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Cannabinoid receptor-mediated antinociception with acetaminophen drug combinations in rats with neuropathic spinal cord injury pain

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Abstract

Pre-clinical evidence demonstrates that neuropathic spinal cord injury (SCI) pain is maintained by a number of neurobiological mechanisms, suggesting that treatments directed at several pain-related targets may be more advantageous compared to a treatment focused on a single target. The current study evaluated the efficacy of the non-opiate analgesic acetaminophen, which has several putative analgesic mechanisms, combined with analgesic drugs used to treat neuropathic pain in a rat model of below-level neuropathic SCI pain. Following an acute compression of the mid-thoracic spinal cord, rats exhibited robust hind paw hypersensitivity to innocuous mechanical stimulation. Fifty percent antinociceptive doses of gabapentin, morphine, tramadol or memantine were combined with an ineffective dose of acetaminophen; acetaminophen alone was not antinociceptive. The combination of acetaminophen with either tramadol or memantine resulted in an additive antinociceptive effect. Acetaminophen combined with either morphine or gabapentin, however, resulted in supra-additive (synergistic) efficacy. One of the analgesic mechanisms of acetaminophen is inhibiting the uptake of endocannabinoids from the extracellular space. Pre-treatment with AM251, a cannabinoid receptor subtype-1 (CB1) antagonist, significantly diminished the antinociceptive effect of the acetaminophen+gabapentin combination. Pre-treatment with AM630, a cannabinoid receptor subtype-2 (CB2) antagonist, did not have an effect on this combination. By contrast, both AM251 and AM630 reduced the efficacy of the acetaminophen+morphine combination. None of the active drugs alone were affected by either CB receptor antagonist. The results imply that modulation of the endocannabinoid system in addition to other mechanisms mediate the synergistic antinociceptive effects of acetaminophen combinations. Despite the presence of a cannabinoid mechanism, synergism was not present in all acetaminophen combinations. The combination of currently available drugs may be an appropriate option in ameliorating neuropathic SCI pain if single drug therapy is ineffective.

Keywords

allodynia; cannabinoid receptor; combination drug therapy; paracetamol; synergism

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1. Introduction

A significant percentage of spinal cord injury (SCI) patients suffer chronic pain in addition to motor function loss and visceral dysfunction (Dijkers et al., 2009; Widerstrom-Noga, 2009). Persistent pain, characterized as burning or shooting, may be experienced above or below the level of the spinal lesion and could be musculoskeletal in origin, the result of peripheral or central nervous system damage or a combination of all of these (Finnerup and Jensen, 2004). Hypersensitivity to cutaneous stimulation has also been reported in SCI patients below the level of the lesion. Similar to peripheral neuropathic pain, there are multiple converging post-injury neurological and inflammatory events mediating chronic neuropathic SCI pain (Yeziarski, 2009). The numerous neuropathological events make effective treatment of neuropathic SCI pain highly challenging.

Although novel pain-related targets are continuously being identified, the development of useful clinical treatments from these targets will take considerable time and effort. In the mean time, currently marketed drugs, such as a tricyclic antidepressants and anticonvulsants, could be used to treat SCI pain (Kroenke et al., 2009). However, drugs that show efficacy for peripheral neuropathic pain are not entirely effective in SCI pain (Finnerup and Jensen, 2004). In addition, some drugs may be contraindicated for SCI patients, since their side-effects may lead to further complication of existing health problems (Finnerup and Jensen, 2004; Ragnarsson, 1997). There is a need, then, for effective pharmacological treatments for SCI pain without the liabilities observed in currently available analgesics.

Drugs with divergent mechanisms may be combined to utilize the phenomenon of synergism with the goal of increasing efficacy beyond that of the efficacies of the constituents alone (Raffa et al., 2003; Tallarida, 2001). Reduced doses of the constituents are combined to yield an additive or a supra-additive effect. Conversely the decrease doses of the constituents would lead to reduced incidence of adverse side-effects. The non-opiate analgesic acetaminophen has been used as an adjunct with opiates for the treatment of moderate to severe pain, decreasing total consumption of opiates and their associated side-effects (Christo and Mazloomdoost, 2008). Some SCI patients have reported modest temporary pain relief with acetaminophen alone (Cardenas and Jensen, 2006; Widerstrom-Noga and Turk, 2003). Acetaminophen lacks the adverse side-effects observed with centrally acting drugs like anticonvulsants, which could in part explain the substantial minority of SCI patients who are currently taking this drug for pain (Cardenas and Jensen, 2006). Thus, acetaminophen could be combined with other pain drugs to treat neuropathic SCI pain.

Chronic SCI pain patients have reported significant analgesia with exogenous cannabinoids such as those found in *Cannabis sativa* (Cardenas and Jensen, 2006; Karst et al., 2003). The use of exogenous cannabinoids, however, for medical purposes is socially controversial. Alternatively, increasing endogenous cannabinoids such as *N*-acyl ethanolamine (anandamide) leads to analgesia which is mediated via activation of the cannabinoid-1 (CB1) receptor (Bertolini et al., 2006; Hohmann, 2002; Lichtman et al., 2004; Russo et al., 2007b). Increased extracellular endocannabinoids may also be obtained by inhibiting their enzymatic degradation or cellular uptake. Coincidentally, one of acetaminophen's analgesic mode of action is to increase CNS endocannabinoid levels by blocking cellular uptake of anandamide (Bertolini et al., 2006; Hogestatt et al., 2005). Acetaminophen could be used an alternate means of modulating the endocannabinoid system for pain relief.

One analgesic drug with multiple mechanisms that is currently marketed for short-term acute pain management is Ultracet®, an acetaminophen and tramadol combination. Tramadol alone has at least three mechanisms that could be working in concert: inhibition of norepinephrine and serotonin neural uptake, similar to a tricyclic antidepressant, and a metabolite with high

potency for the μ -opioid receptor (Pandita et al., 2003; Raffa et al., 1992). Furthermore, an additional cannabinoid mechanism via acetaminophen could add to the efficacy of tramadol. A possible involvement of the endocannabinoid system with an acetaminophen and tramadol combination has not been reported. Other mechanisms (e.g. μ -opioid receptor) of this combination of drugs in SCI pain have not been reported.

The current study determined whether various combinations of acetaminophen with analgesic drugs were synergistic in a rat model of neuropathic SCI pain. The test drugs have previously demonstrated antinociceptive effects in chronic pain models, including neuropathic SCI pain, and in clinical pain (De Vry et al., 2004; Finnerup and Jensen, 2004; Grande et al., 2008; Hama and Sagen, 2007c; Kroenke et al., 2009). To confirm a CB receptor-mediated effect of the drug combinations, SCI rats were treated with CB1 and CB2 receptor antagonists prior to combination treatment.

2. Materials and methods

Male Sprague-Dawley rats were used (140-160 g at the time of surgery; Harlan, IN). Experimental procedures were reviewed and approved by the University of Miami Animal Care and Use Committee and followed the recommendations of the "Guide for the Care and Use of Laboratory Animals" (National Research Council). Prior to and after surgical procedures, rats were allowed free access to food and water. Rats were housed two per cage in a room with a 12 hr. light/dark cycle.

2.1 Surgical procedure

An acute compression of the mid-thoracic spinal cord was performed as previously described. Using aseptic technique, a laminectomy was performed to expose spinal segments T₆-T₇ (Hama and Sagen, 2007c). Care was taken not to incise the dura or disturb near-by nerve roots. A micro-vascular clip (Harvard Apparatus, MA) was placed vertically around the spinal cord and allowed to compress the spinal cord for one minute. Following removal of the clip, the musculature was sutured shut and the skin closed with wound clips. Spontaneous voiding returned to these rats in 1-2 days following surgery and rats were able to feed and drink unaided.

2.2 Hind paw sensitivity to tactile stimulation

To determine cutaneous hind paw sensitivity to innocuous mechanical stimulation, the up-down method with von Frey filaments was used (Chaplan et al., 1994). Rats were placed in Plexiglas boxes resting on an elevated mesh floor. A filament was placed vertically on the plantar surface of the hind paw and gently pushed, resulting in a slight bowing of the filament. If the rat did not respond with a hind paw withdrawal from the filament, the next higher force filament was used. If the rat responded, then the lower force filament was used. A pattern of six responses was used to calculate the 50% withdrawal threshold (g). The hind paw withdrawal threshold in a sham-operated rat was 15 g.

Rats were tested four weeks after spinal cord compression. To be included in the study, the hind paw withdrawal thresholds needed to be 4 g or less. (In this study, the average inclusion rate was 8 out of 10 rats.) Following baseline threshold measurements, rats were injected with either drug or vehicle and tested once every 30 min up to 120 min post-injection.

2.3 Drugs

Drug vehicles, injection volumes, routes of administration and sources are summarized in Table 1.

The highest dose of acetaminophen (*N*-acetyl-para-aminophenol; APAP) used in combination with other drugs was 100 mg/kg, which was combined with the 50% antinociceptive dose (A_{50}) of effective drugs determined from a previous study (Hama and Sagen, 2007c). Fractions of the highest combination dose were used to construct the dose response curve. For example, the doses of the combination of APAP+gabapentin were 100+26 mg/kg, 50+13 mg/kg, 25+6.5 mg/kg and 12.5+3.25 mg/kg. The drugs were injected separately but at the same time.

In the antagonist studies, the antagonist was injected 30 min prior to the injection of the highest dose of the combination. There were four treatment groups for all antagonist studies (pre-treatment/post-treatment): vehicle/vehicle; vehicle/APAP+active drug; antagonist/vehicle; antagonist/APAP+active drug. (For active drugs combined with APAP, see Table 2.) Rats were tested 60 or 90 min after the second injection of either vehicle or combination, depending on the particular combination.

The antagonists used were: AM251 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide), AM630 (6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone) and naloxone HCl. The doses of AM251 (CB1 receptor antagonist; 3 mg/kg) and AM630 (CB2 receptor antagonist; 1 mg/kg) have been previously shown to block the effects of a cannabinoid receptor agonist in vivo and the dose of naloxone (5 mg/kg) has previously been demonstrated to block the effect of tramadol (Di Filippo et al., 2004; Hama and Sagen, 2007a, 2007b).

2.4 Statistical analysis

To plot the dose-response curve, the withdrawal thresholds following drug treatment were converted into a percent maximum possible effect (MPE %):

$$\text{MPE \%} = ((\text{Post-drug threshold} - \text{Baseline threshold}) \div (15 \text{ g} - \text{Baseline threshold})) * 100$$

In a typical case of determining synergism of a two drug combination, both drugs are active and the 50% antinociceptive doses are used to calculate the theoretical A_{50} of the combination. An isobologram is constructed by plotting the A_{50} of each drug on an x- and y-axis. The line connecting the A_{50} values is the theoretical additive line. If the experimentally determined A_{50} value falls below the line of additivity, the combination is supra-additive (synergistic) whereas if the A_{50} value falls on the line, the combination is merely additive (Tallarida et al., 1989).

However, in the current study, there is a lack of efficacy of one of the drugs, APAP, so a modified isobolar analysis was used. Synergism is still defined as a significant change of the experimentally determined potency of the combination compared to theoretically calculated potency of the combination. To calculate the theoretical additive A_{50} :

$$\text{additive } A_{50} = \text{active drug } A_{50} / P$$

where P is the proportion of the combination that is the active drug. The A_{50} of the combinations (and the 95% confidence limits) were calculated from the dose response curves of the combinations using a computer program (Tallarida and Murray, 1981). The experimentally determined and additive A_{50} were compared using a *t*-test. If experimental $A_{50} < \text{additive } A_{50}$ (at $p < 0.05$), then the combination is synergistic (Hama et al., 2001; Porreca et al., 1990; Raffa et al., 2003; Raffa et al., 2000, 2001; Tallarida, 2007). Lack of statistical significance indicated additivity.

Statistical analysis of the effects of antagonist pre-treatment on the combinations was performed using a 2-way ANOVA with Student-Newman-Keuls method for post-hoc analysis. The level of significance was $p < 0.05$.

3. Results

Four weeks after spinal cord compression, rats exhibited robust hind paw mechanical hypersensitivity. The mean ($\pm$ S.E.M.) baseline withdrawal threshold before injection was 2.2 ± 0.2 g.

3.1 Acetaminophen alone

In the current study, the highest injectable dose of APAP was 100 mg/kg, which was in a clear solution. At 60 min and 90 min post-injection of APAP alone, the MPEs were $27 \pm 8\%$ and $33 \pm 11\%$, respectively (Fig. 1A, B; $p > 0.05$ vs. vehicle).

3.2 Acetaminophen+gabapentin

The doses of APAP+gabapentin tested were 100+26 mg/kg, 50+13 mg/kg, 25+6.5 mg/kg and 12.5+3.25 mg/kg (Fig. 1A). Since peak efficacy of gabapentin alone was previously observed at 90 min post-injection, the theoretical and experimental A_{50} values were calculated at this time point (Hama and Sagen, 2007c). There was a 2.6-fold leftward shift of the APAP+gabapentin dose-response curve compared to that of gabapentin alone and the experimentally determined A_{50} of the combination was significantly less than the theoretical A_{50} (Table 2; $p < 0.05$). Therefore, combination of the two was synergistic.

Pretreatment with AM251 significantly attenuated the antinociceptive effect of APAP+gabapentin (Fig. 2A; $p < 0.05$ vs. veh/APAP+GP). No significant difference was observed between the AM251/vehicle and AM251/APAP+gabapentin treated groups ($p > 0.05$). Pretreatment with AM630 did not significantly attenuate the effect of the APAP+gabapentin combination (Fig. 2B; $p > 0.05$ vs. vehicle/APAP+GP).

3.3 Acetaminophen+memantine

The doses of APAP+memantine tested were 100+8 mg/kg, 50+4 mg/kg and 25+2 mg/kg. The experimentally determined A_{50} of the combination (at 60 min post-injection) was not significantly different from the theoretical A_{50} (Fig. 1B; $p > 0.05$). No significant shift in potency was observed between the combination and memantine alone. Therefore, combination of the two was merely additive (Table 2).

3.4 Acetaminophen+morphine

The doses of APAP+morphine tested were 100+1 mg/kg, 30+0.3 mg/kg and 10+0.1 mg/kg. Since peak efficacy of morphine alone was previously observed 60 min post-injection, the theoretical and experimental A_{50} values were calculated at this time point (Hama and Sagen, 2007c). The experimentally determined A_{50} of the combination was significantly less than the theoretical A_{50} (Fig. 1C, Table 2; $p < 0.05$). There was a 3.4-fold leftward shift in potency of APAP+morphine compared to morphine alone. Therefore, combination of the two was synergistic.

Pretreatment with AM251 significantly attenuated the effect of APAP+morphine (Fig. 3A; $p < 0.05$ vs. veh/APAP+Mor). However, a significant “residual” antinociception was still observed in AM251-pretreated rats, in that the mean withdrawal threshold was still higher than that of vehicle post-treated rats ($p < 0.05$ vs. AM251/veh). Pretreatment with AM630 partially decreased the antinociceptive effect of APAP+morphine (Fig. 3B; $p < 0.05$ vs. veh/APAP

+Mor). Similar to pretreatment with AM251, a “residual” antinociception in the AM630/APAP +morphine group was observed ($p < 0.05$ vs. AM630/veh).

3.5 Acetaminophen+ tramadol

The doses of APAP+tramadol tested were 100+20 mg/kg, 50+10 mg/kg, 25+5 and 12.5+2.5 mg/kg. A 2-fold leftward shift in potency of APAP+tramadol compared to tramadol alone was observed (Fig. 1D). However, the experimentally determined A_{50} of the combination (at 90 min post-injection) was not statistically significant from the experimentally determined A_{50} ($p > 0.05$). Therefore, combination of the two was merely additive (Table 2).

Since this combination is analogous to the analgesic drug Ultram®, further characterization using CB receptor antagonists was performed, despite a merely additive effect of the combination. The μ -opioid receptor could also be crucial to the antinociceptive effect of APAP +tramadol combination, given that tramadol's metabolite binds to the μ -opioid receptor, in this rat SCI model.

Pretreatment with AM251 significantly attenuated the effect of APAP+tramadol (Fig. 4A; $p < 0.05$ vs. veh/APAP+Tram). However, pretreatment with AM630 did not affect APAP +tramadol antinociception (Fig. 4B; $p > 0.05$). Pretreatment with naloxone significantly attenuated the effect of the APAP+tramadol combination (Fig. 5; $p < 0.05$ vs. veh/APAP +Tram).

3.6 Antagonists

Pretreatment with either AM251 (s.c., 3 mg/kg), AM630 (s.c., 1 mg/kg) or vehicle did not significantly affect withdrawal thresholds of SCI rats post-treated with vehicle (Fig. 2-4; $p > 0.05$). Pre-treatment with naloxone (s.c., 5 mg/kg) did not significantly change withdrawal thresholds of rats that were post-treated with vehicle (Fig. 5; $p > 0.05$).

It is possible that the suppressive effect of the CB receptor antagonists on the drug combinations actually decreased the efficacy of gabapentin, morphine and tramadol in place of blocking a hypothesized endocannabinoid-mediated mechanism. In a separate group of SCI rats, AM251 was injected 30 min prior to an injection of either morphine (3 mg/kg), gabapentin (100 mg/kg), or tramadol (100 mg/kg) alone. Also, AM630 was injected 30 min prior to injection of morphine (3 mg/kg) alone. Pre-treatment with the CB receptor antagonists did not significantly change the efficacy of the drugs alone ($p > 0.05$; data not shown).

4. Discussion

The current study demonstrated that combinations of APAP with either gabapentin or morphine induced a robust synergistic antinociception in rats with neuropathic SCI pain. By contrast, the combinations of either memantine or tramadol with acetaminophen were merely additive, suggesting that there may not be a generalized synergistic phenomenon with APAP combinations. The attenuation with CB receptor antagonists of the effects of the APAP combinations indicates a possible mechanism via activation of the endogenous CB system. It is possible that other analgesic drugs combined with APAP may lead to enhanced analgesia utilizing a CB receptor-mediated mechanism. To address the multiple neuropathological events that maintain chronic SCI pain, treatments addressing multiple yet complementary (synergistic) mechanisms could be useful in ameliorating pain. Furthermore, a source of such multi-mechanism treatments could be drugs that are currently available.

There are numerous pre-clinical data that point to robust changes in spinal cord ion channel function and receptor pharmacology following SCI and it is possible that these targets may to the development of novel analgesic drugs (Hulsebosch et al., 2008; Nesic et al., 2005).

However, development of orally active drugs against these targets will take considerable time and effort. By contrast, drugs that are currently marketed, which have extensive safety data, could be immediately evaluated for use in neuropathic SCI pain (Chong and Sullivan, 2007). Furthermore, clinical drugs may be combined in sub-effective doses to obtain synergism with the potential for reduced adverse side-effects. This would be especially beneficial for SCI patients in whom some drugs may aggravate existing urinary retention and gastrointestinal motility, leading to potentially life-threatening acute hypertension (autonomic dysreflexia; (Rabchevsky, 2006)).

Cannabinoid receptor agonists are antinociceptive in animal models of central as well as peripheral neuropathic pain (Hama and Sagen, 2007b; Herzberg et al., 1997). Limited numbers of clinical studies indicate that centrally-mediated pain states, such as pain arising from multiple sclerosis and SCI have reported significant pain relief with exogenous cannabinoids (Cardenas and Jensen, 2006; Karst et al., 2003; Russo et al., 2007a). A major psychoactive and analgesic component of *C. sativa* is Δ -9-tetrahydrocannabinol, the effect of which *in vivo* is attenuated by a CB1 receptor antagonist (Smith et al., 1998; Welch et al., 1998). However, access to *C. sativa* for general medical use is controversial and the centrally-mediated adverse psycho-motor effects observed with CB1 receptor agonists may not be tolerable for all patients.

In addition to activation of CB1 receptors with exogenous ligands, it is also possible to evoke analgesia by enhancing levels of endocannabinoids, by either blocking the degradative enzyme or blocking cellular uptake. Enhancement of endocannabinoid concentrations via these methods may be an alternate means of engaging CB receptors for pain relief without the adverse side-effects observed with full agonists (Piomelli et al., 2006). Compounds designed to increase endocannabinoids have been found to be efficacious in various rat chronic pain models but the effects of these compounds have yet to be evaluated in a SCI pain model (Jayamanne et al., 2006; La Rana et al., 2006).

Complex changes in CB receptor expression and endocannabinoid levels (e.g. anandamide) in the spinal cord following SCI have been reported (Garcia-Ovejero et al., 2009). A rapid increase in endocannabinoid was observed in rats following a mid-thoracic spinal contusion injury, which decreased to sham-operated levels four weeks after injury (Garcia-Ovejero et al., 2009). Observations were limited to the injury site and to the spinal tissue immediately rostral to the injury, which could be a key neuroanatomical focus mediating at-level or below-level SCI pain (Finnerup and Jensen, 2004). Four weeks post-injury, a decrease in CB1 receptor mRNA and an opposite increase in CB2 receptor mRNA were observed. The significance of these changes to below-level neuropathic SCI pain is not entirely clear, but this suggests that a loss of CB1-mediated tonic inhibition in the spinal dorsal horn, allowing abnormal activity of dorsal horn neurons. In the current study, hind paw hypersensitivity was measured as a surrogate of below-injury level pain. There is currently no data on the state of the endocannabinoid system below the injury, where abnormal lumbar dorsal horn neural activities have been reported (Wang et al., 2005; Yezierski and Park, 1993). In evaluating compounds that increase endocannabinoid levels for below-level pain, it will be important to measure endocannabinoids in lumbar spinal cord before and after compound treatment.

The mechanism of APAP's analgesic action is currently not well defined despite discovery over 100 years ago. Unlike typical nonsteroidal anti-inflammatory analgesics, APAP has no significant inhibitory activity on cyclooxygenase, thus has little analgesic effects through an anti-inflammatory pathway (Bertolini et al., 2006). In the hot plate test in rats, modest efficacy (about 45% MPE) is observed at high systemic doses (e.g. 800 mg/kg). Associated with this antinociception is an increase in CNS levels of serotonin released from brainstem serotonergic neurons, suggesting central mediation (Courade et al., 2001; Pini et al., 1997; Raffa et al., 2000). The antinociceptive effect of a high dose of APAP is attenuated with naloxone, a CB1

receptor antagonist and in CB1 receptor knockout mice, suggesting that APAP could act either directly as an opioid or CB1 receptor ligand or indirectly by increasing endogenous opioid or CB receptor ligands (Bertolini et al., 2006; Mallet et al., 2008). However, the high doses used in acute pain tests will lead to acute hepatotoxicity in the rat (see below), which could lead to a significantly altered bio-distribution of APAP and its metabolites and complicate interpretation of the pharmacodynamic data.

Another intriguing CB-related possibility is that the metabolism of APAP in brain and in dorsal root ganglia leads to the formation of *N*-(4-hydroxyphenyl)-arachidonamide (AM404), which inhibits cellular reuptake of endocannabinoids (Bertolini et al., 2006). This compound has demonstrated efficacy in neuropathic and inflammatory pain models but has no effect in acute pain models (La Rana et al., 2006). A low but measurable concentration of AM404 is found in the brain following systemic injection of 100 mg/kg APAP (Hogestatt et al., 2005). AM404 could have a significant role in the antinociceptive effect of the currently evaluated combinations.

Alternatively, the efficacy of APAP or its metabolite in acute pain and peripheral neuropathic pain could be due to activity on other pain-related targets such as the Transient Receptor Potential (TRP) receptors found on primary afferent nociceptors (Hogestatt et al., 2005). AM404, but not APAP, activates TRPV1 (TRP vanilloid subtype 1) receptors, which leads to antinociception possibly due to desensitization of nociceptors (Knotkova et al., 2008). Topical application the TRPV1 receptor agonist capsaicin attenuates at-level neuropathic SCI pain (Sandford and Benes, 2000). The importance of activating TRPV1 receptors in below-level neuropathic SCI pain is not clear. The TRPV1 receptor is upregulated in the lumbar spinal cord dorsal horn following a thoracic-level transection (Zhou et al., 2002). As opposed to an agonist, systemic injection of a TRPV1 *antagonist* ameliorated hind paw heat hyperalgesia in spinally contused rats (Rajpal et al., 2007). Further studies on the role of primary afferents below the spinal lesion should clarify the relevance of these receptors in SCI pain and whether or not nociceptor de-sensitization would significantly attenuate neuropathic SCI pain.

Although APAP itself has no effect on the cyclooxygenases that generate inflammatory prostaglandins, AM404 inhibits these enzymes (Hogestatt et al., 2005). The importance of this activity on nociception in these SCI rats probably is probably minimal since the cyclooxygenase-inhibiting drugs naproxen and rofecoxib are not antinociceptive (Hama and Sagen, 2007c). However, up to 50% of SCI patients are currently taking such drugs, possibly to ameliorate musculoskeletal pain that is sensitive to cyclooxygenase inhibitors.

The 50% MPE dose of APAP in SCI rats was estimated to be greater than 300 mg/kg (i.p., data not shown), but robust efficacy has been observed in rat models of peripheral neuropathic pain (Lynch et al., 2004). Injection of APAP into the hind paw ipsilateral to a sciatic nerve injury leads to a reversal of neuropathic mechanical hypersensitivity, which is mediated through both CB1 and CB2 receptors (Dani et al., 2007). This suggests that APAP may be modulating the CB system in peripheral neuropathic pain. The difference in APAP efficacy across models suggests a distinction between the role of the endocannabinoid system in peripheral and central neuropathic pains. In general, it is likely that analgesic treatments may not be equally efficacious in both pain states – clinical studies appear to support this contention (Finnerup and Jensen, 2004).

It is possible that the effects of the APAP combinations in the current study could be due to increased concentrations of the active drug as a consequence of an inhibitory effect of APAP on liver (drug metabolism) or kidney (drug excretion) function. High doses of APAP could in fact lead to hepatotoxicity and nephrotoxicity. Serum alanine aminotransferase (ALAT) and blood urea nitrogen (BUN) levels at 750 mg/kg (i.p.) were normal in 3 mo old rats (Tarloff et

al., 1996). Doses over 1,000 mg/kg, however, increased ALAT activity and BUN. Significantly elevated ALAT suggest hepatic damage and elevated BUN suggests moderate to severe renal failure, which are clinically observed after ingesting extremely high doses of acetaminophen. In younger rats (about 1.5 mo) a dose of 500 mg/kg (i.p.) induced visible “necrosis” in rat liver tissue and significantly increased serum ALAT and liver concentrations of reduced glutathione, a tripeptide that conjugates and neutralizes the toxic metabolite of APAP responsible for liver necrosis (Chen et al., 2009). Resistance to hepatotoxicity in rodents appears to be age and sex-dependent (Davis et al., 1974; Tarloff et al., 1996). In the current study, rats were 2-2.5 mo old and the dose of 100 mg/kg (i.p.) is well below that used by Chen et al. (2009). It is unlikely that highest dose used in the current study compromised rat liver or kidney function.

The clinical efficacy of APAP combinations cannot be explained by a general increase of drug levels either. In humans, acute administration of Ultracet® did not affect plasma concentrations of either tramadol or its metabolites and APAP (McClellan and Scott, 2003). Similarly, given a combination of APAP+codeine, morphine (the product of *O*-demethylation of codeine) did not affect the metabolism of APAP and *vice versa* (Somogyi et al., 1991; Sonne et al., 1988). A drug interaction between gabapentin and APAP that could alter either's metabolism has not been reported, but gabapentin is not metabolized in humans, making the possibility of such a metabolic interaction unlikely. Thus, the antinociceptive effects of the combinations in the rat were likely due to pharmacodynamic mechanisms rather than a change in drug pharmacokinetics.

In contrast to the frequently used clinical combinations of APAP with opiates, there is a paucity of studies on the efficacy of other drugs combined with APAP. Gabapentin is approved for use in treating pain due to post-herpetic neuralgia. A short-term study has been reported with gabapentin in combination with APAP to treat acute post-operative pain, based on the hypothesis that blocking or attenuating post-injury neural sensitization will reduce the intensity and duration of pain (Durmus et al., 2007). Pre-operative APAP+gabapentin decreased post-operative morphine analgesic use and decreased both movement-associated pain and pain at rest to a greater extent than either gabapentin alone or placebo. Although the study was not designed to detect synergism, the results suggest improved analgesia with an APAP combination. Effects on long-term pain with a similar combination have yet to be reported, but the current results suggest efficacy of APAP+gabapentin in chronic SCI pain.

The effect of an APAP+tramadol combination has not been reported in either pre-clinical or clinical SCI pain studies. Previous pre-clinical studies have found that tramadol is antinociceptive in rat SCI pain models (Hama and Sagen, 2007c; Singh et al., 2006). A recent double-blinded placebo-controlled study in neuropathic SCI pain patients confirmed the pre-clinical findings (Norrbrink and Lundeberg, 2009). The combination in the current study was merely additive but it is possible that other ratios of APAP to tramadol could be synergistic – such as those observed in tests of acute nociception (Tallarida and Raffa, 1996). The current study indicates that an Ultracet®-like combination could also be useful in clinical SCI pain. Attenuation of the antinociceptive effect of APAP+tramadol with naloxone indicates involvement of opioid receptors, via a tramadol metabolite with μ -opioid receptor affinity (Raffa et al., 1992). The effect of APAP+tramadol is also mediated by CB1, but not CB2, receptors. Thus, an Ultracet®-like combination would have multiple analgesic mechanisms, including cannabinoid, opioid and serotonergic, working in concert, without the obvious motoric effects and sedation observed with full CB and opioid agonists.

The current study pointed out a merely additive effect of APAP+memantine. Although it is presumed that memantine, an NMDA receptor antagonist, and APAP have distinct analgesic mechanisms, it is possible that one drug could be antagonizing the effect of the other or that both drugs have a redundant, common mechanism. It has been suggested that NMDA receptor-

mediated antinociception is mediated through endocannabinoids -- anandamide can inhibit NMDA receptor function (Forman, 2003; Hampson et al., 1998). However, it also appears that anandamide can also increase NMDA receptor function through a non-CB receptor mediated mechanism. Which of these could be occurring in the current study following treatment is unknown, but the presence of apparently conflicting functions of endocannabinoids suggests that antinociceptive synergism involving the CB system may not be ubiquitous. In designing synergistic drug combinations, each constituent should not have mechanistic overlap (Tallarida, 2001). The constituents of APAP+memantine in the current study may not have been distinct, thus the lack of synergism.

Decreased efficacy following AM630 administration was found only in the APAP+morphine combination. AM630 administered peripherally but not centrally attenuated the antinociceptive effect of morphine (da Fonseca Pacheco et al., 2008; Pacheco Dda et al., 2009). This suggests that the effects of the APAP+morphine could be mediated through peripheral as well as central CB receptors (Catheline et al., 1996; Dani et al., 2007). Endocannabinoids, CB receptors and metabolism of APAP to AM404 can be readily observed outside of the CNS, such as in primary afferent nociceptors (Hogestatt et al., 2005; Mitirattanakul et al., 2006; Wotherspoon et al., 2005). Synergistic interactions between opioids and cannabinoids have been demonstrated both within the CNS and in nociceptors (Haller et al., 2008; Smith et al., 1998; Tham et al., 2005; Yesilyurt et al., 2003). Thus, such a combination could be administered peripherally (e.g. as a patch), further minimizing potential adverse effects of APAP and morphine. The hypothesis of a peripheral, CB receptor-mediated effect of morphine should be approached cautiously, however, as there was no change in efficacy of systemic morphine following 1 mg/kg (s.c.) AM630 treatment and morphine was fully efficacious in CB2 knockout mice (Ibrahim et al., 2006). The expression of CB receptors appears to be greatly altered in the injured spinal cord but the disposition of these receptors in peripheral nociceptors and uninjured lumbar dorsal horn has not been reported (Garcia-Ovejero et al., 2009).

The antinociceptive effects of the combinations in the current study were markedly attenuated with the CB1 receptor antagonist AM251. However, AM251 did not diminish the antinociception of the active drugs alone (i.e. gabapentin, morphine, tramadol), indicating that the efficacy of these drugs individually is not derived from modulating the endogenous CB system. An interaction between the gabapentin binding site (alpha-2-delta subunit of the voltage-gated calcium channel) and cannabinoids has not been reported in the literature. Previous studies have demonstrated functional interaction between the opioid and CB receptor systems (Haller et al., 2008; Smith et al., 1998). Evidence, however, demonstrating that morphine, tramadol or the metabolite of tramadol binds directly to CB receptors or that an increase in endocannabinoids is crucial in morphine's effect in SCI rats is lacking. Thus, the most likely source of the CB receptor sensitive component of the antinociceptive effects of the combinations in the current experiment is APAP (or its metabolite).

The present data points out the potential utility of combination analgesic therapy utilizing APAP for neuropathic SCI pain. Clinical guidelines for general chronic pain treatment have endorsed combination analgesic treatment (Christo and Mazloomdoost, 2008; Gilron and Max, 2005). Adjuvants combined with "first-line" analgesics have been proposed for SCI pain despite the lack of extensive experimental evidence to support such treatments (Siddall and Middleton, 2006). Although APAP is generally a safe drug, it will be important to identify combinations that may have potential toxicity prior to widespread clinical use (Prescott, 2000; Virani et al., 1997). Further clinical studies could uncover other useful combinations and optimal dose ratios.

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References

- Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: new vistas of an old drug. *CNS Drug Rev* 2006;12:250–275. [PubMed: 17227290]
- Cardenas DD, Jensen MP. Treatments for chronic pain in persons with spinal cord injury: A survey study. *J Spinal Cord Med* 2006;29:109–117. [PubMed: 16739554]
- Catheline G, Kayser V, Guilbaud G. Further evidence for a peripheral component in the enhanced antinociceptive effect of systemic morphine in mononeuropathic rats: involvement of kappa-, but not delta-opioid receptors. *Eur J Pharmacol* 1996;315:135–143. [PubMed: 8960876]
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994;53:55–63. [PubMed: 7990513]
- Chen YH, Lin FY, Liu PL, Huang YT, Chiu JH, Chang YC, Man KM, Hong CY, Ho YY, Lai MT. Antioxidative and hepatoprotective effects of magnolol on acetaminophen-induced liver damage in rats. *Arch Pharm Res* 2009;32:221–228. [PubMed: 19280152]
- Chong CR, Sullivan DJ Jr. New uses for old drugs. *Nature* 2007;448:645–646. [PubMed: 17687303]
- Christo PJ, Mazloomdoost D. Cancer pain and analgesia. *Ann N Y Acad Sci* 2008;1138:278–298. [PubMed: 18837907]
- Courade JP, Caussade F, Martin K, Besse D, Delchambre C, Hanoun N, Hamon M, Eschaliere A, Cloarec A. Effects of acetaminophen on monoaminergic systems in the rat central nervous system. *Naunyn Schmiedeberg Arch Pharmacol* 2001;364:534–537. [PubMed: 11770008]
- da Fonseca Pacheco D, Klein A, de Castro Perez A, da Fonseca Pacheco CM, de Francischi JN, Duarte ID. The mu-opioid receptor agonist morphine, but not agonists at delta- or kappa-opioid receptors, induces peripheral antinociception mediated by cannabinoid receptors. *Br J Pharmacol* 2008;154:1143–1149. [PubMed: 18469844]
- Dani M, Guindon J, Lambert C, Beaulieu P. The local antinociceptive effects of paracetamol in neuropathic pain are mediated by cannabinoid receptors. *Eur J Pharmacol* 2007;573:214–215. [PubMed: 17651722]
- Davis DC, Potter WZ, Jollow DJ, Mitchell JR. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci* 1974;14:2099–2109. [PubMed: 4847802]
- De Vry J, Kuhl E, Franken-Kunkel P, Eckel G. Pharmacological characterization of the chronic constriction injury model of neuropathic pain. *Eur J Pharmacol* 2004;491:137–148. [PubMed: 15140630]
- Di Filippo C, Rossi F, Rossi S, D'Amico M. Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol* 2004;75:453–459. [PubMed: 14657208]
- Dijkers M, Bryce T, Zanca J. Prevalence of chronic pain after traumatic spinal cord injury: a systematic review. *J Rehabil Res Dev* 2009;46:13–29. [PubMed: 19533517]
- Durmus M, Kadir But A, Saricicek V, Ilksen Toprak H, Ozcan Ersoy M. The post-operative analgesic effects of a combination of gabapentin and paracetamol in patients undergoing abdominal hysterectomy: a randomized clinical trial. *Acta Anaesthesiol Scand* 2007;51:299–304. [PubMed: 17257177]
- Finnerup NB, Jensen TS. Spinal cord injury pain--mechanisms and treatment. *Eur J Neurol* 2004;11:73–82. [PubMed: 14748766]
- Forman LJ. The effect of cannabinoid receptor antagonism with SR141716A on antinociception induced by cocaine and the NMDA receptor antagonist, MK-801. *Brain Res Bull* 2003;61:153–158. [PubMed: 12832001]

- Garcia-Ovejero D, Arevalo-Martin A, Petrosino S, Docagne F, Hagen C, Bisogno T, Watanabe M, Guaza C, Di Marzo V, Molina-Holgado E. The endocannabinoid system is modulated in response to spinal cord injury in rats. *Neurobiol Dis* 2009;33:57–71. [PubMed: 18930143]
- Gilron I, Max MB. Combination pharmacotherapy for neuropathic pain: current evidence and future directions. *Expert Rev Neurother* 2005;5:823–830. [PubMed: 16274339]
- Grande LA, O'Donnell BR, Fitzgibbon DR, Terman GW. Ultra-low dose ketamine and memantine treatment for pain in an opioid-tolerant oncology patient. *Anesth Analg* 2008;107:1380–1383. [PubMed: 18806055]
- Haller VL, Stevens DL, Welch SP. Modulation of opioids via protection of anandamide degradation by fatty acid amide hydrolase. *Eur J Pharmacol* 2008;600:50–58. [PubMed: 18762181]
- Hama A, Sagen J. Altered antinociceptive efficacy of tramadol over time in rats with painful peripheral neuropathy. *Eur J Pharmacol* 2007a;559:32–37. [PubMed: 17207479]
- Hama A, Sagen J. Antinociceptive effect of cannabinoid agonist WIN 55,212-2 in rats with a spinal cord injury. *Exp Neurol* 2007b;204:454–457. [PubMed: 17045264]
- Hama A, Sagen J. Behavioral characterization and effect of clinical drugs in a rat model of pain following spinal cord compression. *Brain Res* 2007c;1185:117–128. [PubMed: 17935699]
- Hama AT, Lloyd GK, Menzaghi F. The antinociceptive effect of intrathecal administration of epibatidine with clonidine or neostigmine in the formalin test in rats. *Pain* 2001;91:131–138. [PubMed: 11240085]
- Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM, Leonoudakis DJ, Bickler PE. Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* 1998;70:671–676. [PubMed: 9453561]
- Herzberg U, Eliav E, Bennett GJ, Kopin IJ. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci Lett* 1997;221:157–160. [PubMed: 9121688]
- Hogestatt ED, Jonsson BA, Ermund A, Andersson DA, Bjork H, Alexander JP, Cravatt BF, Basbaum AI, Zygmunt PM. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J Biol Chem* 2005;280:31405–31412. [PubMed: 15987694]
- Hohmann AG. Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. *Chem Phys Lipids* 2002;121:173–190. [PubMed: 12505699]
- Hulsebosch CE, Hains BC, Crown ED, Carlton SM. Mechanisms of chronic central neuropathic pain after spinal cord injury. *Brain Res Rev*. 2008
- Ibrahim MM, Rude ML, Stagg NJ, Mata HP, Lai J, Vanderah TW, Porreca F, Buckley NE, Makriyannis A, Malan TP Jr. CB2 cannabinoid receptor mediation of antinociception. *Pain* 2006;122:36–42. [PubMed: 16563625]
- Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW. Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *Br J Pharmacol* 2006;147:281–288. [PubMed: 16331291]
- Karst M, Salim K, Burstein S, Conrad I, Hoy L, Schneider U. Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain: a randomized controlled trial. *Jama* 2003;290:1757–1762. [PubMed: 14519710]
- Knotkova H, Pappagallo M, Szallasi A. Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? *Clin J Pain* 2008;24:142–154. [PubMed: 18209521]
- Kroenke K, Krebs EE, Bair MJ. Pharmacotherapy of chronic pain: a synthesis of recommendations from systematic reviews. *Gen Hosp Psychiatry* 2009;31:206–219. [PubMed: 19410099]
- La Rana G, Russo R, Campolongo P, Bortolato M, Mangieri RA, Cuomo V, Iacono A, Raso GM, Meli R, Piomelli D, Calignano A. Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404 [N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide]. *J Pharmacol Exp Ther* 2006;317:1365–1371. [PubMed: 16510698]
- Lichtman AH, Shelton CC, Advani T, Cravatt BF. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain* 2004;109:319–327. [PubMed: 15157693]

- Lynch JJ, Wade CL, Zhong CM, Mikusa JP, Honore P. Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model. *Pain* 2004;110:56–63. [PubMed: 15275752]
- Mallet C, Daulhac L, Bonnefont J, Ledent C, Etienne M, Chapuy E, Libert F, Eschalier A. Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. *Pain* 2008;139:190–200. [PubMed: 18485596]
- McClellan K, Scott LJ. Tramadol/paracetamol. *Drugs* 2003;63:1079–1086. discussion 1087–1078. [PubMed: 12749738]
- Mitrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H, Mackie K, Faull KF, Spigelman I. Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. *Pain* 2006;126:102–114. [PubMed: 16844297]
- Nesic O, Lee J, Johnson KM, Ye Z, Xu GY, Unabia GC, Wood TG, McAdoo DJ, Westlund KN, Hulsebosch CE, Regino Perez-Polo J. Transcriptional profiling of spinal cord injury-induced central neuropathic pain. *J Neurochem* 2005;95:998–1014. [PubMed: 16219025]
- Norrbrink C, Lundeberg T. Tramadol in neuropathic pain after spinal cord injury: a randomized, double-blind, placebo-controlled trial. *Clin J Pain* 2009;25:177–184. [PubMed: 19333166]
- Pacheco Dda F, Klein A, Perez AC, Pacheco CM, de Francisci JN, Reis GM, Duarte ID. Central antinociception induced by mu-opioid receptor agonist morphine, but not delta- or kappa-, is mediated by cannabinoid CB1 receptor. *Br J Pharmacol* 2009;158:225–231. [PubMed: 19594755]
- Pandita RK, Pehrson R, Christoph T, Friderichs E, Andersson KE. Actions of tramadol on micturition in awake, freely moving rats. *Br J Pharmacol* 2003;139:741–748. [PubMed: 12812997]
- Pini LA, Vitale G, Ottani A, Sandrini M. Naloxone-reversible antinociception by paracetamol in the rat. *J Pharmacol Exp Ther* 1997;280:934–940. [PubMed: 9023309]
- Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, Dasse O, Monaghan EP, Parrott JA, Putman D. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev* 2006;12:21–38. [PubMed: 16834756]
- Porreca F, Jiang Q, Tallarida RJ. Modulation of morphine antinociception by peripheral [Leu5] enkephalin: a synergistic interaction. *Eur J Pharmacol* 1990;179:463–468. [PubMed: 2364995]
- Prescott LF. Therapeutic misadventure with paracetamol: fact or fiction? *Am J Ther* 2000;7:99–114. [PubMed: 11319578]
- Rabchevsky AG. Segmental organization of spinal reflexes mediating autonomic dysreflexia after spinal cord injury. *Prog Brain Res* 2006;152:265–274. [PubMed: 16198706]
- Raffa RB, Clark-Vetri R, Tallarida RJ, Wertheimer AI. Combination strategies for pain management. *Expert Opin Pharmacother* 2003;4:1697–1708. [PubMed: 14521480]
- Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an ‘atypical’ opioid analgesic. *J Pharmacol Exp Ther* 1992;260:275–285. [PubMed: 1309873]
- Raffa RB, Stone DJ Jr, Tallarida RJ. Discovery of “self-synergistic” spinal/supraspinal antinociception produced by acetaminophen (paracetamol). *J Pharmacol Exp Ther* 2000;295:291–294. [PubMed: 10991992]
- Raffa RB, Stone DJ Jr, Tallarida RJ. Unexpected and pronounced antinociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine. *Eur J Pharmacol* 2001;412:R1–2. [PubMed: 11165231]
- Ragnarsson KT. Management of pain in persons with spinal cord injury. *J Spinal Cord Med* 1997;20:186–199. [PubMed: 9144608]
- Rajpal S, Gerovac TA, Turner NA, Tilghman JJ, Allcock BK, McChesney SL, Miranpuri GS, Park SW, Resnick DK. Antihyperalgesic effects of vanilloid-1 and bradykinin-1 receptor antagonists following spinal cord injury in rats. *J Neurosurg Spine* 2007;6:420–424. [PubMed: 17542507]
- Russo EB, Guy GW, Robson PJ. Cannabis, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. *Chem Biodivers* 2007a;4:1729–1743. [PubMed: 17712817]
- Russo R, Loverme J, La Rana G, Compton TR, Parrott J, Duranti A, Tontini A, Mor M, Tarzia G, Calignano A, Piomelli D. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamate acid 3'-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. *J Pharmacol Exp Ther* 2007b;322:236–242. [PubMed: 17412883]

- Sandford PR, Benes PS. Use of capsaicin in the treatment of radicular pain in spinal cord injury. *J Spinal Cord Med* 2000;23:238–243. [PubMed: 17536293]
- Siddall PJ, Middleton JW. A proposed algorithm for the management of pain following spinal cord injury. *Spinal Cord* 2006;44:67–77. [PubMed: 16116488]
- Singh VP, Patil CS, Kulkarni SK. Analysis of interaction between etoricoxib and tramadol against mechanical hyperalgesia of spinal cord injury in rats. *Life Sci* 2006;78:1168–1174. [PubMed: 16182319]
- Smith FL, Cichewicz D, Martin ZL, Welch SP. The enhancement of morphine antinociception in mice by delta9-tetrahydrocannabinol. *Pharmacol Biochem Behav* 1998;60:559–566. [PubMed: 9632241]
- Somogyi A, Bochner F, Chen ZR. Lack of effect of paracetamol on the pharmacokinetics and metabolism of codeine in man. *Eur J Clin Pharmacol* 1991;41:379–382. [PubMed: 1804656]
- Sonne J, Poulsen HE, Loft S, Dossing M, Vollmer-Larsen A, Simonsen K, Thyssen H, Lundstrom K. Therapeutic doses of codeine have no effect on acetaminophen clearance or metabolism. *Eur J Clin Pharmacol* 1988;35:109–111. [PubMed: 3220092]
- Tallarida RJ. Drug synergism: its detection and applications. *J Pharmacol Exp Ther* 2001;298:865–872. [PubMed: 11504778]
- Tallarida RJ. Interactions between drugs and occupied receptors. *Pharmacol Ther* 2007;113:197–209. [PubMed: 17079019]
- Tallarida RJ, Murray RB. *Manual of Pharmacological Calculations with Computer Programs*. Springer-Verlag; New York, NY: 1981.
- Tallarida RJ, Porreca F, Cowan A. Statistical analysis of drug-drug and site-site interactions with isobolograms. *Life Sci* 1989;45:947–961. [PubMed: 2677570]
- Tallarida RJ, Raffa RB. Testing for synergism over a range of fixed ratio drug combinations: replacing the isobologram. *Life Sci* 1996;58:PL 23–28.
- Tarloff JB, Khairallah EA, Cohen SD, Goldstein RS. Sex- and age-dependent acetaminophen hepato- and nephrotoxicity in Sprague-Dawley rats: role of tissue accumulation, nonprotein sulfhydryl depletion, and covalent binding. *Fundam Appl Toxicol* 1996;30:13–22. [PubMed: 8812206]
- Tham SM, Angus JA, Tudor EM, Wright CE. Synergistic and additive interactions of the cannabinoid agonist CP55,940 with mu opioid receptor and alpha2-adrenoceptor agonists in acute pain models in mice. *Br J Pharmacol* 2005;144:875–884. [PubMed: 15778704]
- Virani A, Mailis A, Shapiro LE, Shear NH. Drug interactions in human neuropathic pain pharmacotherapy. *Pain* 1997;73:3–13. [PubMed: 9414051]
- Wang J, Kawamata M, Namiki A. Changes in properties of spinal dorsal horn neurons and their sensitivity to morphine after spinal cord injury in the rat. *Anesthesiology* 2005;102:152–164. [PubMed: 15618799]
- Welch SP, Huffman JW, Lowe J. Differential blockade of the antinociceptive effects of centrally administered cannabinoids by SR141716A. *J Pharmacol Exp Ther* 1998;286:1301–1308. [PubMed: 9732392]
- Widerstrom-Noga EG. Pain: a multidimensional problem of national priority. *J Rehabil Res Dev* 2009;46:vii–ix. [PubMed: 19533515]
- Widerstrom-Noga EG, Turk DC. Types and effectiveness of treatments used by people with chronic pain associated with spinal cord injuries: influence of pain and psychosocial characteristics. *Spinal Cord* 2003;41:600–609. [PubMed: 14569261]
- Wotherspoon G, Fox A, McIntyre P, Colley S, Bevan S, Winter J. Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. *Neuroscience* 2005;135:235–245. [PubMed: 16084654]
- Yesilyurt O, Dogrul A, Gul H, Seyrek M, Kusmez O, Ozkan Y, Yildiz O. Topical cannabinoid enhances topical morphine antinociception. *Pain* 2003;105:303–308. [PubMed: 14499448]
- Yeziarski RP. Spinal cord injury pain: spinal and supraspinal mechanisms. *J Rehabil Res Dev* 2009;46:95–107. [PubMed: 19533523]
- Yeziarski RP, Park SH. The mechanosensitivity of spinal sensory neurons following intraspinal injections of quissqualic acid in the rat. *Neurosci Lett* 1993;157:115–119. [PubMed: 8233021]

Zhou Y, Wang Y, Abdelhady M, Mourad MS, Hassouna MM. Change of vanilloid receptor 1 following neuromodulation in rats with spinal cord injury. *J Surg Res* 2002;107:140–144. [PubMed: 12384077]

Abbreviations

AM404	<i>N</i> -(4-hydroxyphenyl)-arachidonylamide
APAP	<i>N</i> -acetyl-para-aminophenol (acetaminophen)
CB	cannabinoid
MPE	maximum possible effect
SCI	spinal cord injury
TRPV1	Transient Receptor Potential Vanilloid subtype 1

Figure 1.

Comparison of the potencies of gabapentin (A), memantine (B), morphine (C) and tramadol (D) alone and in combination with acetaminophen (APAP). The horizontal axis is the dose of the active drug (in mg/kg) and the vertical axis is percent maximum possible effect (%). For comparison, the effects of 100 mg/kg APAP alone (∇) at 90 min (A) and 60 min (B) post-injection are shown. Data are expressed as mean $\pm$ S.E.M. $n = 6-8$ /group.

A.

B.

Figure 2.

Effect of CB receptor antagonists on the antinociceptive effect of acetaminophen+gabapentin. Rats were pretreated with either vehicle (veh) or antagonist 30 min prior to a second injection of either vehicle or APAP+gabapentin combination. Rats were tested 90 min post-injection of either acetaminophen+gabapentin (APAP+GP) or vehicle. (A) A robust antinociception was observed in APAP+GP rats pretreated with vehicle. However, pretreatment with AM251 significantly reduced the antinociceptive effect of APAP+GP. (B) Pretreatment with AM630 did not affect APAP+GP antinociception. Data are expressed as mean $\pm$ S.E.M. * $p < 0.05$ vs. veh/veh, # $p < 0.05$ vs. veh/APAP+GP; $n = 7-8$ /group.

A.

B.

Figure 3.

Effect of CB receptor antagonists on the antinociceptive effect of acetaminophen+morphine. Rats were pretreated with either vehicle (veh) or antagonist 30 min prior to a second injection of either vehicle or APAP+morphine combination. Rats were tested 60 min post-injection of either acetaminophen+morphine (APAP+Mor) or vehicle. (A) A robust antinociception was observed in APAP+Mor rats pretreated with vehicle. Pretreatment with AM251 significantly reduced the antinociceptive effect of APAP+Mor, however, withdrawal thresholds were still significantly elevated compared to the AM251/veh group. (B) Pretreatment with AM630 significantly reduced the antinociceptive effect of APAP+Mor, however, withdrawal thresholds were still significantly elevated compared to the AM630/veh group. Data are

expressed as mean $\pm$ S.E.M. * p < 0.05 vs. veh/veh; # p < 0.05 vs. veh/APAP+Mor; ^ p < 0.05 vs. antagonist/veh; n = 7/group.

A.

B.

Figure 4.

Effect of CB receptor antagonists on the antinociceptive effect of acetaminophen+tramadol. Rats were pretreated with either vehicle (veh) or antagonist 30 min prior to a second injection of either vehicle or acetaminophen+tramadol combination. Rats were tested 90 min post-injection of either acetaminophen+tramadol (APAP+Tram) or vehicle. (A) A robust antinociception was observed in APAP+Tram rats pretreated with vehicle. However, pretreatment with AM251 significantly reduced the antinociceptive effect of APAP+Tram. (B) Pretreatment with AM630 did not significantly change the antinociceptive effect of APAP+Tram. Data are expressed as mean $\pm$ S.E.M. * $p < 0.05$ vs. veh/veh; # $p < 0.05$ vs. veh/APAP+Tram; $n = 7$ /group.

Figure 5.

Effect of a μ -opioid receptor antagonist on the antinociceptive effect of acetaminophen +tramadol. Rats were pretreated with either vehicle (veh) or antagonist 30 min prior to a second injection of either vehicle or acetaminophen+tramadol combination. Rats were tested 90 min following the second injection of either APAP+Tram or vehicle. Pretreatment with naloxone significantly reduced the antinociceptive effect of APAP+Tram. Data are expressed as mean $\pm$ S.E.M. * p < 0.05 vs. veh/veh; # p < 0.05 vs. veh/APAP+Tram; n = 7/group.

Table 1

List of drugs used

	Vehicle	Route	Volume	Source
Acetaminophen	10% Tween-80 in saline	i.p.	3 ml/kg	Sigma-Aldrich, Co.
Gabapentin	Saline	s.c.	1 ml/kg	Toronto Research Chemicals, Inc.
Memantine HCl	Saline	s.c.	1 ml/kg	Sigma-Aldrich, Co.
Morphine sulfate	Saline	s.c.	1 ml/kg	Sigma-Aldrich, Co.
Tramadol HCl	Saline	s.c.	1 ml/kg	Sigma-Aldrich, Co.
<i>Antagonists</i>				
AM251	10% DMSO, 10% Tween-80 in saline	s.c.	2 ml/kg	Tocris Bioscience
AM630	10% DMSO, 10% Tween-80 in saline	s.c.	2 ml/kg	Tocris Bioscience
Naloxone HCl	Saline	s.c.	1 ml/kg	Spectrum Chemicals

s.c., subcutaneous

i.p., intraperitoneal

Table 2

Summary of effect of drugs alone and in combination

Drugs alone:	Theoretical A ₅₀	Experimentally determined A ₅₀
Acetaminophen	-	> 300
Gabapentin ^a	-	26 (12)
Memantine ^a	-	8 (6)
Morphine ^a	-	1 (0.5)
Tramadol ^a	-	20 (11)
Combination of acetaminophen... ^b		
+ gabapentin	122 (59)	48 (24)*
+ memantine	102 (81)	85 (29)
+ morphine	126 (55)	37 (13)*
+ tramadol	121 (67)	63 (50)

A₅₀, 50% antinociceptive dose in mg/kg (95% C.L.).^a Previously determined A₅₀ values (Hama and Sagen, 2007).^b A₅₀ were calculated 90 min post-injection for gabapentin and tramadol and 60 min post-injection for memantine and morphine.* Synergistic interaction, $p < 0.05$ compared to the theoretically determined A₅₀.