REVIEW

Systematic Review of Systemic and Neuraxial Effects of Acetaminophen in Preclinical Models of Nociceptive Processing

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Abstract: Acetaminophen (APAP) in humans has robust effects with a high therapeutic index in altering postoperative and inflammatory pain states in clinical and experimental pain paradigms with no known abuse potential. This review considers the literature reflecting the preclinical actions of acetaminophen in a variety of pain models. Significant observations arising from this review are as follows: 1) acetaminophen has little effect upon acute nociceptive thresholds; 2) acetaminophen robustly reduces facilitated states as generated by mechanical and thermal hyperalgesic end points in mouse and rat models of carrageenan and complete Freund's adjuvant evoked inflammation; 3) an antihyperalgesic effect is observed in models of facilitated processing with minimal inflammation (eg, phase II intraplantar formalin); and 4) potent anti-hyperpathic effects on the thermal hyperalgesia, mechanical and cold allodynia, allodynic thresholds in rat and mouse models of polyneuropathy and mononeuropathies and bone cancer pain. These results reflect a surprisingly robust drug effect upon a variety of facilitated states that clearly translate into a wide range of efficacy in preclinical models and to important end points in human therapy. The specific systems upon which acetaminophen may act based on targeted delivery suggest both a spinal and a supraspinal action. Review of current targets for this molecule excludes a role of cyclooxygenase inhibitor but includes effects that may be mediated through metabolites acting on the TRPV1 channel, or by effect upon cannabinoid and serotonin signaling. These findings suggest that the mode of action of acetaminophen, a drug with a long therapeutic history of utilization, has surprisingly robust effects on a variety of pain states in clinical patients and in preclinical models with a good therapeutic index, but in spite of its extensive use, its mechanisms of action are yet poorly understood.

Keywords: intrathecal, paracetamol, cannabinoid, serotonin, anandamide, analgesia

Introduction

Acute and chronic pain imposes a burden on society, not only because of the associated human suffering, but also because of the cost of medical treatment, loss of productivity and disability payments, which has been estimated to be up to $650 billion per year in the USA alone. According to the recently posted National Institutes of Health (NIH) Pain Management fact sheet, pain affects more Americans than diabetes, heart disease and cancer combined.[1] Opiates are widely used but are associated with a significant risk of addiction and diversion, while other types of analgesics [eg, nonsteroidal anti-inflammatory drug (NSAIDs), antidepressants and anti-epileptics] are hampered by limited efficacy and varying side effects.[2–5]

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Acetaminophen, (also known generically as APAP or paracetamol) was synthesized by Morse in 1873 via the reduction of p-nitrophenol with tin in glacial acetic acid. It was then discovered as a metabolite of phenacetin in the 1890s.[6,7] It is a small molecule (151 Da). Its commercial development occurred in the USA in the 1950s. Here, the product went on sale in the United States in 1955 under the brand name "Tylenol".[8]

Unlike opioid analgesics, it was early appreciated that acetaminophen does not generate euphoria or mood alteration. It has, over decades of use, been appreciated to be without risks of sedation, addiction, dependence, tolerance and withdrawal when administered alone.[9–12] The principal limitation is that in large amounts over time, acetaminophen can result in the formation of hepatotoxic metabolites.[13,14] Its general tolerability, lack of abuse potential and, as discussed below, efficacy has led to its appreciation as a therapeutic with an important role in pain management. As such, it is one of the most commonly employed analgesic molecules with over 25 billion doses delivered annually in the US alone.[15,16] In spite of its widespread use, its mechanisms of action remain controversial.[17]

This narrative review looks to consider issues related to site and mechanism of action. It is organized into three parts: 1) an overview of the clinical efficacy of acetaminophen; 2) a review of the effects of acetaminophen in animal models of acute nociception, after tissue injury and nerve injury following per os (PO), intravenous (IV), intrathecal (IT), intracerebroventricular (ICV) and peripheral routes of delivery; and 3) a summary of mechanisms by which acetaminophen (and/or its metabolites) exert its analgesic actions.

Analgesic Efficacy of Acetaminophen in Humans

While acetaminophen is often times considered dismissively as a weak or minimally efficacious therapeutic, clinical studies often show that it compares favorably to other analgesic regimens in the management of a variety of pain states. Clinically, acetaminophen is available for oral, rectal and IV delivery. Not surprisingly, higher plasma concentrations are achieved with the shortest latency after IV delivery[18,19] and peak cerebrospinal fluid concentrations are significantly greater with IV administration than with PO.[20]

Experimental Pain

The analgesic effects of acetaminophen have been studied in experimental pain models in humans. In double-blinded and placebo-controlled trials, electrical stimulation at high current densities induced spontaneous acute pain and distinct areas of hyperalgesia for painful mechanical stimuli (pinprick hyperalgesia). IV acetaminophen (650 mg), tramadol (75 mg) or a combination of both (325/37.5 mg, respectively) was administered. Tramadol led to a maximum pain reduction of 12% with negligible antihyperalgesic properties. In contrast, acetaminophen led to a similar pain reduction (10%), but surprisingly displayed a sustained antihyperalgesic effect.[21]

Inflammatory and Postoperative Pain

PO doses of 1000 mg of acetaminophen are reported to result in modest, but statistically significant reductions in mild to moderate acute pain states with etiologies ranging from acute soft tissue injury (sprain),[22,23] major surgeries,[24,25] headache,[26] total hip or knee replacements[27] and hip or knee osteoarthritis.[28,29] The use of IV acetaminophen has been reported to be well tolerated and efficacious in well-powered, prospective and retrospective trials and to result in significant decreases in postoperative morphine consumption and pain intensity after a variety of surgical interventions including orthopedic,[30–33] dental,[34] spinal fusion,[35] abdominal laparoscopic interventions,[36] post bariatric,[37] C-section and hysterectomies,[38–42] cardiac surgery,[43] and head and neck cancer surgery.[44] IV acetaminophen reduced the likelihood of readmission after 30 days following knee arthroscopy.[45] This raises the possibility that the actions of acetaminophen reflect disease modifying actions that alter the transition from an acute to a chronic pain state hypothesized to reflect a role of innate/adaptive immune systems.[46] Less promising results have also been reported,[47] concerns of toxicity remain[48] and the superiority of IV vs PO acetaminophen has been questioned.[49]

Neuropathic Pain

While there are surprisingly few studies reporting efficacy of acetaminophen alone in mono and poly neuropathic pain states, work has emphasized that opiate–acetaminophen combinations reveal synergy yielding increased utility in a variety of pain states including, not only in osteo and rheumatoid arthritis,[50,51] but in diabetic neuropathy.[52] In contrast, in cancer pain, addition of acetaminophen did not affect the analgesic effect of methadone or morphine.[53,54]

Alternate Routes in Human Acetaminophen Delivery

While meta-analyses often emphasize the lack of "high quality evidence" in many of the effects of acetaminophen (by any route), particularly for persistent pain states (eg, back pain, hip or knee osteoarthritis and cancer),[28,44,55,56] IV acetaminophen has been formally approved for mild-to-moderate pain and moderate-to-severe pain with adjunctive opioid analgesics in Europe and more recently in the USA.[57] Thus, the efficacy of IV acetaminophen relative to placebo has been shown in controlled postoperative trials where patient satisfaction with acute postoperative pain control was rated through 24 h after dosing. Patients receiving IV acetaminophen reported excellent satisfaction more often than those receiving placebo and was the strongest predictor of patient satisfaction.[58,59] Of note, clinical trials on the use of intrathecal acetaminophen in knee and hip procedures (ClincialTrials.gov, 2016) prepared in a novel supersaturated formulation are currently registered, though no data have as yet been reported.[60]

Key Points Regarding Human Action of Acetaminophen

Four clinical observations are of potential significance as to the activity and mechanisms of action of acetaminophen:

1) CSF sampling after acetaminophen reveal high levels within 10 min after an IV delivery with peak concentrations being lower and delayed after PO administration.[20,61]

2) Acetaminophen reduced the areas of secondary hyperalgesia to pinprick and touch otherwise observed after peripheral electrical stimulation. These results suggest an effect independent of the presence of peripheral injury/inflammation leading to a hyperalgesic state and suggest an effect upon central processes underlying sensitization.[62]

3) Importantly, brain imaging has further confirmed the effects of analgesic doses of acetaminophen on brain responses to aversive stimulation. Human studies with fMRI have revealed that acetaminophen reduces the response to noxious thermal stimulation as compared to placebo in prefrontal cortices, insula, thalami, anterior cingulate cortex and periaqueductal gray matter, leading to the suggestion of an inhibitory effect of acetaminophen on spinothalamic outflow.[29] Similarly, in subjects with knee osteoarthritis, acetaminophen reduced blood oxygenation level dependent signal activation in the sensory

cortex and supramarginal gyrus, prefrontal and frontal cortex, and insula.[63] Thus, while acetaminophen efficacy has been questioned in the management of osteoarthritis pain,[64] it is clear that it has state dependent effects upon neuraxial processing.

4) Systematic analysis of plasma levels after acetaminophen dosing and the magnitude of the opioid sparing effects in postoperative pain point to a ceiling effect of the effect of even IV acetaminophen on postoperative pain.[65]

5) While acetaminophen has minimal effects other than altering nociception, recent work has suggested that, in addition, acetaminophen may have broader psychological effects. Thus, acetaminophen has been shown to reduce affective responses to both negative and positive emotional images[235] and reduced the neural responses associated with experiences of acute applic pain.[236,237]

Analgesic Efficacy of Acetaminophen in PreClinical Models

Routes of Delivery

As presented in Tables 1–3, we found 99 preclinical studies on analgesia of acetaminophen when retrieved on an electronic database using the following search phrases: (("acetaminophen"[MeSH Terms] OR "acetaminophen"[All Fields] OR "paracetamol"[All Fields]) OR ("acetaminophen"[MeSH Terms] OR "acetaminophen"[All Fields])) NOT ("liver"[MeSH Terms] OR "liver"[All Fields]) AND "animals"[MeSH Terms: noexp]. The systematic search was undertaken through 9 September 2019. These are summarized by pain models in the tables. Among them, 41 studies involved oral administration of acetaminophen,[66–105] 38 studies with intraperitoneal injection,[73,76,86,102,106–140] 13 studies with intrathecal administration,[74,87,121,132,141–147] 12 studies with intravenous administration,[82,87,107,134,143,145,148–153] 5 studies with intracerebroventricular administration[89,126,135,146,154] and with local subcutaneous in the paw,[82,126,135,155,156] 7 studies with subcutaneous administration[124,141,157–161] and 1 study with rectal administration.[98]

Formulation

Acetaminophen is freely soluble in alcohol; soluble in methanol, ethanol, dimethylformamide, ethylene dichloride, acetone, ethyl acetate; slightly soluble in ether; and practically insoluble in petroleum ether, pentane, and benzene.[162] Solubility in water at 25 °C is 14 mg/mL, and water solubility is markedly increased with heat.[162,163]

Table I Preclinical Analgesic Effect of Acetaminophen by PO, IT, IP/SC, IV, IT, ICV Administration in Acute Nociception

	Study	Test Model	Nociceptive State	Vehicle	Specie Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
1.	Ginger rhizome enhances anti-inflammatory and anti-nociceptive effects of APAP in mouse fibromyalgia	Paw pressure Hot plate Tail flick	Fibromyalgia syndrome	Saline	Mice/F	Increased threshold	400					Montserrat-de la Paz et al 2018[66]
2.	Enhanced analgesic effects of nefopam with APAP	Tail flick Hot plate	Normal	Saline	Mice/F, M	No change		42–168				Li et al 2018[107]
		Tail flick Hot plate	Normal	Saline	Mice/F, M	No change			60–240			
3.	Central dopaminergic system plays a role in analgesic action of APAP	Hot plate	Normal	Saline	Mice/M	Increased escape latency	100					Bhagyashree et al 2018[68]
4.	Effect of modulating 5-HT on analgesic action of APAP	Hot plate	Normal	N/A	Albino mice/F, M	Increased latency		200				Karandikar et al 2016[110]
5.	Interactions with codeine and APAP in mice	Hot plate	Normal mice	Saline	Mice/F, M	Increased latency		20				Raskovic et al 2015[111]
6.	Synergic effects of pregabalin–APAP combination in somatic and visceral nociceptive reactivity	Tail flick	Normal mice	Saline	Mice/M	Increased latency	200					Mititelu et al 2014[101]
7.	APAP administration during neonatal brain development affects cognitive function and alters analgesic and anxiolytic response in adult	Hot plate	Normal mice	Saline	Mice/M	Increased latency	30–60					Viberg et al 2014[91]
8.	Cav3.2 calcium channels in supraspinal effect of APAP	Tail flick Von Frey Paw immersion tests	Normal mice or Cav3.2 knockout mice	Saline	Mice/M	Increased latency	200					Kerckhove et al 2014[70]

No.	Description	Method	Group	Vehicle	Species/Sex	Effect	Dose 1	Dose 2	Dose 3	Reference
9.	TRPA1 mediates spinal antinociception induced by APAP and a cannabinoid.	Hot plate Paw pressure	Normal mice or Trpa1 $^{-/-}$ mice	Saline	Mice/M	Effects of spinal/systemic APAP lost in Trpa1 $^{-/-}$		300		Andersson et al 2011[141]
		Hot plate Paw pressure	Normal mice or Trpa1 $^{-/-}$ mice	Saline	Mice/M	Effects of spinal/systemic APAP lost in Trpa1 $^{-/-}$	50 or 100			
10	Phenazopyridine on rat bladder primary afferent activity, and comparison with lidocaine and APAP	Bladder primary afferent activity	Normal	Saline	Rat/F	Decreased activity			1–10	Aizawa et al 2010[92]
11	Different mechanisms underlie analgesic actions of APAP and dipyrone in inflammatory pain	Von Frey	Normal	Saline	Rat/M	Increased threshold Dose dependent		60–360		Rezende et al 2008[124]
12.	Endocannabinoid and serotonergic systems needed for APAP-induced analgesia	Von Frey Hot plate	Normal	DMSO and saline	Rat/M	Increased threshold			300	Mallet et al 2008[95]
13.	Differential involvement of opioid and 5-HT systems in the antinociceptive activity of AM404: comparison with APAP	Hot plate Paw pressure	Normal	12.5% 1,2-propanediol and saline	Rat/M	Increased threshold		400		Ruggieri et al 2008[76]
14.	Effect of acute and repeated administration of APAP on opioid and 5-HT systems	Hot plate	Normal	Saline	Rat/M	Increased threshold		400		Sandrini et al 2007[139]
15.	Modulation of APAP by caffeine and by adenosine A2 receptor antagonists.-	Von Frey Hot plate	Normal	PO4 buffer saline	Mice/N/A	Increased threshold		10–200		Godfrey et al 2006[116]
16.	The analgesic activity of APAP is prevented by CB1 receptor blockade	Hot plate	Normal	Saline	Rat/M	Increased threshold Dose dependent			250–1000	Ottani et al 2006[77]
17.	Opioid receptor antagonists on action of APAP	Paw pressure	Normal	Saline	Rat/M	Increased threshold			400	Bujalska 2004[81]
18.	APAP involves spinal tropisetron-sensitive, non–5-HT3 receptor	Paw pressure	Normal	Saline	Rat/M	Increased threshold			400	Libert et al 2004[173]

(Continued)

Table 1 (Continued).

	Study	Test Model	Nociceptive State	Vehicle	Specie Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
19.	Involvement of central 5-HT1B and 5-HT3 receptors in effect of APAP	Hot plate Paw pressure	Normal	1,2-propanediol/saline	Rat/M	Increased latency/threshold		400				Sandrini et al 2003[117]
20.	Role of 5-HT1A/B autoreceptors in antinociceptive effect of APAP	Hot plate	Normal	Propylene glycol/water	Mice/M	Increased latency Dose dependent		300–800				Roca-Vinardell et al 2003[140]
21.	NCX-701 (nitroAPAP) is an effective antinociceptive agent in rat withdrawal reflexes and wind-up	Electrical stimulation Von Frey	Normal	DMSO/polyethylene glycol	Rat/M	Increased threshold				22–724		Romero-Sandoval et al 2002[150]
22.	COX and NOS inhibitors on action of APAP	Paw pressure	Normal	Saline	Rat/M	Increased threshold Dose dependent	100–800					Bujalska and Gumulka 2001[84]
23.	5-HT receptor subtypes involved in spinal antinociceptive effect of APAP	Paw pressure test	Normal	Trisodic citrate, 0.02 g/mL	Rat/M	Increased threshold			200			Courade et al 2001[151]
24.	APAP-induced antinociception via central 5-HT2A receptors	Tail flick	Normal rat	12.5% of 1,2-propanediol in saline	Rat/M	Increased latency		300 or 400				Srikiatkhachorn et al 1999[127]
25.	APAP exerts spinal antinociceptive effect involving interaction with 5-HT3 receptors	Paw pressure	Normal	Solvent trisodic citrate, 0.02 g/mL	Rat/M	Increased threshold	200–800					Pelissier et al 1996[87]
		Paw pressure	Normal	Solvent trisodic citrate, 0.02 g/mL	Rat/M	Increased threshold			50–300			

No.	Study	Method	Condition	Solvent	Animal/Sex	Effect	Dose A	Dose B	Dose C	Dose D	Reference
		Paw pressure	Normal	Solvent trisodic citrate, 0.02 g/mL	Rat/M	Increased threshold				100–200	
26.	Antinociceptive action of APAP associated with changes in 5-HT system in brain	Hot plate	Normal	1,2-propanediol in saline	Rat/M	Increased latency Dose dependent		200–400	200–400		Pini et al 1996[128]
27.	Central APAP effect involving spinal 5-HT3 receptors	C fiber-evoked reflex	Normal	N/A	Rat/M	Increased threshold		200–400			Pelissier et al 1995[153]
28.	Increasing-temperature hot plate test	Hot plate	Normal	Propanediol in NaCl	Rat or mice/M	Increased latency			200–400		Tjølsen et al 1990[119]
29.	Depression by morphine, metamizol (dipyrone), lysine acetylsalicylate and APAP, of thalamus neurons evoked by electrical stimulation	Behavioral response and evoked activity in thalamus	Normal	Saline	Rat/M	Increased threshold Dose dependent		5–25			Carlsson et al 1988[174]
30.	Central effect of APAP depresses nociceptive activity in thalamic neurons	Behavioral response	Normal	Saline	Rat/M and F	Increased threshold			50–150		Carlsson and Jurna 1987[134]
31.	Acute toxicity and analgesic action of a combination of buclizine, codeine and APAP in tablet/suppository form	Tail flick	Normal	Saline	Mice/M and F	Increased latency Dose dependent	97–772				Behrendt et al 1985[98]
32.	Blood levels and analgesic effects of APAP	Paw pressure	Normal	Water	Mice/M	Increased threshold Dose dependent			149–360		Shibasaki et al 1979[152]
		Paw pressure	Normal	Water	Mice/M	Increased latency Dose dependent		111–360			
33.	Inhibition of prostaglandin synthetase in the brain by APAP	N/R	N/R	N/R	Rabbit/NR	Increased threshold	ID50 14 μg/mL				Flower and Vane 1972[105]

Table 2 Preclinical Analgesic Effect of Acetaminophen by PO, IT, IP/SC, IV, IT, ICV Administration in Tissue Injury and Inflammation

	Study	Test Model	Nociceptive State	Vehicle	Species Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
1.	ATP-sensitive K$^+$ channels and μ-opioid receptors in antinociceptive synergism of APAP-tapentadol	Formalin test	Formalin	Saline	Mice/M	Decreased behavior Dose dependent		56–562				Zapata-Morales et al 2018[106]
2.	Synthesis and antinociceptive evaluation of bioisosteres and hybrids of naproxen, ibuprofen and APAP	Formalin test	Formalin	Saline	Rat/M	Decreased behavior Phase 2		0.6–3.2				González-Trujano et al 2018[157]
4.	Enhanced analgesic effects of nefopam with APAP	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior Dose dependent		42–168				Li et al 2018[107]
5.	APAP relieves inflammatory pain through CB1 in rostral ventromedial medulla	Von Frey	ITSP extract IPLT zymosan	Saline	Mice/M	Increased threshold Dose dependent	30–300					Klinger-Gratz et al 2017[67]
7.	Supraspinal TRPV1 desensitization induced by ICVT resiniferatoxin	Tail flick Formalin test	Resiniferatoxin agonist, hind paw)	Propylene glycol	Mice/M	Increased latency Decreased behavior		100 or 300				Fukushima et al 2017[108]
8.	Imidazoline receptor and APAP schedule-controlled responses	Von Frey Thermal	Freund's adjuvant	20% DMSO and saline	Rat/M	Increased threshold/latency Dose dependent		56–178				Siemian et al 2016[109]
9.	Modulating 5-HT system on the analgesic action of APAP in mice	Formalin test	Formalin	N/A	Mice/M	Decreased behavior Phase 1/2		200				Karandikar et al 2016[110]
10.	Antinociceptive effects of sinomenine in postoperative pain	Von Frey	Incisional surgery model (Brennan)	N/A	Rat/M	Increased threshold Dose dependent	30–180					Zhu et al 2016[69]

No.	Title	Test	Model	Vehicle	Species/Sex	Effect				Reference
11.	Acetaminophen interacts with morphine and tramadol analgesia for the treatment of neuropathic pain	Von Frey	Carrag paw	Saline	Rat/M	Increased threshold Dose dependent			20–1000	Shinozaki et al 2015[104]
12.	Supra-spinal FAAH required for APAP analgesia	Hot plate Von Frey	Carrag paw	Saline	Mice/M	Increased latency/threshold			200	Dalmann et al 2015[90]
13.	Antinociception by MT(2) melatonin receptor partial agonists	Hot plate	MT(2) melatonin receptor partial agonists	70% dimethyl and 30% saline	Rat/M	Normalized threshold		200		López-Canul et al 2015[158]
14.	Synergy of pregabalin-APAP in somatic/visceral pain	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior			200	Mtitelu et al 2014[101]
15.	Adamantyl analogues of APAP as potent analgesic drugs via inhibition of TRPAI	Visceral pain	Acetic acid (IP, 2%)	N/A	Mice/M	Decreased behavior Dose dependent		100–200		Fresno et al 2014[112]
16.	Cav3.2 calcium channels in supra- spinal APAP effect	Formalin test	Formalin	Saline	Mice/M	Decreased behavior			200	Kerckhove et al 2014[70]
17.	Arsenic decreases activity of APAP. Involvement of 5-HT and CB receptors	Formalin test	Formalin	Saline	Rat/M	Decreased behavior phase I			400	Vijayakaran et al 2014[71]
18.	APAP involves spinal serotonin 5-HT7 and adenosine AI receptors, and peripheral adenosine AI receptors	Formalin test	Formalin	Saline	Mice/M	Decreased behavior Phase 2		300		Liu et al 2013[120]
19.	Naltrexone did not change synergism between APAP and tramadol	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior Dose dependent	4.0			Miranda et al, 2012[121]
20.	APAP-induced analgesic and antihyperalgesic effects by 5-HT pathways and spinal 5-HT7 receptors	Tail flick	Plantar incision	20:1:1:78 mixture of DMSO: ethanol: Tween saline	Mice/M	Increased latency Dose dependent			200–600	Dogrul et al 2012[72]

(Continued)

Table 2 (Continued).

	Study	Test Model	Nociceptive State	Vehicle	Species Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
21.	Caffeine inhibits APAP antinociception by spinal adenosine A_1 receptors	Formalin test	Formalin	20% DMSO	Mice/M	Decreased behavior phase 2 Dose dependent		100–300				Sawynok and Reid 2011[123]
22.	Systematic evaluation of nefopam–APAP combination in rodent models of antinociception	Writhing test	Acetic acid	1% Tween 80 and saline	Mice/M	Decreased behavior Dose dependent		25–200				Girard et al 2011[159]
		Formalin test	Formalin	1% Tween 80 and saline	Mice/M	Decreased behavior Dose dependent		100–600				
		Hot plate	Carrag in hind paw	1% Tween 80 and saline	Rat/M	Normalized threshold		300–600				
23.	Ondansetron does not block APAP-induced analgesia	Von Frey Hot plate	Fracture pain model	N/A	Mice/M	Increased threshold/ Latency		300				Minville et al 2011[113]
24.	Synergy of APAP and oxcarbazepine	Paw pressure	Carrag hind paw	Tween 80 and saline	Rat/M	Increased threshold Dose dependent		50–200				Tomić et al 2010[114]
		Writhing test	Acetic acid	Tween 80 and saline	Rat/M	Decreased behavior Dose dependent		60–180				
25.	Synergy between ibuprofen, APAP and codeine	Tail flick	Rat tail Ischaemia	DMSO and saline	Rat/M	Increased threshold Dose dependent		11–88				Mitchell et al 2010[115]

No.	Study	Test	Model	Vehicle	Species/Sex	Result	Dose	Dose	Dose	Reference
26.	TRPV1 in brain is involved in APAP-antinociception	Formalin test Hot plate Von Frey	FAAH and TRPV1 knockout mice	10% DMSO/2.5% Tween 80/Saline	Mice/M	Decreased behavior Increased latency/threshold	200			Mallet et al 2010[73]
		Formalin test Hot plate Von Frey	FAAH and TRPV1 knockout mice	10% DMSO/2.5% Tween 80/Saline	Mice/M	Decreased behavior Increased latency/threshold		100		
		Active movement	FAAH and TRPV1 knockout mice	10% DMSO/2.5% Tween 80/Saline	Mice/M	Decreased behavior Dose dependent		100–300		
27.	Modulation of APAP and nefopam antinociception by 5-HT(3) receptor	Formalin test	Formalin	1% solution of Tween 80	Mice/M	Decreased behavior		400		Girard et al 2009[161]
28.	Synergy between rilmenidine/APAP	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior Dose dependent	127–445			Soukupová et al 2009[93]
29.	Differential effects of APAP LPS induced hyperalgesia in various mouse pain models	Formalin test	Formalin	20% DMSO	Mice/M	Decreased behavior Phase 1/2 Dose dependent	25–300			Seo et al 2008[146]
		Formalin test	Formalin	20% DMSO	Mice/M	Decreased behavior Dose dependent			25–100	
		Formalin test	Formalin	20% DMSO	Mice/M	No effect			25–100	
30.	Different mechanisms for APAP and dipyrone	Von Frey	Carrag hindpaw	Saline	Rat/M	Increased threshold		60–360		Rezende et al 2008[124]

(*Continued*)

Table 2 (Continued).

	Study	Test Model	Nociceptive State	Vehicle	Species Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/ IPLT mg/kg	IV mg/kg	IT μg	ICV μg	Reference
31.	CB and 5HT systems needed for APAP-analgesia	Formalin test	Formalin	DMSO and saline	Rat/M	Decreased behavior Phase I/2.	300					Mallet et al 2008[95]
35	APAP prevents hyperalgesia in central pain cascade	Hot plate	Spinal substance P	5% DMSO	Rat/M	Normalized threshold	300					Crawley et al 2008[74]
		Hot plate	Spinal substance P	5% DMSO	Rat/M	Increased latency Dose dependent				10–200		
32.	Morphine and ABT-594 (a nicotinic acetylcholine agonist) exert central analgesia	Abnormal postures and eye closure)	Cyclophosphamide to induce bladder inflammation	N/A	Rat/M	No change	44–480					Joshi et al 2008[75]
33.	Different mechanisms underlie actions of APAP and dipyrone	Paw pressure	Carrag hindpaw	Saline	Mice/M	Increased threshold		60–360				Rezende et al 2008[124]
34.	Isobolographic interactions between ketoprofen and APAP	Writhing test	Acetic acid	0.5% carboxymethylcellulose	Mice/M	Decreased behavior Dose dependent	ED 50 = 129 mg/kg					Qiu et al 2007[94]
35.	Orofacial formalin test in the mouse: a behavioral model for studying physiology and modulation of trigeminal pain	Formalin test	Formalin	Saline	Rat/M	Decreased behavior Dose dependent		25–200				Luccarini et al 2006[125]
36.	Analgesic effects of nonsteroidal anti-inflammatory drugs, APAP, and morphine	Von Frey	Bone cancer pain model	Methylcellulose 0.5% solution	Mice/M	Increased threshold	300					Saito et al 2005[78]
37.	Spinal 5-HT1A influence nociceptive processing and effects of APAP, venlafaxine	Formalin test Paw pressure	IT administration of substance P	Saline containing 0.02 g/mL trisodium citrate	Rat/M	Decreased behavior Normalized threshold			50–300			Bonnefont et al 2005[149]

#	Title	Test	Treatment	Vehicle	Species/Sex	Effect	Dose	Reference
38.	Effect of aspirin and APAP on proinflammatory cytokine-induced pain	Scratch biting episodes	IT TNF-α, IL-β or IFN-γ	20% DMSO	Mice/M	Decreased behavior Dose dependent	100–300	Kwon et al 2005[79]
39.	IPLT APAP does not act locally	Formalin test	Formalin	Saline	Rat/M	Decreased behavior phase I	10–20 (IPLT)	Bonnefont et al 2003[82]
		Formalin test	Formalin	Saline	Rat/M	Decreased behavior phase 1/2 Dose dependent	100–400	
		Formalin test	Formalin	Saline	Rat/M	Decreased behavior Phase 1/2 Dose dependent	100–300	
		Formalin test	Formalin	Saline	Rat/M	Decreased behavior Phase 2	200	
40.	Time course of progression of allodynia and efficacy of analgesics	Von Frey Hot plate Vocal	Freund's adjuvant	Suspended in 0.5% methylcellulose	Rat/M	No change	100–600	Nagakura et al 2003[83]
41.	APAP exerts a spinal, tropisetron-reversible effect	Von Frey	Carrag treated	Trisodic citrate, propacetamol	Rat/M	Normalized threshold Dose -dependent	100–300	Alloui et al 2002[143]
		Von Frey	Carrag treated	Trisodic citrate, propacetamol	Rat/M	Normalized threshold Dose dependent	50–200	
42.	Antinociceptive profiles of aspirin and APAP in formalin, substance P and glutamate pain models	Formalin test	Formalin	20% DMSO	Mice/M	Decreased behavior Dose dependent	10–300	Choi et al 2001[85]
		Licking/biting	Intrathecal Substance P Glutamate pain	20% DMSO	Mice/M	Decreased behavior Dose dependent	10–300	

(*Continued*)

Table 2 (Continued).

	Study	Test Model	Nociceptive State	Vehicle	Species Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/ IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
43.	Antinociceptive synergy between spinal APAP and phentolamine	Abdominal irritant test	IP acetylcholine bromide	5% ethanol/water	Mice/M	Decreased behavior				137		Raffa et al 2001[144]
44.	"Self-synergistic" spinal/supraspinal antinociception produced by APAP	Abdominal irritant test	Injected IP with acetylcholine bromide	5% ethanol/water	Mice/M	Decreased behavior				137		Raffa et al 2000[154]
		Abdominal irritant test	Injected IP with acetylcholine bromide	5% ethanol/water	Mice/M	Decreased behavior					45–150	
45.	Phenacetin, APAP and dipyrone: analgesic and rewarding effects	Formalin test	Formalin	Ethanol/water/10% Tween	Rat/M	Decreased behavior Phase 2 Dose dependent		25–400,				Abbott and Hellemans 2000[126]
		Formalin test	Formalin	Ethanol/water/10% Tween	Rat/M	Decreased behavior, low > high dose		5–100 µg (IPLT)				
		Formalin test	Formalin	Ethanol/water/10% Tween	Rat/M	Decreased behavior, phase 2 No dose dependency					3–50	
46.	NitroAPAP exhibits anti-inflammatory and anti-nociceptive activity	Von Frey	Carrag hind paw	Saline	Mice/M	Normalized threshold Dose-dependent		25–100				Al-Swayeh et al 2000[86]
		Abdominal irritant test	Acetic acid induced abdominal constrict	Saline	Mice/M	Normalized behavior Dose dependent	25–100					
47.	Potentiation of APAP plus morphine involves 5-HT system	Formalin test	Formalin	12.5% of 1,2-propanediol in saline	Rat/M	Decreased behavior Phase 1/2		100				Sandrini et al 1999[118]

No.	Study	Test	Model/Stimulus	Vehicle	Species/Sex	Result	Dose	Reference
48.	APAP is associated with changes in the serotonergic system in the rat brain	Formalin test	Formalin	12.5% of 1,2-propanediol in saline	Rat/M	Decreased behavior	200–400	Pini et al 1996[128]
49.	The dose-related effects of APAP on hyperalgesia and nociception in the rat	Von Frey Paw pressure	IPLT Brewer's yeast	0.25% methocel in 0.9% NaCl	Rat/M	Normalized threshold Dose dependent	25–100	Bianchi et al 1996[96]
50.	Central antinociceptive effects of non-steroidal anti-inflammatory drugs and APAP	Biting, scratch licking	Intrathecal SP, NMDA, AMPA	Saline	Rat/M	Decreased behavior	0.01–10	Björkman 1995[129]
51.	APAP blocks spinal hyperalgesia induced by NMDA and substance P	Biting, scratch licking	Intrathecal SP, NMDA, AMPA	Saline	Rat/M	Decreased behavior	200	Björkman et al 1994[130]
52.	Morphine, nefopam and APAP	Formalin test	Formalin	12.5% of 1,2-propane-diol in 0.9% sterile saline	Rat/N/A (Naked mole rat)	Decreased behavior Phase 2, not phase 1	200–400	Kanui et al 1993[131]
53.	Intrathecal APAP on visceral noxious stimulation in rabbits	Visceral	Colonic distension Cutaneous electrical	N/R	Rabbit/M	Colonic: increased thresholds Electrical increased threshold Dose dependent	0.5–5 mg	Jensen et al 1992[145]
		Visceral	Colonic distension	N/R	Rabbit/M	Colonic No change Electrical No change	10–50	
54.	APAP plasma levels and analgesic effect	Impairment	Joint uric acid	Saline	Rat/M	Increased threshold Dose dependent	178–562	Granados-sato et al 1992[88]

(*Continued*)

Table 2 (Continued).

Study	Test Model	Nociceptive State	Vehicle	Species Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/ IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
55. Spinal nonsteroidal anti-inflammatory agents	Formalin test	Formalin	5% ethanol/distilled water	Rat/M	Decreased behavior (phase 2) Dose dependent				163–405		Malmberg and Yaksh 1992[132]
56. Anti-inflammatory effects of a low dose of APAP following surgery	Applied pressure	Postoperative pain	NA	Dog/M and M	Normalized threshold	500					Mburu 1991[97]
57. APAP effect is partly dependent on spinal 5-HT systems	Formalin test	Formalin	5% ethanol/distilled water	Rat/M	Normalized behavior		400				Tjølsen et al 1991[133]
58. Acetylsalicylic acid, APAP and morphine inhibit behavioural responses to substance P or capsaicin	Biting, licking and scratch	Intrathecal SubsP/ Capsaicin	12.5% 1,2-propanediol/ saline	Mice/M	Decreased behavior Dose dependent		300–400				Hunskaar et al 1985[136]
59. Aspirin, mefenamic acid, dihydrocodeine, dextropropoxyphene and APAP on respiration and prostaglandin biosynthesis	Writhing test	Acetic acid	1% tragacanth and saline	Rat/M	Decreased behavior	213					Sewell et al 1984[99]
60. Analgesic drugs in chronic inflammatory pain: possible central analgesic action of NSAIDs	Electrical stimulus Vocalize	Heat-killed adjuvant	Saline	Rat/M	Increased threshold Dose dependent	50–400				25–400	Okuyama and Aihara 1984[89]
	Electrical stimulus Vocalize	Heat-killed adjuvant	Saline	Rat/M	Increased threshold Dose dependent					25–400	
61. Aspirin/APAP on naloxone potency induced by morphine	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior		10 or 20				Wong et al 1980[160]
62. Butorphanol and APAP combination	Writhing test	Acetic acid	Saline	Mice/M	Decreased behavior Dose dependent	137–171					Pircio et al 1978[100]

No.	Description								Reference
63.	Central/peripheral action of aspirin-like drugs	Paw pressure	Carrag Hind paw	Saline	Mice/M	Increased threshold Dose dependent	100–400		Ferreira et al 1978[135]
		Paw pressure	Carrag Hind paw	Saline	Mice/M	Increased threshold Dose dependent	50–400 µg (IPLT)	50–400 µg	
		Paw pressure	Carrag Hind paw	Saline	Mice/M	Increased threshold Dose dependent			
64.	Comparison of analgesic and anti-inflammatory activities of aspirin, phenacetin and APAP in rodents	Paw pressure	IPLT Trypsin Hind paw	N/A	Rat/NA	No change	ED 50 > 360 mg/kg		Vinegar et al 1976[102]
		Paw pressure	IPLT Kaolin Hind paw	N/A	Rat/NA	Increased threshold Dose dependent	ED 50 = 305 mg/kg		
		Paw pressure	IPLT Carrag Hind paw	N/A	Rat/NA	Increased threshold Dose dependent	ED 50 = 110 mg/kg		
		Paw pressure	Acetic acid	N/A	Rat/NA	Increased threshold Dose dependent	ED 50: = 305 mg/kg		

Table 3 Preclinical Analgesic Effect of Acetaminophen by PO, IT, IP/SC, IV, IT, ICV Administration in Neuropathic Models

	Study	Test Model	Nociceptive State	Vehicle	Specie Sex (M/F)	Effect of APAP	PO mg/kg	IP/SC/IPLT mg/kg	IV mg/kg	IT µg	ICV µg	Reference
1.	Antinociception effect of acetaminophen in model of diabetic chronic constriction injury pain	Weight bearing Tail flick	Chronic constriction injury	Saline	Rat/M	Decreased Behavior Dose dependent		100–400				Munro et al 2016[138]
2.	Acetaminophen interact with morphine and tramadol analgesia for the treatment of neuropathic pain	Von Frey	Tibial neuroma transposition model	Saline	Rat/M	No change	20–1000					Shinozaki et al 2015[104]
3.	Antinociception effect of acetaminophen in model of diabetic neuropathy	Von Frey Tail flick	Streptozotocin induced diabetic model	Saline	Mice/M	Increased threshold Dose dependent	5–100					Micov et al 2015[103]
4.	Antihyperalgesia by codelivery of N-palmitoylethanolamide and APAP	Formalin test	Streptozotocin induced diabetic model	Saline	Rat/M	Decreased Behavior Dose dependent		3–300 µg (IPLT)				Déciga-Campos and Ortiz-Andrade 2015[156]
5.	Antinociceptive effect of APAP in neuropathic pain	Hot plate Von Frey Cold allodynia	Chung ligation	Saline	Rat/M	Increased threshold Dose dependent		25–300				Im et al 2012[122]
6.	Cannabinoid receptor-mediated antinociception with neuropathic spinal cord injury pain	Von Frey	Neuropathic spinal cord injury pain	Saline	Rat/M	Increased threshold		100				Hama and Sagen 2010[137]
7.	NCX-701 (nitro-APAP) and co-administration with gabapentin	Von Frey Electrical stimuli	Seltzer model	Saline	Rat/M	No change				15–960 µmol/kg		Curros-Criado and Herrero 2009[148]
8.	COX3 inhibitors not attenuate streptozotocin-induced mechanical hyperalgesia	Von Frey	Streptozotocin induced diabetic model	Saline	Rat/M	No change				1, 7 mg		Matsunaga et al 2007[147]
9.	Local antinociceptive effects of APAP and cannabinoid receptors	Von Frey Hot plate	Neuropathic pain (Seltzer)	Saline	Mice/M	Increased threshold		100 ng (IPLT)				Dani et al 2007[155]
10.	Allodynia and hyperalgesia suppression by a novel analgesic in experimental neuropathic pain	Von Frey Hot plate	Neuropathic pain (Bennett)	45% 2-cyclodex in saline	Rat/M	Increased threshold				300		Cui et al 2006[142]
11.	Attenuation of mechanical allodynia chemotherapy-induced neuropathic pain	Von Frey	Vincristine-induced neuropathic pain animal model	20% DMSO	Rat/M	Increased threshold	151–2265					Lynch et al 2004[80]

While acetaminophen is very stable at room temperature, acetaminophen degrades rapidly to p-aminophenol and undergoes oxidative modifications at elevated temperatures in the presence of oxygen.[164–166] The development of a stable supersaturated concentration of acetaminophen has been described.[60] The normal limited solubility of acetaminophen in water typically has required utilization in preclinical studies of a variety of vehicles and additive to increase solubility or wettability including dimethyl sulfoxide (DMSO), dimethylformamide (DMF), propylene glycol (PG), ethanol, Cremophor and Tween 20 with varying degrees of biological compatibility and typically with poorly described pharmacokinetics. Control data, baseline responses were typically examined with the respective vehicle.[167]

Acute Nociception

The acute application of thermal or mechanical stimulus above a threshold intensity will evoke a homotopic withdrawal or escape response (eg, stimulation of the left hind paw will result in a withdrawal of the left hind paw). The effects of APAP delivered by various routes are summarized in Table 1.

Thermal
Behavioral Model
Thermal responsivity may be assessed by applying a thermal stimulus to the body surface and assessing the latency to withdraw the stimulated part or escape. Application of heat to the rodent tail leads to a "tail flick", a model with a strong reflex component.[168] Withdrawal of the hind paw is typically assessed by the placing of the animal on a uniformly heated surface (hot plate) maintained at a surface temperature typically between 48* and 55 °C with the measured end point being latency to a licking of the hind paw or a jumping from the surface.[169] An alternate model, referred to as the Hargreaves model, employs focusing a light source under one or the other hind paws of an animal placed on a glass plate, with the latency to withdrawal of that paw being the measured end point.[170] These thermal models are considered to reflect the acute activation of high threshold nociceptive afferents.[171]

Drug Effect
Significant increases in hind paw thermal escape latencies over baseline were observed over a range of dose 500–850 mg/kg in the rat or mouse.[68,73,76,77] Tail flick latencies were significantly elevated in mice after PO administration of acetaminophen (400 mg/kg).[66] Orally administered acetaminophen inhibited tail flick in a concentration-dependent manner (97–772 mg/kg).[98] IT acetaminophen (50 and 100 μg/rat) showed significant elevations in hind paw escape latencies on the hot plate test.[74,141]

Mechanical
Behavioral Model
Mechanical compression of the paw is accomplished by a progressively increasing pressure progressively applied through a blunt probe to the paw situated on a non-compliant surface. This model referred to as the Randall–Selitto test is a classical way to measure mechanical thresholds.[172] The pressure which leads to hind paw withdrawal is the measured end point. In this case, the threshold response generated by the blunt stimulus surface is considered to be mediated by high threshold nociceptors lying in the muscle and bone of the compressed tissue.[171] A variant stimulus involves the application of a small diameter probe (von Frey hairs) to the plantar surface and the stimulus resulting in withdrawal is considered to be the threshold. In the absence of inflammation it is considered that the withdrawal, in contrast to the Randall–Selitto paradigm, is mediated by the activation of superficial terminals of high threshold cutaneous afferents.[172]

Drug Effect
Pressures leading to paw withdrawal were significantly elevated in mice and rats with acetaminophen in doses in the range of 200–800 mg/kg.[76,81,84,87,173] Increases in thresholds were found in rats at 200 mg/kg.[73] IV acetaminophen (22–724 mg/kg) did not alter by mechanical stimulation thresholds in rats,[150] and IV acetaminophen (50–300 mg/kg) resulted in a dose dependent elevation in the compression required to produce a withdrawal response.[87,149] Similar results were observed in rats after a single IV dose of acetaminophen (200 mg/kg).[151] IT acetaminophen (100 or 200 μg/rat) produced a significant increase in baseline paw compression thresholds,[87] a finding confirmed at lower IT acetaminophen doses (50 and 100 μg/rat).[141]

Electrical Stimulation
Behavioral Model
An electrical stimulus may be applied through subcutaneous electrodes (typically in the paw). The threshold electrical stimulus which evokes one of several specified responses (vocalization, flinch, escape) is the measured variable.[171]

Drug Effect

The stimulus evoked vocalization was reduced by PO (50,400 mg/kg) and ICV (50–400 µg) acetaminophen in both normal and adjuvant arthritic rats. The equipotent doses were less in the inflamed than the normal rat.[89] ICV administration of acetaminophen at 25–400 µg dose-dependently inhibited the withdrawal initiated by hind paw stimulation electrical stimulation in both normal rats and adjuvant arthritic rats.[89] Consistent with these effects of acetaminophen, experiments were carried out on rats under urethane anesthesia in which neuraxial activation was elicited by supramaximal electrical stimulation of sural nociceptive afferents and the activation of single neurons in the dorsomedial part of the ventral nucleus (VDM) of the thalamus was observed. IV acetaminophen (5–25 mg/kg) reduced nociceptive-evoked activation of these neuron in the VDM thalamus.[174] ICV administration of acetaminophen displayed potent analgesic actions in rats.[89]

Facilitated States of Nociceptive Processing

A common observation is that certain interventions may lead to an enhanced response to mildly noxious stimuli (hyperalgesia) or a significant pain response to an otherwise innocuous stimulus (allodynia). In the latter case, the common end point is the behavioral response to a low intensity tactile stimulus (as applied through application of von Frey hairs) and hence referred to as tactile allodynia. These facilitated states may occur as a result of direct central sensitization (as with an intrathecal or neuraxial treatment) or after tissue inflammation or nerve injury.

Neuraxially Evoked Spontaneous and Facilitated Behavioral Pain States
Spontaneous Behaviors

Behavioral Model. The intrathecal (spinal) delivery of a variety of afferent transmitters (eg, substance P and glutamate) and pro-inflammatory cytokines (TNF-α, IL-β or IFN-γ) will initiate acute pain behaviors as biting of the body surface, agitation or vocalization as well as robust facilitated states. The behavioral relevance of these facilitated states is evidenced by an increased sensitivity to mechanical (touch) and thermal stimulation. As this enhanced responsiveness is initiated by an action within the spinal cord, systemic drugs blocking that hyperalgesia are considered to reflect their central effect upon neuraxial mechanisms of nociceptive processing.

Drug Effect. Biting and scratching in the rat after intrathecal substance P and glutamate and the response time were reduced in a dose dependent fashion by PO acetaminophen (10–300 mg/kg).[85] The scratching and biting episodes evoked in the mice by IT TNF-α, IL-β or IFN-γ were reduced in a dose dependent fashion by PO acetaminophen (100–300 mg/kg).[79]

Facilitated Pain States

Behavioral Model. The intrathecal delivery of agents such as substance P will evoke an increased response to an otherwise innocuous or mildly aversive stimulus.

Drug Effect. Thermal hyperalgesia evoked by IT substance P examined in rats was significantly reduced after PO acetaminophen (30–300 mg/kg).[74] These results support the assertion that a component of the effects of systemic acetaminophen reflect an effect upon neuraxial nociceptive processing.

Inflammation Induced Facilitated States in Somatic Tissues

Generation of inflammation in soft tissue and joints routinely leads to a hyperalgesic state. This enhanced responsiveness reflects the appearance of a variety of proinflammatory products in the local injury milieu. These products, often acting through eponymous receptors expressed on the terminals of the primary afferent, serve to depolarize the terminal and through the activation of local kinases enhance the sensitivity of the terminal to subsequent stimulation.[175,176] These changes lead to ongoing afferent traffic, which leads to the initiation of a state of spinal sensitization. Such a state leads to an enhanced input–output function of the dorsal horn and a state of spinal facilitation.[177] This scenario is common to virtually all of the inflammatory motifs in soft tissue and joint.

Local Inflammation and Somatic Pain

Behavioral Model. Intraplantar or intra-articular injections of carrageenan, uric acid or injections of adjuvants such as complete Freund's adjuvant (CFA) result in a robust ipsilateral (if local) or bilateral, if systemic, inflammation and an associated thermal and mechanical hyperalgesia and tactile allodynia.

Drug Effect. The hyperpathia, but not the inflammation, is significantly reduced by PO acetaminophen.[8] In rats rendered hyperalgesic with adjuvant arthritis, PO acetaminophen (50–

400 mg/kg) attenuated the withdrawal response otherwise evoked by stimulation of electrical stimulation in adjuvant arthritic rats.[89] In rats rendered arthritic with intra-articular injection of uric acid in the knee, PO acetaminophen (178–562 mg/kg) resulted in a significant analgesic effect, quantified as the recovery of a functionality index.[88] On the other hand, PO acetaminophen (100–600 mg/kg) had only modest effects in the CFA-induced arthritic rat model, as measured by tactile or thermal escape latencies.[83,104] IV acetaminophen (100–300 mg/kg) showed a significant reduction in the tactile with no change in paw swelling, for about 60 minutes compared to the control (vehicle). After ICV delivery, acetaminophen produced a dose dependent (50–400 µg) reduction in the hyperalgesia evoked by intraplantar carrageenan in mice.[135] IT acetaminophen (50–200 µg) resulted in a dose dependent normalization of tactile allodynia in the carrageenan rat paw.[143] Intraplantar acetaminophen (50 and 400 µg) of acetaminophen in mice rendered hyperalgesic with intraplantar carrageenan reversed the hyperalgesia in a dose dependent manner in the paw pressure test.[135]

Plantar Incision

Behavioral Model. The creation of an incision of the plantar surface of the rodent is considered to mimic a postoperative state where there has been a skin incision and an underlying muscle retraction with an associated inflammation secondary to the wounding.[178]

Drug Effect. PO acetaminophen resulted in a robust dose dependent analgesic effect in mice (200–600 mg/kg)[72] and rats (30–180 mg/kg) after plantar incision.[69]

Formalin Evoked Flinching

Behavioral Model. Injection of a small volume of formalin into the dorsum of the rodent paw results in a biphasic flinching. The first phase is considered to reflect behavior evoked by the acute afferent drive from the injected paw while the second phase represents a response mediated by the low-level input arising from the injected paw and a state of spinal facilitation initiated by the first conditioning barrage.[179]

Drug Effect. Systemic acetaminophen typically results in a robust dose dependent suppression of phase II formalin and to a lesser degree phase I in mice (10–300 mg/kg)[73] (60) and rats (100–400 mg/kg)[82] but[71] IV acetaminophen shows analgesic effect in phase I only at high concentration (300 mg/kg) in rats. Phase II shows analgesic effects

at doses of 100–300 mg/kg.[82,149] ICV acetaminophen had no effect upon phase I but significantly reversed phase II in rats.[126] IT administration of acetaminophen (4.5–45 µg) in the rat was observed to be effective in phase II.[132] IPL acetaminophen (5–200 µg) had no effect upon either phase I or phase II formalin flinching in the rat formalin test in rats.[82,126]

Inflammation Induced Facilitated States in Visceral Tissues

Mechanical distention of hollow organs, eg colon/bladder, application of irritants to the peritoneal cavity or the hollow organs, will lead in a pressure dependent fashion to ongoing pain behavior characterized by vocalization, autonomic responses increased abdominal muscle tone and pressing of the abdomen against the floor of the chamber. Such inflammation will lead to a sensitization of the system such that even minor mechanical stimulation will lead to enhanced responses.[138,180]

Peritoneal Irritants

Behavioral Model. Delivery of irritants such as dilute acetic acid, capsaicin or phenyl benzoquinone into the peritoneal space will result in abdominal constriction in mice and rats,[86,107,121] reflecting activation of polymodal nociceptive afferent innervating the peritoneal wall. Distention of hollow organs will yield similar somatomotor response, and the threshold for generating these responses is lowered in the face of colonic inflammation.[181]

Drug Effect. IP acetaminophen delivered in mice treated with IP acetic acid (ED 50 = 49.5 mg/kg) significantly reduced abdominal constriction in a dose dependent fashion.[121] ICV acetaminophen in mice (45–150 µg) suppressed the abdominal constriction response generated by IP acetylcholine.[154] IT acetaminophen in mice produced a dose dependent antinociceptive activity in the acetic acid-induced writhing model.[121,154] In rabbits, intestinal distension of the distal colon led to a somatic-affective pain response that was reduced in a dose dependent fashion by IT acetaminophen (500–5000 µg).[145]

Bladder Irritants

Behavioral Model. Acute inflammation of the bladder generated by intra-vesicular installation of an irritant such as cyclophosphamide, or a bacterial infection, leads to an

ongoing biting and scratching and a hyperalgesic state with a robust referred somatic pain component.[182] This hyperpathic state is mediated by a significant in-migration of inflammatory cells and degranulation of intrinsic mast cells with an associated release of proinflammatory cytokines. Over extended periods, sprouting of bladder afferents is observed and there is a hypertrophy of the urothelium. In the dorsal horns there is an associated activation of astrocyte and microglia,[182,238] and PO acetaminophen (45–450 mg/kg). A model of bladder inflammation-induced hyperalgesia displayed no analgesic effect.[75]

Bone Cancer Pain

Behavioral Model. Bone cancer models typically employ placement of syngeneic cancer cells into the marrow of either the femur or the tibia.[183] These osteolytic sarcoma cells initiate remodeling of the bone, a significant sprouting of polymodal nociceptors into the intramedullary environment and an associated reorganization of the spinal dorsal horn that provides the sensory to innervation of the cancerous bone and this leading to a neurochemical profile resembling a robust neuropathic pain state.[184]

Drug Effect. PO acetaminophen (300 mg/kg) attenuated the tactile allodynia otherwise noted in the mouse bone cancer pain model.[78]

Nerve Injury Induced Facilitated States
Mononeuropathies

Behavioral Model. These nerve injuries reflect an injury restricted to a nerve trunk or its distribution (eg, L5 ligation (spinal nerve ligation – Chung model), hemiligation of the sciatic nerve (Seltzer model), or loose ligatures placed around the sciatic nerve (chronic compression injury – Bennett model), or ligation of the distal branches of the sciatic (spared nerve injury)). Commonly, rats or mice with these interventions will display a robust tactile and cold allodynia. The underlying mechanism of this hyperpathic state reflects upon the development of neuromas at the site of injury and reactive changes in the dorsal root ganglion of the injured nerve, leading to the development of ongoing afferent traffic from the neuroma and the DRG. These events are accompanied by prominent activation of dorsal horn neurons and glia.[137,185]

Drug Effect. IP acetaminophen (25–300 mg/kg) dose-dependently, suppressed induced pain in thermal

hyperalgesia, mechanical, and cold allodynia in L5 ligated rats.[122] Neither IV not IT acetaminophen (2.3–145 mg/kg) significantly reversed the allodynia otherwise observed in the nerve ligated rat.[148] Of note, IPLT acetaminophen in the rat Seltzer model ligation neuropathy dose-dependently decreased mechanical allodynia and lowered nociceptive scores associated with hyperalgesia testing[155] (see Table 3).

Polyneuropathy

Behavioral Effect. Clinical syndromes such as diabetes and chemotherapeutics can give rise to a distal-symmetric sensory neuropathy with sensory loss, paresthesia and dysesthesia, and is only incompletely reversible.[186] Painful diabetic neuropathy is characterized by spontaneous tingling, lancinating, pain that frequently occurs in conjunction with touch-evoked pain and numbness.[187] The mechanisms underlying these events are multifocal and include changes in peripheral terminals and DRG morphology and trophic changes in dorsal horn connectivity leading to a facilitated response to otherwise innocuous stimuli.[188,189]

Drug Effect. PO acetaminophen (151–2265 mg/kg) showed an antinociceptive effect on the vincristine-induced neuropathic pain rat model.[80] In addition, oral administration of acetaminophen (5–100 mg/kg) has an antinociceptive effect on the streptozotocin induced diabetic model.[103]

Preclinical to Clinical Dose Comparisons

The focus in this review has been characterizing the profile of acetaminophen based on published literature. An important point of consideration related to whether these observed effects of acetaminophen dosing are relevant to the human conditions. The issue of dose comparability across species has particular relevance in drug development. In the present case it is evident that dosing in the rodent reveals activity at substantial dose levels. A nominal dose level that is common in those studies where acetaminophen was shown to be efficacious is on the order of 300 mg/kg which would appear substantial. It is beyond the scope of this review, but we would note that there is significant support for the assertion that allometric scaling across species might be more closely approximated through comparisons based on body surface area.[190,191]

Thus, the typical systemic acetaminophen dose in the rat is on the order of 300 mg/kg. Converting the rat mg/kg dose to a human mg/kg dose on the basis of a body surface area conversion of the rat dose where the rat dose is divided by the body surface area scaling factor (6.2) yields a predicted human dose of 48 mg/kg.[192] It is noteworthy that the approved IV dose in humans is 4000 mg/70 kg = 57 mg/kg. Such scaling has been shown to be useful for drawing first order comparisons across species.

Mechanisms of Acetaminophen Action
Metabolism
Acetaminophen (pKa: 9.5) at physiological pH is essentially neutral and is rapidly absorbed after PO delivery. In humans, blood half-life ranges from 1.5–3 h at therapeutic half-lives to 8 h at dose up to 4 g/day.[193] Similarly, in the rat half-lives are dose dependent and range from approximately 15 min at low doses and up to 120 min after high (therapeutic) doses (300 mg/kg).[194] In all species, elimination largely occurs in the liver, where the majority of the drug undergoes conjugation catalyzed by a variety of transferases and then excretion in urine. The formation of toxic metabolites, such as N-acetyl-p-benzoquinone imine (NAPQI), occurs secondary to this sequence.[195] More recently it has been shown that acetaminophen, following deacetylation to its primary amine (4-aminophenol), is conjugated with arachidonic acid through the actions of fatty acid amide hydrolase in the brain and spinal cord to form a variety of biologically active molecules.[196] This will be discussed further below.

Mechanisms of Analgesic Action
A number of mechanisms have been hypothesized for the actions of acetaminophen in modulating pain transmission. Broadly speaking, these effects can be considered in terms of an effect of the drug itself or an action mediated by a metabolite. In the human, acetaminophen is known to be metabolized in the liver into p-aminophenol and then converted to N-(4-hydroxyphenyl)-arachidonamide (AM404) via fatty acid amide hydrolase (FAAH) and into N-acetyl-p-benzoquinone imine (NAPQI) via cytochrome P450 (CYP) enzymes.[196–198] While this conversion typically occurs in the liver, recent work has shown that acetaminophen can be converted into the AM404 metabolite within the neuraxis.[61]

Membrane Target
Measurement of acetaminophen levels in the brain revealed significant tissue/blood ratios that were essentially the same across the brain.[199] Quantitative autoradiography has failed to demonstrate that acetaminophen binds specifically to any area of the murine brain or spinal cord.[200] Competition studies with a variety of targets failed to show interactions with a variety of monoamine receptors or opioid receptors.[201]

Cyclooxygenase Inhibition
Prostaglandins play an important central and peripheral role in sensitizing systems which are involved in nociceptive transmission.[202] The prostanoids are formed by the actions of constitutive (COX1) and inducible forms (COX2) of cyclooxygenase. Common non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid, or ibuprofen, block activity of these isoforms.[203] While a literature has suggested acetaminophen may inhibit cyclooxygenase, minimal evidence supports a direct effect.[147,204–206] This assertion is consistent with the side effect profile for acetaminophen, which does not include effects commonly associated with COX1 inhibition known on the gastrointestinal tract, platelet activation, and kidney functions and with COX2 on cardiovascular, gastrointestinal and kidney function.[207,208] One explanation for this distinction is that acetaminophen may act as a CNS-COX inhibitor, whereas the other COX inhibitors act both centrally and peripherally. In this regard, acetaminophen acts to interfere with the peroxidase activity of COX2. This interaction is particularly manifest when the cellular environment is low in arachidonic acid and peroxides and may reflect the "central" effects where constitutively expressed COX2 at the spinal level plays a principal role in initiating facilitated states,[209,210] and why it appears to display less activity in inflamed tissues (where peroxides and arachidonic acid are highly concentrated). In this regard, acetaminophen inhibits conversion of arachidonic acid to Prostaglandin (PG) E2, PGF2 and thromboxane-A2 in microglia exposed to lipopolysaccharide[211] at 3-fold lower concentrations in microglia than in peripheral macrophages. A further point is that recent work has suggested that AM404, the downstream metabolite of acetaminophen, can inhibit isolated cyclooxygenase (COX)-1 and COX-2 enzymes and prostaglandin synthesis in macrophage cultures and in brain slices.[196,212,213] AM404 is also a potent inhibitor of T cell activation and inhibited TNF gene transcription and protein synthesis,

thereby regulating activation of several transcription factors including nuclear factor-kappa B (NF-κB).[214]

TRP Signaling

The acetaminophen metabolite AM404 can bind to the vanilloid binding site and activate the transient receptor potential vanilloid 1(TRPV1) channel in dorsal root ganglia (DRG) neurons.[215,216] Moreover, the antinociceptive effects of acetaminophen are absent in TRPV(1) knockout mice.[73] Activation of TRPV1 can lead to inhibition of downstream excitatory T-type calcium channels[70]. NAPQI, the toxic liver metabolite of acetaminophen, has been shown to activate TRPV1.[216] Further, acetaminophen and the metabolite NAPQI sensitizes and activates transient receptor potential ankyrin 1 (TRPA1) slowly but directly by interacting with distinct intracellular cysteine residues.[217,218] This TRPA1 activation paradoxically serves to reduce voltage-gated calcium and sodium currents in DRG neurons, while intrathecal acetaminophen and NAPQI produced anti-nociception that was absent in TRPA1 KO mice.[141]

An important caveat is that the activation of TRP receptors is generally associated with adverse events including activation of pain signaling. It should be noted that NAPQI will result in neurogenic inflammation, which indeed suggest that such metabolites would serve to activate small peptidergic nociceptive afferents.[218] If the actions of acetaminophen action are indeed mediated through such signaling, then one would similarly anticipate adverse pain components, which to date have not been noted.

Cannabinoid Signaling

The effects of acetaminophen are reportedly blocked by Cannabinoid (CB) 1 receptor antagonism and by CB1$^{-/-}$ transgenic mice.[77,95] This effect is believed to reflect the fact that acetaminophen, by its metabolite AM404, also exerts a possible effect on the endocannabinoid system by acting as a ligand at cannabinoid CB1 receptors, or as an inhibitor of anandamide uptake, an endogenous agonist for CB1 receptors.[219,220]

Anandamide (AEA) is an endocannabinoid that activates CB1.[221,222] AEA is degraded by fatty acid amide hydrolase (FAAH), the enzyme that metabolizes acetaminophen into AM404. Thus, AM404 could act as a competitive inhibitor of FAAH and indirectly activate CB1. Alternatively, AM404 has been shown to inhibit FAAH-like anandamide transporter, a membrane-bound variant of

FAAH lacking its hydrolase activity, which is present on neurons and glia and is thought to aid in intracellular transport of AEA.[223] By inhibiting FAAH, AM404 could increase levels of AEA available in the synaptic cleft. The insensitivity of CB1 knockout mice and rats pretreated with the CB1 antagonist (AM251) to acetaminophen supports the involvement of CB1 in the mechanism of acetaminophen.[95,224] AEA has been reported to activate CB1 receptors on cultured DRG neurons.[225,226]

As with the hypothesis that acetaminophen alters COX formation while the molecule is not associated with many biological effects associated with COX inhibition, cannabinoids have been shown to have both robust negative and positive reinforcing properties and to produce dependence.[227,228] In marked contrast, it is of note that in animals[229] and in humans, acetaminophen alone has no established positive or adverse rewarding properties or diversion potential.[230]

Serotonin Signaling

Several lines of investigation have suggested that acetaminophen may exert its effects through serotonin transmission. As acetaminophen displays little or no affinity for 5-HT receptors, or for neuronal reuptake sites,[201] these actions have been argued to reflect an indirect effect notably by a brainstem action altering activity in bulbospinal serotonergic projections. Thus, destruction of bulbospinal 5-HT projections is reported to attenuate the antinociceptive action of acetaminophen.[128,133] An important issue is whether the effects of acetaminophen reflect an increase or a decrease in the activation of the bulbospinal projection. It has been reported that acetaminophen increases 5-HT levels in rat brain.[128] Such an increase may be interpreted as having increased serotonin to be released. It is equally consistent with the notion that acetaminophen is decreasing terminal release leading to accumulation. In this regard, bulbospinal serotonin projections have been said to facilitate dorsal horn processing leading to hyperalgesia, likely mediated through an excitatory 5-HT receptor such as 5-HT3.[231] Others have argued that activation of the descending pathway may lead to a block of dorsal processing by activating a G protein coupled inhibitory 5-HT receptor (eg, 5-HT1 isotypes). Other possibilities are that serotonin, through an excitatory receptor (5-HT2, 3, 7), may activate an inhibitory interneuron such as GABA[232] or encephalin,[233] as suggested by the report of naloxone sensitivity of acetaminophen antinociception to naloxone, an opioid.[234] In the case of acetaminophen, the literature is

complex and controversial. Thus, IT 5-HT1B, 5-HT2A/C and 5-HT7 antagonism has been reported to reduce acetaminophen actions.[72,151] IT 5-HT3 antagonism has been reported to inhibit the antinociceptive effects of acetaminophen in various pain models[87,143,151,173] and specific knock down with 5-HT3 receptor antisense did not alter acetaminophen antinociception.[173] In humans, pain reports by median nerve stimulation were significantly reduced by acetaminophen, and this effect was reduced vs control by 5-HT receptor antagonists.[235]

Concluding Comments

"The current evidence supports the assertion that acetaminophen has a behaviorally relevant, but delimited, analgesic effect in humans and in a variety of preclinical models." While mechanisms of action remain arguably controversial, the effects of acetaminophen on centrally evoked facilitated states (as after IT sP) and on aversive electrical stimulation strongly support the conclusion that at least a component of the effects of systemic acetaminophen reflect an effect upon CNS nociceptive processing that engage both spinal and supraspinal systems in humans and animals. The long history of acetaminophen in showing a highly desirable side effect profile and the absence of abuse potential is consistent with its wide use as an over-the-counter medication. Though it may possess deleterious effects upon liver function with persistent high-level use, it is surprisingly well tolerated in this regard in humans. A number of proposed mechanisms exist involving serotonin, cannabinoids and TRP signaling, while these mechanisms appear interesting and relevant, they are noteworthy in the absence of effects such as rewarding potential and absent any activating effects that such actions would endow the parent compound. It is the authors' opinion that the mechanisms of this profoundly interesting compound remain to be fully understood.

Significance Statement

Acetaminophen, a drug with a long therapeutic history of utilization, has surprisingly robust effects on a variety of pain states in clinical patients and in preclinical models with a good therapeutic index. In spite of its extensive use, its mechanisms of action are yet poorly understood.

Acknowledgments

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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