, in a repeated-dose, randomized, double-blind, placebo-controlled, three-parallel group study of orthopaedic surgery patients that compared 1000 mg i.v. acetaminophen (at 6-h intervals over 24 h) to placebo (23), pain intensity (measured using a 100-mm visual analogue scale) and pain relief (measured using a five-point verbal scale), and rescue i.v. patient-controlled morphine use (time to use and quantity) were significantly decreased in the paracetamol group compared with the placebo group. The median time to first rescue medication (patient-controlled i.v. morphine) was 3 h for paracetamol compared with <1 h for placebo and the amount of rescue morphine was significantly lower in the paracetamol group than in the placebo group.

Several studies have reported paracetamol to be effective in a variety of models of hyperalgesia (enhanced sensitivity to pain) (24–36) (Table 1). By systemic or local administration, paracetamol has been reported to be antinociceptive against chemical-induced hyperalgesia in mice and rats (26, 27, 29, 30, 34–37), formalin-induced pain-related behaviour (30) and thermal hyperalgesia (33), although Bianchi (27) reported that paracetamol was only effective in preventing hyperalgesia, not reversing it. Direct injection into the brain (intracerebroventricular administration) suppresses thermal and mechanical hyperalgesia (25). Direct injection into the spinal cord (intrathecal administration failed to reduce streptozotocin-induced hyperalgesia when given 1-month post-induction (38). Paracetamol (1000 mg) given i.v. significantly reduced electricity-induced hyperalgesia in healthy young adults (32).

Several studies have also reported paracetamol to be effective in a variety of models of allodynia [painful sensation from normally non-painful stimuli (24)] when given systemically or intrathecally to mice or rats (39–42) (Table 2).

Paracetamol has been tested for the treatment of migraine headaches [e.g. (43, 44)], and it is widely used, but its effectiveness is not so notable as to give particular insight into its mechanism of action.

Likewise, there has not been substantial evidence showing paracetamol to be particularly advantageous in treating menstrual pain. In a randomized, active-controlled, single-blind, parallel-designed study, subjects treated with a heat wrap experienced a greater decrease in pain, cramping, fatigue and mood swings than did the 1000 mg paracetamol group (45).

CLINICAL SPECTRUM

As possible insight into paracetamol's analgesic mechanism of action, it is instructive to examine its spectrum of other pharmacological activities. Specifically, with regard to fever (and body temperature), inflammation and platelets.

Fever and body temperature

It is well known that paracetamol is antipyretic. It reduces fever in multiple species (46–51). A central site of antipyretic action against induced fever was demonstrated in rabbits by direct injection into the organum-vasculosum-lamina-terminalis (OVLT) located in the anterior wall of the third intracerebral ventricle (49).

It is less well known that paracetamol can also lower afebrile body temperature (47, 52–56) (Table 3). For example, several studies, employing different methods and routes of administra-

Table 1. Test for analgesic activity of paracetamol against hyperalgesia

Subject[a] Route[b] Dose	Study design	Results	Reference
Hyperalgesia			
mR i.c.v. 0·05–0·5 mg/kg	(1) *Noxious thermal stimulus.* Tail hyperalgesia induced by 49 °C water. Tail flick latency measured (2) *Mechanical noxious stimulus.* Tail ischaemia induced by inflatable cuff at the base of tail. Tail flick latency measured pretourniquet and post-tourniquet release	In the absence of ischaemia, APAP had no effect on latencies of ischaemia/tail flick. In the presence of ischaemia, APAP at minimum 0·2 mg/kg abol ished reperfusion hyperalgesia. i.c.v. dosing 2–3 times less than systemic dosing	25
mR oral 25–100 mg/kg	*Chemical-induced hyperalgesia*. Brewer's yeast intraplantar injection. APAP given 1 h prior yeast. Inflammatory hyperalgesia, edema and nociceptive threshold measured	Central hyperalgesia reduction at 25 mg/kg. Peripheral hyperalgesia reduction at 50–100 mg/kg and in creased in threshold of non-inflammed paws. Paw edema and tail threshold not affected	26
mR oral 25 mg/kg	*Chemical-induced hyperalgesia*. Formalin intradermal injection to tail. Effect on hindpaw nociception threshold by thermal stimulation measured	APAP prevented hyperalgesia, but not effective when hyperalgesia already established	27
mR i.p. 400 mg/kg	*Chemical/heat-induced hyperalgesia.* Opioid antagonists pretreatment. APAP injected 15-min post-antagonists. Hyperalgesia triggered with formalin or hotplate. Dynorphin-A level measured by brain autopsy radio immunoassay	Dynorphin level in frontal cortex significantly decreased with APAP. Naloxone reversed APAP effect on hotplate, but not in formalin test	28
mM oral 10–300 mg/kg	*Chemical-induced hyperalgesia.* Intraplantar formalin, intrathecal substance P and glutamate. Measured duration (s) of nociceptive response	APAP showed dose-dependent antinociception in both phases (0–5; 20–40 min) in formalin only. Glutamate and substance P nociception reversed in phase 2	29
mR oral 100–400 mg/kg i.v. 1 mL/kg intraplantar–20 mg/kg i.t. 200 μg	*Chemical-induced hyperalgesia.* Intraplantar formalin to rats. Oral APAP 40 min prior formalin, i.v. 5 min prior, Intraplantar 30 min prior, s.c. in the back 30 min prior and IT 10 min prior	APAP oral (300–400 mg/kg) reduced nociceptive behaviour (biting/licking) in both phases (0–5, 20–40 min) more in phase 2, post-formalin. Same with i.v. Intraplantar required very high dose to inhibit behaviour	30
mR oral 100–600 mg/kg	*Chemical-induced hyperalgesia.* Induce arthritis and neuropathic pain in rats with Freund's adjuvant intraplantar in hindpaw Mechanical allodynia, thermal and joint hyperalgesia mea sured. APAP 30 min prior measure using von Frey filaments and radiant heat source	APAP at 300 mg/kg had very weak activity in reduction of thermal hyperalgesia and mechanical allodynia	31

Table 1. (Continued)

Subject[a] Route[b] Dose	Study design	Results	Reference
mR i.t. 1–7 mg	*Chemical-induced hyperalgesia.* 75 mg/kg streptozotocin intraperito neal to mimic diabetic neuropathy. Measured hind-paw-withdrawal threshold from anesthesimeter tip (hyperalgesia developed in 3–6 weeks)	APAP given 4 weeks after streptozotocin. APAP did not ↓ decrease hyperalgesia	38
fR i.p. 30 mg/kg	*Thermal-induced hyperalgesia.* Hindpaws of rats immersed in water bath tem perature 51 °C for 20 s. Hyperalgesia detected after 60 min. APAP 20-min post-injury. Noxious heat threshold measured	APAP inhibited the drop of heat threshold dose-dependently	33
mR oral 65 mg/kg 2×d	*Freund's adjuvant-induced inflammatory hyperalgesia*. Adjuvant intraplantar to left hindpaw to induce PGE2 and TNF alpha release in spinal cord. Randall-Sellito-paw-withdrawal test measured withdrawal latency. Measured PGE2 and TNF alpha in spinal fluid	APAP increased nociceptive threshold. APAP was weakest among other investigative drugs including tramadol. APAP decreased PGE2 release compared with adjuvant alone. APAP alone did not decrease TNF alpha level compared with placebo ($P = 0{\cdot}007$)	34
mR oral 300 mg/kg i.t. 10–200 μg	*Chemical-induced hyperalgesia.* i.t. Sub stance P-induced hyperalgesia. APAP 60 min prior substance P	Oral APAP decreased magnitude of hyperalgesia, thermal response latency, and spinal PGE2 level. APAP i.t. suppressed hyperalgesia dose-dependently.	35
mR i.p. 500 mg/kg	*Chemical-induced hyperalgesia.* Carra geenan (CG) intraplantar to hind paw. APAP intraplantar to hind paw 30 min prior or 2 h post-CG	APAP reversed hyperalgesia and increased threshold above basal level. APAP also raised threshold in non-inflammed paw. Effect reversed by naloxone	36
H i.v. 1000 mg	*Electric noxious stimulation* pain in duced electrically and secondary hyperalgesia induced by von Frey filaments	APAP significantly reduced hyperalgesia	32

[a]M, mouse; R, rat; Rb, rabbit; H, human; f, female; m, male.
[b]i.p., intraperitoneal; s.c., subcutaneous; i.v., intravenous; i.c.v., intraverebroventricular; i.t., intrathecal; supp, suppository; APAP = APAP; ASA, aspirin; OVLT, organum vasculosum of the lamina terminalis.

tion, have shown that paracetamol produces hypothermia in mice when the drug is administered intravenously (160 mg/kg, 2·5 °C decrease) (47), intraplantarily (100–300 mg/kg with 0·4–2 °C decrease respectively) (53) or intracerebrovascularly (dose, 0·25 °C decrease) (52).

The data in humans are mixed. Effective and rapid reduction in brain temperature (2 °C) was reported with a single 1000 mg dose of paracetamol in patients with subarachnoid haemorrhage or head trauma (57) and oral or suppository paracetamol given 6 g daily to stroke patients lowered afebrile body temperature by 0·3 °C (56), a decrease attributed with reducing relative risk by 10–20%. However, oral paracetamol (650–1300 mg) was reported to not lower core body temperature in

Table 2. Test for analgesic activity of paracetamol against allodynia

Subject Route Dose	Study design	Results	Reference
Allodynia			
mM s.c. 25–400 mg/kg	*Chemical-induced allodynia.* Formalin injected to upper lip. APAP injected in the neck 20 min prior formalin. Rubbing frequency measured	Rubbing behaviour ↓ in 1st phase (0–3 min) and 2nd phase (15–39 min) after formalin. Dose-dependent only during 2nd phase	37
mR i.v. 1–5 mL/kg	*Chemical-induced allodynia.* Vincristine via osmotic pump 30 μg/kg/day for 14 days. Measured thermal, mechanical nociception, cold and mechanical allodynia	APAP ED50 = 1100 μM/kg in mechanical allodynia. Analgesic efficacy 72%. Therapeutic index 12, better performance than ibuprofen, aspirin and celecoxib	42
mM oral 0·3, 3, 30, 300, 3000 mg/kg	*Cancer-induced allodynia.* Osteolytic murine sarcoma cells injected into distal femur. APAP 2 weeks post-tumour-implant. Level of cancer pain measured	Oral APAP decreased minimum number of pain related responses in dose-dependent manner from 0·3 to 3000 mg/kg. Plateau effect at 3000 mg/kg	41
mR i.t. 20 mM	*Post-surgical (left sciatic nerve exposition and gut ligatures).* APAP IT to hindpaw. Von Frey filaments or hot plates (28 °C) measured withdrawal threshold	APAP suppressed tactile allodynia and thermal hyperalgesia	39
mR s.q. 100 mg	*Post-surgical (partial sciatic nerve ligation).* Induced neuropathy. APAP SQ to hindpaw 15 min prior von Frey test of mechanical allodynia	APAP produced dose-dependent anti-allodynic effect and antihyperalgesic effect	40

See Table 1 for abbreviations.

normothermic cardiac (54) or stroke (3900 mg daily) patients (55). Thus, paracetamol-induced hypothermia appears to be clinically insignificant when given at therapeutic daily dose <4 g.

Inflammation

Paracetamol has been reported to suppress various inflammation-related substances in animals [e.g. (58–61)] and in inflamed dental tissue (1000 mg pretreatment and 4000 mg post-surgery in patients with two-third molar extractions) (61), but paracetamol is generally not considered to display very effective anti-inflammatory action in the clinical setting (58–64) (Table 4). For example, paracetamol given i.p. or orally at 100 mg/kg (62), i.v. at 100–300 mg/kg or intrathecally at 200 μg/kg (63) reduced inflammatory pain, but had no effect on edema and in a randomized, double-blind, placebo-controlled trial no significant improvement was seen in the paracetamol (1000 mg four times daily) group when assessed 2 and 12 weeks into treatment (65). The relatively poor anti-inflammatory effect of paracetamol is a characteristic distinction from the NSAIDs and might be a reflection of different mechanism of action.

Platelet aggregation

Because of the common impression that paracetamol lacks clinically relevant antiplatelet action, it is often used to avoid the bleeding risk associated with aspirin and other NSAIDs.

Table 3. Test for analgesic activity of paracetamol against hypothermia

Subject Route Dose	Study design	Results	Reference
Hypothermia			
mM i.c.v. 500 mg/kg	Mice were pretreated with APAP hepatotoxic inducers. Crossover was done. Brain, liver, blood samples collected. Rectal temp recorded. Brain and liver APAP levels compared with to degree of hypothermia	Significant temperature decrease APAP 20 min post-ICV and lasted for 10 min. An approximate drop of −0·25 °C. Correlation between APAP level and tissue was most significant in plasma and brain, not in liver. Hypothermia is induced by APAP parent drug in brain and not metabolites in liver	52
mM intraplantar 100–300 mg/kg	Basal body temperature and brain PGE2 levels were measured before and after administration of APAP in control mice and COX-1/COX-2 KO mice	One hour post-APAP: 100, 200, 300 mg/kg lowered basal body temperature by 0·4, 0·8 and 2 °C respectively. No hypothermic action is seen in the KO mice	53
PGHS-1 weight, PGHS-1 KO mice i.v. 80, 120, 140, 160 mg/kg	Fever induced by LPS (from *Escherichia coli*). APAP injected into wild-type and knock-out mice preplacebo and post-placebo/LPS administration. Core temp recorded before and after LPS. Blood and brain samples col lected and monitored hourly	APAP pre-LPS: <160 mg/kg – no effect, >160 mg/kg –2·5 °C drop in 60 min. LPS induced 1 °C increase similar to APAP-untreated APAP post-LPS: (80 mg/kg) temperature return to baseline after 30 min then re bounded to febrile state after 30 min, (160 mg/kg) temperature dropped 4 °C below baseline and lasted until end of experiment. Brain and plasma PGE2 level in placebo and LPS mice unchanged by APAP 160 mg/kg	47
H oral 650, 1300 mg	Study patients underwent hypothermic cardiopulmonary by pass APAP given to all study patients experience hypothermia, normothermia and hyperthermia during post-operative period	APAP did not affect core temperature when patients are normothermic. APAP did not affect onset of hyperthermia	54
H oral 3900 mg	*Stroke-induced hyperthermia*. Patients were randomized to receive APAP. CBT measured every 30 min for 24 h	For ischaemic stroke patients, hypothermia is more prevalent in APAP patients and the amt of time of hyperthermia is reduced. However, compared with placebo, difference is statistically insignificant (−0·16 °C)	55
H oral, supp 3000–6000 g	*Acute ischaemic stroke hyperthermia*. (two randomized, double-blinded, phase II trials). High dose APAP with low dose APAP vs. placebo in patients with acute ischaemic stroke. High dose APAP with ibuprofen (2400 mg/day) with placebo in patients with acute ischaemic stroke. Majority of patients received txs within 12 h onset. NIHSS measured severity of stroke	High dose APAP decreased baseline temperature by 0·27 °C in 24 h. A 0·3 °C drop correlates to 10–20% risk reduction. Fewer patients in high-dose APAP suffered from severe stroke than in high-dose ibuprofen	56

See Table 1 for abbreviations.

Table 4. Test for antiinflammatory activity of paracetamol

Subject Route Dose	Study design	Results	Reference
Inflammation			
mR i.v. 150 mg/kg oral 150, 500 mg/kg	*Chemical-induced polyarthralgia.* Intradermal Freund's adjuvant to tail. Pressure applied to ankle joint after 3 weeks. Fos-LI neurons number in lumbar spinal cord measured. Number correlates to signs/symptoms of polyarthritic pain	i.v.: No change in number of Fos-LI neuron with APAP 150 mg/kg. Oral (10 days): decrease in number, no symptoms improvement. Oral (14 days): no change in number After 3 weeks Oral (14 days) 41% decrease in number in gray matter, 38% dorsal horn, 46% in ventral horn	59
mR i.v. 75–150 mg/kg	*Chemical-induced inflammation.* Carrageenixn intraplantar to paws. APAP 15 min prior carrageenin. Number of c-Fos-LI neurons measured	APAP reduced more number of c-Fos neurons at higher dose than at lower dose. More marked effect in deeper laminae than superficial at the dorsal horn	58
mR i.p. 2·5–100 mg/kg oral 25–100 mg/kg	*Chemical-induced inflammation* carra geenin i.p. (edema), 150 μL (hyperalgesia) into hindpaw of rats. Edema size/volume and nociception threshold measured	Non-nitrated APAP – no edema reduction, minimum of 100 mg/kg for antinociception. ED50 = 62 Nitrated APAP– reduced edema, minimum of 2·5 mg/kg for antinociception. ED50 = 44	62
mR i.v. 100–300 mg/kg i.t. 50, 100, 200 μg	*Chemical-induced hyperalgesia.* Give APAP 2 h post-carrageenin. Nociception assessed with mechanical noxious stimulation. Vocalization threshold (squeak) and edema volume measured before and after carrageenin and treatment	i.t.: APAP increased vocal threshold dose-dependently, highest at 200 μg/kg. i.v.: APAP increased vocal threshold simi larly across the three doses. APAP failed to reduce edema volume if given >2 h post-injury	63
H oral 4000 mg	*Osteoarthritis Pilot Study.* APAP compared with NSAIDs in reduction of anti-inflammation in OA patients. WOMAC osteoarthritis index pain score used (5–25). Total effusion volume measured during knee pain with each APAP withdrawal/resumption	APAP withdrawal resulted in pain score of 18 and after resuming APAP it dropped to 9 (50% decrease). Both APAP and NSAIDs have similar outcomes	64
H oral 1000 mg	*Surgical removal of 2 impacted mandibular third molar.* Pretreat with APAP 1 hour prior surgery. Continue to treat post-surgery same dose q6h. Microdialysis biopsy done to measure PGE2 (COX-1, 2) and TXB2 (COX-1)	APAP suppressed PGE2 significantly after 80–180 min. APAP had nearly no effect on TXB2 suppression	61
fR tube feed 100 mg/kg	*Chemical-induced inflammation.* Zymosan induced. Air pouch created on back APAP given 1 h later. Exudates (IL-1 beta, TNF alpha, PGE2) in pouch collected and measured. APAP plasma conc. measured	APAP significantly increased exudates TNF alpha level and not exudates IL-1 beta level	60

See Table 1 for abbreviations.

There is some evidence of antiplatelet activity of paracetamol in human blood samples using *in-vitro* and *ex-vivo* assays [e.g. (66–71)], but other studies suggest a lack of antiplatelet action (66–74) (Table 5). Such an action, when present, is believed to be reversible (shorter acting), in contrast to the irreversible action of aspirin and NSAIDs (66, 70). At least two recent clinical trials report that paracetamol did not interrupt platelet aggregation when given at 1000 mg (73) or 3000 mg i.v. (74). Paracetamol might (68) or might not (72) interact with NSAIDs on this endpoint.

The relatively poor inhibition of platelet aggregation by paracetamol is another characteristic distinction from the NSAIDs that might be a reflection of a different mechanism of action.

TARGETS FOR IMPROVEMENT

Efficacy and potency

Despite the demonstration of some degree of effectiveness against a very wide variety of pains, paracetamol's common clinical application is primarily limited to use against types of pain that are generally described as mild to moderate. It is not clear whether this limitation on clinical analgesic efficacy is imposed by the drug's level of intrinsic analgesic activity/action or whether the limitation is imposed by not being able to administer higher doses (because of the fear of inducing serious adverse effects). A non-opioid, non-NSAID analgesic with greater clinical efficacy than paracetamol would be highly desirable.

Adverse effects

Paracetamol displays an excellent safety profile within its usual therapeutic range. However, the major negative aspect of paracetamol is its ability to induce serious, even fatal, hepatotoxicity above the usual therapeutic range. The mechanism of this toxicity is well known and results from the depletion of endogenous glutathione and subsequent shunting of paracetamol metabolism from benign to toxic pathways (75). The risk is greater when the liver is compromised by disease or excessive alcohol use. Importantly, the mechanism of paracetamol's toxicity appears to be distinct from its mechanism of analgesia, so that greater separation (larger Therapeutic Index) might be possible.

More speculatively, paracetamol has been associated with asthma. Because the prevalence of asthma in developing countries (76) increased in parallel with increased paracetamol use, a causal relationship has been postulated. The Peer Education in Pregnancy Study (76), which examined women at high risk of having a child with asthma, reported that use of paracetamol by these women during mid to late pregnancy was significantly related (after control for potential confounders) to wheezing in the offspring during the first year of life. The Danish National Birth Cohort, a population-based study of 100 000 newborns recruited between 1996 and 2003, reported that prenatal exposure to paracetamol was associated with wheezing and asthma in the offspring at 18 months and 7 years of age. The use of paracetamol during pregnancy was associated with an increase in rate of hospitalizations for asthma up to 18 months of age and the use of paracetamol during the first trimester was associated with increased severity of asthma attacks at 7 years of age (77).

The Nurses Health Study reported that women who used paracetamol more than 1 day/month had a significantly higher risk of developing hypertension (78) (it is not clear why the women were taking paracetamol every day, but it suggests that there was already an underlying health condition). A subgroup of the Physicians Health Study (79) examined the risk of hypertension in 8229 male physicians (ages 53–97) without prior hypertension who reported analgesic use. After adjusting for potential confounding variables, there was no significant increase in hypertension (defined as BP 140/90 mmHg) associated with use of acetaminophen.

Whether or not asthma or hypertension is related to paracetamol use, high-dose hepatotoxicity is sufficient reason to desire an alternative. Unfortunately, without knowledge of the mechanism of paracetamol's analgesic action, it cannot be known if the maximum possible separation between therapeutic and toxic doses has been achieved already in paracetamol.

POSTULATED MECHANISMS OF ACTION

The major mechanisms that have been proposed to account for the analgesic action of paracetamol are

Table 5. Test for antiiplatelet activity of paracetamol

Subject Route Dose	Study design	Results	Reference
Antiplatelet			
Human blood *In-vitro* 1, 3, 6 mM	Aspirin inhibition of COX and platelet function. Measured COX-inhibition interference by APAP	High dose APAP did not show interference with ASA-induced inhibition of COX or platelet function	72
Human blood *In-vitro* 0–1·3 mM *Ex-vivo* 650, 1000 mg	*In-vitro*: collagen, epinephrine, arachidonate, ionophore A23187 were added to induce platelet aggregation *Ex-vitro*: blood collected prior and post-APAP administration to measure changes in platelet aggregation and secretion of 14 °C-5HT	Arachidonate-induced aggregation inhibited by low dose APAP reflected by decreased TXB2 production. APAP did not inhibit norepinephrin-induced aggregation *Ex-vivo* platelet aggregation and 14 °C-5HT secretion reduced only when APAP reached high plasma concentration *APAP may be a reversible platelet aggregation inhibitor*	66
H i.v. 30 mg/kg	Randomized, double-blinded and cross-over. Venous blood collected at 0 h prior APAP, and at 2, 24 and 48 h post-APAP. PT, aPTT, factor V/VII, Hg, Haematocrit, Platelet, and bleeding time, TXB2 were measured. APAP i.v. compared with ketorolac	Adrenaline-induced platelet aggregation inhibited by APAP for 2 h compared with ketorolac for 24 h. Platelet dysfunction lasted longer with ketorolac than with APAP *APAP caused reversible inhibition of platelet aggregation* and decrease in maximal TXB2 level	70
H i.v. 15 mg/kg	*Arachidonate-induced platelet aggregation.* Platelet function measured after 5, 90 min and 24 h. TXB2 levels measured	After 90 min, plasma TXB2 level shows Diclofenac alone vs. APAP + Diclofenac: 44·1 vs. 10·6 ($P < 0·003$) Difference abolished after 22 h ($P < 0·90$). Similarly in ADP level	68
Human blood *Ex-vivo* 0·05–150 mM	*Lipopolysaccharide (LPS)-induced PGE2 and TXB2 production.* APAP at different conc. added to blood samples of each individual	APAP inhibited PGE2 and TXB2 production dose-dependently, 44 and 94 respectively. At therapeutic plasma conc. 100–300 μM inhibited more PGE2 than TXB2	71
H i.v. 15, 22·5, 30 mg/kg	*Randomized, placebo, double-blinded study.* Arachidonate-induced platelet aggregation. TXB2, arachidonate, and APAP plasma concentration measured	10 min post-i.v. at 15 mg/kg, arachidonic acid level and TXB2 levels dropped. APAP dose-dependently inhibited platelet aggregation	67
H i.v. 15, 22·5, 30 mg/kg	Platelet function (photometric aggregometry) and TXB2 level measured	APAP at high conc. prolongs PFA-100. APAP demonstrated dose-dependent TXB2 inhibition from conc. 10 μg/mL and up	69
H i.v. 3000 mg	*Surgically induced pain (& bleeding).* APAP, diclofenac given prior surgery. Photometric aggregometer measured platelet aggregation, APAP plasma level, LFTs, and TXB2 also measured	APAP alone did not affect platelet aggregation instead increase in LFTs. Both APAP and diclofenac decreased TXB2 in 1 h but not direct platelet aggregation	74
H i.v. 1000 mg		Single rescue dose of APAP has no effect on platelet aggregation indicated by signs of bleeding	73

LFT, liver function test.
See Table 1 for abbreviations.

the subject of a recent comprehensive review and details can be found in it (80). There is evidence for and against each proposed mechanism. Our purpose is to present a succinct overview of the available evidence and to identify what questions remain to be addressed.

Cyclooxygenase (EC 1.14.99.1, COX) inhibition

The first well known proposal of a mechanism of action for paracetamol was made by Sir John Vane, who discovered that the mechanism of action of aspirin involved inhibition of cyclooxygenase (81). Ever since, paracetamol has periodically been proposed to inhibit one or more of the cyclooxygenase (prostaglandin synthase, PGHS) enzymes COX-1 (PGHS-1), COX-2 (PGHS-2) and COX-3 (PGHS-1b). COX enzymes catalyse conversion of arachidonic acid to prostanoids and other chemical mediators involved in inflammation, fever, pain, platelet aggregation and mucous production in the gastrointestinal tract. COX-1 is constitutively active. COX-2 is inducible and quickly upregulated in areas of inflammation and during fever. Inhibitors of COX-1 and COX-2 (aspirin and other NSAIDs) inhibit pain, inflammation and fever (82). At some point, each of the three COX enzymes has been proposed to be the target of paracetamol's analgesic action.

Paracetamol generally lacks the clinically meaningful anti-inflammatory and antiplatelet effects displayed by NSAID COX inhibitors. A common hypothesis to explain this difference is that paracetamol acts centrally as a COX inhibitor, whereas the other COX inhibitors act both centrally and peripherally (83). Support for this theory includes evidence that paracetamol inhibits the conversion of arachidonic acid to PGE2, PGF2 and thromboxane-A2 in microglia exposed to lipopolysaccharide (84) at concentrations 3-fold lower in microglia than in peripheral macrophages; evidence that paracetamol is more potent centrally than peripherally as a COX inhibitor. However, paracetamol does not affect 24-h body temperature elevation during the luteal phase of the menstrual cycle in humans, a process thought to be prostaglandin mediated. This suggests that paracetamol's effect on fever/temperature does not involve inhibition of prostaglandins (and so might not its analgesic effect) (85).

COX-1

The antinociceptive (animal equivalent of analgesic) activity of paracetamol is diminished in COX-1 knockout mice (mice that are genetically modified such that they do not produce COX-1), but not in COX-2 knockout mice (83), suggesting that the analgesic activity of paracetamol requires COX-1 but not COX-2. This is seemingly strong evidence in support of inhibition of COX-1 as the mechanism of analgesic action of paracetamol. In the same study, it was shown that unlike diclofenac, which reduces prostaglandin synthesis both centrally and peripherally, paracetamol only reduces prostaglandin synthesis centrally. Even if paracetamol inhibits peripheral COX-1, it is argued that it has too low a potency to produce pharmacological effects (86). In contrast to the antinociceptive endpoint, hypothermic and antipyretic activity of paracetamol is not affected in COX-1 knockout mice (47), suggesting that these effects of paracetamol are not related to COX-1.

However, the argument that paracetamol inhibits COX-1 centrally, but not peripherally, raises some challenging questions about distribution to the CNS vs. the periphery (Table 6) and is this different from the NSAIDs; the local conditions (e.g. pH) at the sites of distribution within the CNS; whether there is a difference between 'central' and 'peripheral' COX-1; and the site of paracetamol's hypothermic and antipyretic effects. These questions and others make it difficult to conclude that paracetamol's mechanism of action is direct inhibition of COX-1.

COX-2

There have been suggestions that COX-2, but not COX-1 or COX-3, is the isozyme involved in the antipyretic and hypothermic properties of paracetamol, as there is no loss of antipyretic activity in COX-1 knockout mice (47). A recent study in humans reports that paracetamol inhibited COX-2 in *ex vivo* whole blood samples to a comparable extent as NSAIDs and COX-2 specific inhibitors. An affect of COX-2 inhibitors that paracetamol does not display is alteration of fluid balance in the kidneys, which may point to a mechanism of action separate from selective COX-2 inhibitors (82). However, despite the intense interest in COX-2 as

an analgesic mechanism following the discovery of VIOXX and other 'selective COX-2 inhibitors', the connection to paracetamol's analgesic action has not been established. For this postulated mechanism, a difficult question to answer is: if paracetamol shares the same mechanism of analgesic action as the COX-2 inhibitors, why does it not have the same adverse effect profile?

'COX-3'

A cyclooxygenase enzyme identified in canines was originally thought to be encoded by a separate gene than either COX-1 or COX-2 and thus represented a new COX isozyme, named 'COX-3' (87). This new enzyme was shown to be inhibited by paracetamol, implying that it might be involved in paracetamol's mechanism of analgesic action. Because COX-3 is a variant of COX-1, most evidence supporting a central COX-1 mechanism of action also supports the COX-3 theory. However, although it has been shown to be an active COX protein in canines, the 'COX-3' gene sequence of rats appears to be subject to a frame-shift mutation, resulting in the transcription of a protein that is dissimilar to that of COX-1 or COX-2 in both genetic sequence and function.

However, COX-3 does not mediate antinociception in rats, COX-3 is not detected in humans, and the low expression level and kinetics make it is unlikely that COX-3 has clinical relevance for paracetamol's mechanism of analgesic action (88).

Peroxidase

It has been proposed that rather than inhibiting COX enzymes directly, as do the NSAIDs, paracetamol might inhibit the enzymes indirectly. This theory is based on the fact that paracetamol is a phenol, and phenols are powerful reducing agents. According to this view, paracetamol acts as a reducing agent to inactivate COX enzymes by

Table 6. Pharmacokinetic data for paracetamol

Reference	Study design	Results
15	Human	Plasma C_{max} is 20 μg/mL after a 20 mg/kg p.o. dose
18	Human, paediatric; 40 mg/kg administered nasogastric	Cerebrospinal fluid (CSF)/plasma C ratio = 1·18, probably because of low CSF V; CSF C_{max} = 0·1 mmol/L = 15 μg/mL; time lag in CSF C; meta-analysis of other data showed plasma C 0·06–0·13 mmol/L (9–20 μg/mL) associated with antipyretic effect; PD modelling showed CSF C closer to effect C, but still a lag; EC50 = 0·064 mmol/L = 9·6 μg/mL
20	Meta-analysis, human studies	EC50 = 4·63 mg/L for antipyresis EC50 = 9·98 mg/L for analgesia
19	Human, paediatric with scaling to a 70 kg body; 30–40 mg rectally	Cplasma 0–33 mg/L CCSF 0–21 mg/L
16	Rat 0·6 mg/rat i.v.	APAP crosses the BBB Blood conc. 5- to 11-fold higher than brain; highest brain C seen in cerebellum at 120 min and frontal cortex at 15 min post-dose
17	Mice (ddY male), i.v. infusion LD 46·3 mg/kg and MD 12·1 mg/kg for target plasma C 10 μg/mL	Plasma Css = 8·7 μg/mL, brain Css = 5·8 μg/mL

converting them to their inactive oxidized forms. Specifically, paracetamol's reducing capability might disrupt the tyrosyl radical step of the COX pathway (89).

Another mechanism of decreasing peroxide tone can be through scavenging and reducing free peroxides such as peroxynitrate. Paracetamol possesses similar peroxide scavenging potency as other known reducing agents such as uric acid and antioxidants such as ascorbic acid (vitamin C) (90). This mechanism of action might be counteracted (overwhelmed) in the presence of high concentrations of oxidative peroxides, often released in areas of inflammation (91). This hypothesis is supported by evidence that paracetamol is able to efficiently eliminate excessive peroxide tone caused by low-dose peroxynitrate, but not peroxide tone caused by high-dose peroxynitrate, by H_2O_2 or by tert-butyl-OOH (90). This suggests that paracetamol may inhibit mild to moderate peroxide stimulation of PGHS, but is overwhelmed at higher concentrations or by different types of peroxides that are present in inflammatory responses. Another study found that paracetamol inhibits COX-2 in intact cells at concentrations far below the known IC50 in humans, but does not affect COX-2 in broken cell preparations. This effect can be completely blocked by an increase in intracellular hydroperoxides, further supporting the theory the inactivation of paracetamol by overwhelming peroxide tone (92).

The hypothesis that paracetamol produces its analgesic effect by being a phenol and acts as a reducing agent raises the question: are all, or at least some other, phenols and reducing agents analgesic?

Nitric oxide synthase

Inhibition of the enzyme nitric oxide synthase (NOS) has been another hypothesized mechanism of analgesic action of paracetamol. Nitric oxide (NO) is produced in response to activation of the NMDA (*N*-methyl-D-aspartate) receptor and it amplifies neuronal activity and facilitates nociception (93, 94). Inhibition of NO synthesis can attenuate nociception, depending on the pain stimulus (95). There are several subtypes of NOS. One subtype is constitutively active (cNOS) and can be further divided into neuronal NOS (nNOS), located in the central and peripheral nervous systems, and endothelial NOS (eNOS), located in endothelial cells. Another subtype is inducible (iNOS) and plays a role in inflammatory processes (96).

L^G-nitro-L-arginine (L-NO-ARG), a non-specific inhibitor of NOS subtypes, 7-Nitroindazole (7-NI), a specific inhibitor of nNOS and L-N6 (1-Iminoethyl)lysine (L-NIL), a specific inhibitor of iNOS, all potentiate paracetamol antinociception (97), suggesting that paracetamol might be an inhibitor of NOS. L-NO-ARG and 7-NI also potentiate the effects of paracetamol when administered intrathecally, but L-NIL does not, suggesting that iNOS may be involved only in any peripheral actions of paracetamol (98).

Paracetamol has been reported to lack direct inhibitory effect on cNOS or iNOS *in vitro* (99), but perhaps the effect is indirect, as paracetamol inhibits expression of the iNOS gene in response to lipopolysaccharide and interferon gamma in RAW 264.7 macrophages (96).

Cannabinoid

The discovery that a receptor mediates the effects of cannabinoids (marihuana-like compounds) prompted a search for the endogenous ligands (endocannabinoids) (100). This search led to anandamide (101), which is the ethanol amide of arachidonic acid. Further research led to the recognition that the endocannabinoid system is evolutionarily old, occurring in invertebrates as well as in vertebrates (see (102) and references therein). Endocannabinoids are involved in, and exogenous cannabinoid substances can modify, a variety of physiological processes, including pain, motor activity, cognitive function, sleep and appetite (102). In addition, the endocannabinoid system is involved in 'crosstalk' with a variety of other receptor systems, including 5-HT (serotonin), NMDA and vanilloid ($TRPV_1$) (102).

The endocannabinoid system has recently been proposed to be involved in the mechanism of analgesic action of paracetamol. Paracetamol itself does not bind to cannabinoid receptors (103, 104), but one of its metabolites displays cannabinoid-like activity (105). Accordingly, paracetamol could activate the endocannabinoid system by acting as a pro-drug.

Specifically, paracetamol is deacetylated to form a primary amine, *p*-Aminophenol, which subsequently undergoes conjugation with arachidonic

acid in the brain to form *N*-Arachidonoylphenolamine (AM404) (105). The conjugation of arachidonic acid with *p*-Aminophenol is catalysed by the enzyme FAAH (fatty acid amide hydrolase). Inhibition of FAAH has been reported to suppresses the antinociceptive effect of paracetamol in mice (104), suggesting that paracetamol's analgesic action is related to AM404 production. As AM404 lacks significant affinity for cannabinoid receptors, the interaction must be indirect. The nature of such a mechanism was suggested when AM404 was noticed to have structural similarity to agonists at the vanilloid receptor (106) and paracetamol was shown to have binding affinity for vanilloid receptors in human cells (107). Further research has demonstrated that AM404 is an activator of the vanilloid subtype 1 receptor ($TRPV_1$ or previously known as VR1; a proven CB_1 receptor agonist), and an uptake inhibitor of the endocannbinoid anandamide (108, 109). $TRPV_1$ itself is involved in pain (110) and perhaps in thermoregulatory (111) pathways. Acetaminophen's analgesic properties are blocked by the administration of CB_1 receptor antagonists at doses that inhibit known CB_1 receptor agonists (104, 112). This provides further evidence for the cannabinoid hypothesis.

In addition to their antinociceptive action, cannabinoids also reduce body temperature in rats (113). However, administration of a CB_1 receptor antagonist does not change paracetamol's hypothermic effect (Scott Rawls, PhD, Temple University School of Pharmacy, personal communication).

If paracetamol has a significant cannabinoid component, then it is reasonable to expect it to produce effects similar to those of cannabinoid compounds. In an effort to support a cannabinoid hypothesis, it has been claimed that paracetamol can produce feelings of relaxation, tranquility and instill a feeling of well-being (114, 115). However, to date these have not been supported by objective trials and are currently considered anecdotal (116).

5-HT (5-hydroxytryptamine, serotonin)

There is substantial evidence that paracetamol's mechanism of analgesia in some manner involves the descending serotonergical pathway. 5-HT neurons, largely originating in raphe nuclei located in the brain stem (117, 118) send projections down to the spinal cord that synapse on afferent neurons entering the spinal cord. These descending projections exert an inhibitory (analgesic) effect on the incoming pain signal before it is transmited to higher CNS centres.

Although paracetamol does not have affinity for 5-HT receptors or neuronal 5-HT reuptake sites (103, 118–120), there is evidence to suggest an indirect mechanism. Paracetamol administration (100–400 mg/kg p.o. and 200–400 mg/kg i.p.) increases 5-HT levels in various regions of (rat) brain (cortical, pontine, hypothalamus, striatum, hippocampus, brain stem) (121–123) and subsequent down regulation of $5HT_{2A}$ receptors (121, 122, 124, 125). When the spinal 5-HT pathway is lesioned in rats, using 5,6-Dihydroxytryptamine (5,6-DHT) injected intrathecally, paracetamol-induced antinociception in the formalin test is reduced, whereas lesioning the noradrenergic pathway, using 6-Hydroxydopamine (6-OHDA) has no effect (126) on paracetamol antinociception. Likewise, depletion of 5-HT in cortical and pontine regions (12% and 19% of baseline) using *p*-Chlorophenylalanine significantly decreased paracetamol-induced antinociception in rats in the hot-plate test, shown by a decrease in pain threshold from 32·8% to 11% maximum possible effect (121).

Pharmacological approaches have yielded less consistent results. Tropisetron, a $5HT_3$ receptor antagonist, when administered IT inhibits the antinociceptive effects of paracetamol in various pain models, suggesting $5HT_3$ receptor involvement (63, 119, 127, 128). However, granisetron and ondasetron, also $5HT_3$ receptor antagonists, when given IT or SQ, failed to block paracetamol antinociception in rats (118, 120, 129); $5\text{-}HT_3$ receptor antisense deoxynucleotides also failed to inhibit acetaminophen antinoception (120).

Although these results seem counterintuitive, tropisetron, unlike granisetron and ondansetron, has affinity for $5HT_{1B}$, $5HT_{2A}$, $5HT_{2C}$ receptors in addition to $5HT_3$ receptors (129), leading to studies involving these receptor subtypes. One study, using rats and the paw pressure test, concluded acetaminophen antinociception is blocked by IT penbutolol ($5HT_{1B}$ antagonist), ketanserin ($5HT_{2A}$ antagonist) and mesolergine ($5HT_{2C}$ antagonist), but not WAY100635 ($5HT_{1A}$ antagonist) (129). There is conflicting data about the involvement of $5HT_{1A}$ receptor as WAY100635 has also been shown to inhibit acetaminophen antinociception

(30) and to increase acetaminophen antinociception (117). The latter studies utilized different pain assays (paw pressure and hot plate respectively) and species (mouse or rat).

Two studies in human volunteers lend support to a 5-HT-related analgesic mechanism for paracetamol. Both studies utilized volunteers that were rapid metabolizers of tropisetron to ensure homogeneity of the study population and to avoid long washout periods. The first study (n = 26) found that the analgesic effect of 1000 mg oral paracetamol against electrical stimulation of the median nerve was significantly reduced by the 5-HT receptor antagonists tropisetron (5 mg) and granisetron (3 mg) administered i.v. (130). A follow-up study (n = 18) by the same group found that tropisetron completely inhibited the analgesic effect of 1000 mg oral paracetamol in a cold pressor test (131).

Self-synergism

From the beginning, the focus of the search for paracetamol's analgesic mechanism has concentrated on the central nervous system. When administered intraventricularly (i.c.v.), acetaminophen produces no significant analgesia (115, 132). This finding lead to attempts to inject acetaminophen into the spinal cord (i.t.), which produced marked dose-related antinociception (132). It was further demonstrated that combined administration of acetaminophen into the brain and spinal cord produced synergistic antinociception (132). This property of acetaminophen has been dubbed 'self-synergy'. Opioid receptor antagonists administered by i.t. injection attenuate the synergism observed with combined acetaminophen administration (132, 133). Remembering that acetaminophen itself does not bind to opioid receptors (103), this suggests that endogenous opioids contribute to the antinociceptive effects of acetaminophen at the spinal level. A caveat is that acetaminophen binding has been investigated at only 10 μM. As acetaminophen reaches significantly higher concentrations in the CNS at analgesic doses (123), opioid binding at higher concentrations should be tested.

SUMMARY

Paracetamol was already in wide use before the molecular mechanism of the other 'weak' analgesics (NSAIDs) was discovered. It was natural and reasonable to postulate that paractamol's mechanism of analgesic action is the same. The first postulate, inhibition of COX-1, is difficult to reconcile with paracetamol's lack of anti-inflammatory action (but reasonable explanations were, and continue to be, offered), was followed by inhibition of COX-2, then inhibition of the putative COX-3 (subsequently shown to be highly unlikely). When it was recognized that 'weak' is not a helpful designation for analgesic mechanism, other physiological pathways have been proposed. The consensus appears to suggest a primarily central (CNS) site of action (interestingly, this is similar to the earliest proposal, but for a different mechanism). The persistent difficulty in elucidating paracetamol's mechanism of analgesic action might be because of it having more than one mechanism, perhaps acting at multiple sites, and perhaps acting synergistically, neither one of which by itself accounting for the overall effect.

CONCLUSION

Paracetamol, as all drugs, has desirable and undesirable characteristics. It has benefits related to pain relief and it has risks related to adverse effects. Hence, there is reason and motivation to understand how it produces its analgesic action. This knowledge would also be useful for the design or discovery of a similar drug with an even larger therapeutic window.

The mechanism of paracetamol's adverse effects is fairly well understood. In contrast, none of the proposals about paracetamol's mechanism of analgesic action are completely satisfactory. Some proposals have been discounted, others remain promising, but to date lack sufficient evidence to conclude that they are definitive. So far, no single mechanism has been able to describe all of its actions sufficiently. However, it is reasonable to conclude that paracetamol likely has a pharmacological mechanism that interacts with a variety of physiological pathways, likely within the central nervous system.

As long as paracetamol's analgesic mechanism of action remains an enigma, assessment of its benefit/risk ratio and design or discovery of drugs with a similar mechanism, but greater benefit/risk ratio, will be impeded.

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