Distinguishing analgesic drugs from non-analgesic drugs based on brain activation in macaques with oxaliplatin-induced neuropathic pain

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HIGHLIGHTS
● Oxaliplatin-treated macaques demonstrate robust cold hypersensitivity.
● Cold activates insula and secondary somatosensory cortex.
● Duloxetine but not pregabalin and tramadol are antinociceptive.
● Duloxetine blocks cold-evoked brain activation but pregabalin and tramadol do not.
● Findings suggest brain activation delineates analgesic from nonanalgesic drugs.

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ABSTRACT
The antineoplastic agent oxaliplatin is a first-line treatment for colorectal cancer. However, neuropathic pain, characterized by hypersensitivity to cold, emerges soon after treatment. In severe instances, dose reduction or curtailing treatment may be necessary. While a number of potential treatments for oxaliplatin-induced neuropathic pain have been proposed based on preclinical findings, few have demonstrated efficacy in randomized, placebo-controlled clinical studies. This failure could be related, in part, to the use of rodents as the primary preclinical species, as there are a number of distinctions in pain-related mechanisms between rodents and humans. Also, an indicator of preclinical pharmacological efficacy less subjective than behavioral endpoints that is translatable to clinical usage is lacking. Three days after oxaliplatin treatment in Macaca fascicularis, a significantly reduced response latency to cold (10 °C) water was observed, indicating cold hypersensitivity. Cold-evoked bilateral activation of the secondary somatosensory (SII) and insular (Ins) cortex was observed with functional magnetic resonance imaging. Duloxetine alleviated cold hypersensitivity and significantly attenuated activation in both SII and Ins. By contrast, neither clinically used analgesics pregabalin nor tramadol affected cold hypersensitivity and cold-evoked activation of SII and Ins. The current findings suggest that suppressing SII and Ins activation leads to antinociception, and, therefore, could be used as a non-behavioral indicator of analgesic efficacy in patients with oxaliplatin-induced neuropathic pain.

1. Introduction
The platinum-based antineoplastic agent oxaliplatin is a first-line treatment for colorectal cancer. A characteristic symptom of oxaliplatin neurotoxicity reported in most patients is an early-onset, acute hypersensitivity to cold (Attal et al., 2009). While patients recover from cold hypersensitivity within a few days following oxaliplatin treatment, cold hypersensitivity emerges with subsequent treatments, which may be severe enough to lead to dose reduction or discontinuation of potentially beneficial therapy. Furthermore, pain may persist long after

Abbreviations: CIPN, chemotherapy-induced peripheral neuropathy; SII, secondary somatosensory cortex; Ins, insular cortex; fMRI, functional magnetic resonance imaging; NE, norepinephrine; 5HT, 5-hydroxytriptamine
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oxaliplatin treatment cessation (Pachman et al., 2015). A number of pharmacotherapeutics have been proposed for the management of oxaliplatin-induced peripheral neuropathy, including drugs that are approved for the management of other painful peripheral neuropathies (Majithia et al., 2016). Of these, the serotoninergic-norepinephrine reuptake inhibitor duloxetine may be of benefit to patients with chemotherapy-induced peripheral neuropathy (CIPN) and oxaliplatin-induced peripheral neuropathy in particular; currently, however, there are no approved treatments (Smith et al., 2013).

The lack of effective therapeutics for the management of oxaliplatin-induced peripheral neuropathy could be, in part, due to an incomplete understanding of pathophysiology. Currently, there is a singular reliance on rodents as the preclinical model species but there are significant neuroanatomical and phylogenetic differences between rodents and humans (Chen and Hackos, 2015; Friedman et al., 2017; Pan et al., 2010; Verdier et al., 2015). More specifically, pain-related molecular target functionality, such as with voltage-gated and ligand-gated ion channels differ across species (Chen and Hackos, 2015; Han et al., 2015; McIntyre et al., 2001; Zhang et al., 2017). Expression patterns of particular targets within key areas of the CNS involved in pain perception and modulation may also differ between species (Mennicken et al., 2003; Serrano et al., 2012). In addition, there is a lack of pharmacological congruence between rodent CIPN and clinical CIPN. For example, studies utilizing rodent models of oxaliplatin-induced neuropathic pain have demonstrated robust antinociception of the anticonvulsant pregabalin, but a randomized, placebo-controlled clinical study in oxaliplatin-treated patients reported no significant efficacy (de Andrade et al., 2017; Ling et al., 2008). Given that nonhuman primates as a species are closer to humans than rodents, nonhuman primates could be an additional preclinical species in which to obtain in vivo confirmation that a given target is functionally relevant to humans (Chen and Hackos, 2015; Ogawa et al., 2016).

One other possible hindrance of moving potentially novel therapeutics from the laboratory to clinical use is the lack of a robust, quantitative, preclinical biomarker of oxaliplatin-induced peripheral neuropathic pain—one that could be utilized to facilitate the screening of potential therapeutics based on a proposed mechanism (Smith et al., 2017). In chronic neuropathic pain patients assessed with functional magnetic resonance imaging (fMRI), brain areas were activated with innocuous stimuli or mildly noxious stimuli (Boland et al., 2014; Wanigasekera et al., 2018). By contrast, these stimuli did not activate these brain areas in healthy controls. Modulation of brain activation in the neuropathic state could be used as a non-subjective indicator of analgesic treatment efficacy (Smith et al., 2017). While fMRI could be used to observe brain functioning in the neuropathic state and during analgesic treatment, the extent to which brain activation is in fact amenable to analgesic treatments is currently not clear.

A previous study demonstrated robust activation with non-painful cold stimulation in oxaliplatin-treated macaques of the secondary somatosensory cortex and insula (SII/Ins), areas that are not normally activated with non-painful cold, and that brain activation was suppressed with an antinociceptive dose of duloxetine (Chen, 2018; Coghill et al., 1994; Nagasaka et al., 2017). While pregabalin and tramadol, both of which are efficacious in other types of chronic pains, demonstrated no antinociceptive efficacy in oxaliplatin–treated macaques, whether these drugs also changed brain activation similar to that observed with duloxetine is not known; the lack of engagement of the brain mechanism underlying oxaliplatin-induced neuropathic pain could in part explain their lack of efficacy in the current nonhuman primate model and, for pregabalin, in patients (Shidahara et al., 2016). Thus, the goal of the current study was to determine if drugs that are analgesic in other pain states modulate cold-evoked activation of the SII/Ins in macaques with oxaliplatin-induced neuropathic pain. If there is congruence between brain activation and antinociception, then brain activation could be utilized as a physiological marker to aid in the development of treatments for oxaliplatin-induced neuropathic pain.

2. Materials and methods

2.1. Subjects

Male Macaca fascicularis (young adults, 3–5 years old) were used in the current study (EBS Co., Ltd., Hashimoto, Japan). All study procedures were reviewed and approved by the Hamamatsu Pharma Research, Inc. Animal Care and Use Committee (HSTIRB-248) and were carried out in accordance with guidelines within the Guide for the Care and Use of Laboratory Animals (Eighth ed., National Academy of Sciences, 2011). The macaques were housed in adjoining individual primate cages that allowed visual, aural and olfactory interactions. Room conditions, including humidity, temperature, and light were monitored daily by research and animal care staff. Animals had free access to tap water and were fed standard nonhuman primate chow (Oriental Yeast Co., Ltd., Chiba, Tokyo, Japan), which was supplemented weekly with fresh fruits or vegetables. The animals’ home cages were supplied with enrichment devices and positive interaction (e.g. hand-feeding of treats) between research and animal care staff.

In the current study, no behavioral testing of awake macaques was performed. Stimulation of the tail occurred only when the macaques were anesthetized and within the MR scanner.

2.2. Drug treatments

Induction of oxaliplatin-induced peripheral neuropathy in macaques was as previously described (Shidahara et al., 2016). Oxaliplatin (5 mg/kg; Sawai Pharmaceuticals, Osaka, Japan) was dissolved in dextrose 5% in water and intravenously infused over a period of 2 h, as per clinical dosing protocol (package insert). Brain activation to cold was observed with fMRI prior to oxaliplatin infusion and then three days after oxaliplatin infusion as peak cold hypersensitivity appeared three days following oxaliplatin infusion (Shidahara et al., 2016). Three weeks after the first oxaliplatin infusion, macaques received a second oxaliplatin infusion and brain activation was again assessed with fMRI three days after oxaliplatin infusion. All eight macaques underwent two oxaliplatin treatments (or two “cycles” of oxaliplatin treatment).

The study utilized two groups of four oxaliplatin-treated macaques each. In one group, macaques received either vehicle or tramadol in the first oxaliplatin treatment cycle and then were crossed-over to the other treatment in the second oxaliplatin treatment cycle. In a second group, macaques received either pregabalin or duloxetine during the first oxaliplatin treatment cycle and then were crossed-over to the other treatment in the second oxaliplatin treatment cycle. Because of the small number of subjects, and that each subject would get at least two treatments, no randomization was performed.

The doses of drugs (30 mg/kg) used in the current study were taken from previous macaque studies, in which duloxetine was shown to be antinociceptive, whereas pregabalin and tramadol were not (Shidahara et al., 2016). The pharmacological data in the literature generally imply target engagement in the macaque at doses well below those used in the current study. In healthy human subjects, 54% of brain norepinephrine transporters and 82% of serotoninergic transporters are occupied with a clinical used dose of 60 mg duloxetine (Moriguchi et al., 2017). The macaque equivalent dose would be about 3 mg/kg, based on a 60 mg dose in humans (Food and Drug Administration, 2005).

In a clinical study, a single dose of 150 mg pregabalin was shown to reduce herpes zoster pain (Jensen-Dahm et al., 2011). This would be equivalent to 2.5 mg/kg, or a macaque equivalent dose of 7.8 mg/kg (Food and Drug Administration, 2005). In a macaque model of post-operative pain, (p.o.) 30 mg/kg pregabalin suppressed non-noxious pressure evoked activation of the cingulate cortex (Hama et al., 2018).

A pharmacokinetic study in rhesus macaques estimated that antinociceptive doses of tramadol (p.o.) range between 12 and 60 mg/kg (Kelly et al., 2015). A clinically relevant high dose of tramadol, (i.v.) 4 mg/kg, produced greater than 70% in vivo occupancy of brain...
serotonergic and noradrenergic transporters in cynomolgus macaques (Arakawa et al., 2019). High doses of tramadol could lead to seizures—in a pilot study, 60 mg/kg led to brief seizure-like behavior following dosing in the macaque (Ryan and Isbister, 2015). Therefore, 30 mg/kg was the highest tested dose of tramadol. Pregabalin (Kemprotec, Ltd., Cumbria, UK), duloxetine (TCl, Tokyo, Japan) and tramadol (Sigma-Aldrich Japan Co., Tokyo, Japan) were dissolved in distilled water and administered via a gavage tube (p.o.) in a volume of 5 ml/kg.

2.3. Functional magnetic resonance imaging (fMRI)

2.3.1. Effect of cold on brain activity visualized with fMRI

Brain activation was assessed before and 3 days following oxaliplatin treatment using a 3.0T MRI system (Signa HDxt 3.0T MRI system (GE Healthcare, Milwaukee, WI, USA)). The anatomical protocols consisted of a T1-weighted fast spoiled gradient-recalled (FSPGR) sequence (repetition time (TR)/echo time (TE), 15.8/7.0 ms; flip angle, 12°; field of view, 150 mm × 150 mm; matrix, 256 × 224; slice thickness/interval, 1.0/0.5 mm; number of slices, 168). Functional scan sequences consisted of field-echo, echo-planar imaging (TR/TE, 3000/35 ms; flip angle, 90°; field of view, 140 mm × 140 mm; matrix, 64 × 64; slice thickness, 2.4 mm; number of slices, 30).

Macaques were sedated by continuous intravenous infusion of propofol (0.2 mg/kg/min) and heads were fixed within an MR compatible acrylic head holder (Matsui Co., Aichi, Japan). The dose of propofol used has little, if any, analgesic effect (Grounds et al., 1987; Steinbacher, 2001). During one fMRI scan, animals underwent 10 sets of tail stimulations (Fig. 1). One set consisted of 30 s of an “OFF” stimulus, 37 °C stimulation (a warmed gel pack) followed by 30 s of an “ON” stimulus, 10 °C stimulation (a cooled gel pack) applied by hand to rest on the distal portion of the tail. A 30 s interval without stimulation separated each set. Total duration of one scan was 14.5 min.

On the day of MRI scanning for drug treatment effects, oxaliplatin-treated macaques underwent a pre-administration scan and a post-administration scan. Following a baseline scan of 10 sets of temperature stimulations as described earlier, anesthesia was discontinued and oxaliplatin-treated macaques received p.o. either vehicle, duloxetine, pregabalin or tramadol. After induction of anesthesia, MRI scanning was performed again, between 60 and 75 min following dosing, with macaques stimulated with 10 sets of stimuli.

2.3.2. MRI data analysis

All subsequent image analyses were conducted with SPM12 software (Wellcome Trust Centre for Neuroimaging, London, UK). The images were realigned and resliced on to the mean echo-planar imaging (EPI) image to correct for head motion. The EPI images were co-registered to the corresponding T1-weighted anatomical image, and normalized to a macaque brain template (Black et al., 2004). The resulting image was smoothed with a 4 mm × 4 mm × 4 mm full-width at half-maximum Gaussian kernel. Voxel-wise statistical analysis was based on a general linear model. A fixed-effect model was used for group analysis of data from four macaques for vehicle and pregabalin and an additional four macaques for duloxetine and tramadol. Contrast (10 °C vs. 37 °C) was defined to isolate regions responsive to cold stimulation-related signals in the entire brain. Following drug treatment, contrast was defined as (post-treatment vs. pre-treatment) to determine whether treatment altered cold-induced brain activation following drug treatment in oxaliplatin treated macaques. In addition, contrast following drug treatment in oxaliplatin-treated macaques was defined as (vehicle vs. post-drug treatment) to compare activation following drug treatment with vehicle treatment. Peak voxels were considered significant at Z-value > 1.96 (p < 0.05, uncorrected for multiple comparisons, one-tailed t-test).

No statistical determination was used to determine sample sizes prior to the start of the current study—previous behavioral findings guided the selection of animal numbers. For example, previous behavioral findings demonstrated significant cold hypersensitivity in 5–6 oxaliplatin-treated macaques and robust duloxetine antinociception with a n = 4 (Shidahara et al., 2016). (Conversely, no pregabalin efficacy was observed with an n = 4.) Group sizes were similar to those used in previous publications on activation of pain-related brain areas in macaques (Asad et al., 2016; Nagasaka et al., 2017; Seah et al., 2014).

3. Results

Table 1 lists group mean z-values of SII/Ins prior to oxaliplatin treatment and following either vehicle or drug administration in oxaliplatin-treatment macaques. Prior to treatment with oxaliplatin, no significant brain activation was observed in SII/Ins with non-noxious cold. Three days following oxaliplatin treatment, significant cold-evoked activation was observed in SII/Ins (Fig. 2). While individual activation tended to be bilateral, some demonstrated unilateral activation. Oral administration of vehicle to oxaliplatin-treated macaques did not affect group-mean SII/Insula activation (Fig. 3, Table 2). Neither pregabalin (Fig. 3, Table 3) nor tramadol (Fig. 3, Table 4) had any significant effect on cold-evoked brain activation.

In contrast to pregabalin and tramadol, duloxetine suppressed cold-evoked activation of SII/Ins (Fig. 3, Table 5). Mean contrast maps

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SII/Insula Side</th>
<th>Z-value</th>
<th>Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td>Pre-oxaliplatin</td>
<td>Left</td>
<td>1.38</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>1.45</td>
<td>−16</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Left</td>
<td>3.44</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>4.39</td>
<td>−16</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>Left</td>
<td>3.31</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>3.19</td>
<td>−14</td>
</tr>
<tr>
<td>Tramadol</td>
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<td>3.71</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>3.66</td>
<td>−16</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>Left</td>
<td>1.14</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>1.21</td>
<td>−18</td>
</tr>
</tbody>
</table>

Response of SII and Ins to cold stimulation prior to oxaliplatin-treatment (“pre-oxaliplatin”) and following either vehicle or drug treatment in oxaliplatin-treated macaques. Stereotaxic coordinates, according to Horsley-Clarke’s stereotaxic coordinates, of peak voxel group mean Z-values are shown. Peak voxels Z-value > 1.96 were considered significant at p < 0.05. Means derived from four macaques.

Fig. 1. fMRI stimulation block design. An alternating set of temperatures was applied to the distal tail of the macaque with either a heated or cooled gel pack. One temperature stimulation “set” consisted of a 30 s “OFF” stimulus (37 °C) followed by a 30 s “ON” (10 °C) stimulus. Thirty sec. without temperature stimulation separated each set. During pre- and post-treatment imaging, temperature stimulation consisted of a total of 10 sets.

indicated significantly reduced activation compared to vehicle treatment and compared to pre-duloxetine treatment (z-value > 1.96, p < 0.05).

Comparison of group mean contrast maps between duloxetine, tramadol and pregabalin showed that duloxetine “inactivation” was significantly greater compared to that of pregabalin and tramadol (Table 6). Comparison of contrast maps between pregabalin and tramadol showed no difference in a lack of “inactivation” (i.e. neither drug reduced activation).
4. Discussion

The present study confirmed previous findings, in that non-noxious cold stimulation in oxaliplatin-treated macaques evoked significant bilateral activation of the SII and Ins, brain areas known to be associated with pain processing in humans as well as in nonhuman primates and that cold-evoked activation of SII/Ins was reduced by duloxetine (Chen, 2018; Coghill et al., 1994; Nagasaka et al., 2017). To determine whether cold-evoked activation of SII/Ins is sensitive to other pharmacological agents, specifically those which have not demonstrated behavioral efficacy, pregabalin and tramadol were tested (Shidahara et al., 2016). In parallel with previous behavioral findings, neither pregabalin nor tramadol affected cold-evoked brain activation. The current findings provide further support for the role of SII/Ins in oxaliplatin-induced cold hypersensitivity and also suggest that decreasing activation in these areas could be used in the development of therapeutics for oxaliplatin-induced neuropathic pain.

The symptoms commonly reported across painful peripheral neuropathies, such perception of non-noxious stimuli as painful, could be mediated by a common mechanism. It is speculated that, following peripheral nerve injury, central neurons, such as spinal dorsal horn subcortical and cortical somatosensory neurons, undergo “central sensitization,” which is characterized by observable and long-lasting neuropathic and physiological changes, including responding to stimuli that previously did not evoke a response (Millan, 1999). A number of brain areas involved in pain processing that have undergone central sensitization have been visualized using neuroimaging techniques such as fMRI in both humans and nonhuman animals (Borsook et al., 2007). In the current study, non-noxious cold activated SII/Ins in oxaliplatin-treated macaques, but not in untreated macaques, as previously demonstrated, suggesting that neurons within SII/Ins have undergone sensitization (Nagasaka et al., 2017). Functional MRI, then, could be used to uncover pain mechanism in intact organisms at the system level.

The current findings confirms previous findings of activation of SII/Ins in oxaliplatin-treated macaques (Nagasaka et al., 2017). In addition to individual brain areas, it is also possible that oxaliplatin treatment enhanced connectivity between SII/Ins and, for example, the somatosensory thalamus and changes below the level of the cortex such as in dorsal horn spinothalamic neurons and primary afferent nociceptors. Possible changes in connectivity, however, will need to be directly confirmed as changes in connectivity were not specifically explored in the current study. In addition, possible changes in functioning of other CNS pain-processing regions, such as the spinal dorsal horn, and peripheral nerve function will need confirmation, perhaps through invasive methods such as extracellular recordings.

Functional MRI could also be used to define treatment mechanism based on the treatment’s effect on particular pain-processing areas (Nagasaka et al., 2017; Smith et al., 2017; Upadhyay et al., 2013). Activation of a number of brain areas in a rat model of chronic osteoarthritic pain was reduced with a cyclooxygenase-2 (COX-2) inhibitor. The mechanism of the analgesic effect of anti-inflammatory drugs such as COX-2 inhibitors is ostensibly via reducing peripheral inflammation and peripheral nerve activation, thereby decreasing peripheral input to central neurons, but additional mechanisms of action have been suggested, such as inhibition of pain processing areas through enhanced activity of the endogenous central opioid system (Upadhyay et al., 2013).

To fully validate fMRI as a tool for both elucidating treatment mechanism and developing novel treatments, drugs that are not analgesic but nonetheless pharmacologically active should also be tested in relevant pain states in order obtain an accurate association between changes in brain activation and the presence of pain or analgesia. Upadhyay et al. (2011) demonstrated differential patterns of activation between the neurokinin-1 (NK1) receptor antagonist aprepitant, a non-analgesic, and the opioid buprenorphine in response to acute, noxious stimulation in awake healthy subjects. However, whether the effect of the drugs on acute pain in healthy subjects can be extended to chronic pain states remains to be confirmed. The current study utilized pregabalin, a drug that failed to demonstrate efficacy in an oxaliplatin neuropathy clinical trial and failed to show efficacy in oxaliplatin-treated macaques, as a negative control and found a lack of effect on SII/Ins activation (de Andrade et al., 2017). While a clinical trial of tramadol in oxaliplatin neuropathy has yet to been reported, it is expected, based on the lack of effect on brain activation and lack of behavioral effect, that it will lack clinical efficacy. Other treatments that have failed clinical trials could be used to further test the validity of both the macaque behavioral model and brain imaging as a physiological marker of pain and analgesia.

In vivo neuroimaging could be used to further elucidate potential mechanisms of actions of pharmacological agents and suggest potential areas of further investigation.

4.1. Duloxetine mechanism of action

Duloxetine is used to manage a number of chronic pain states and the primary mechanism of duloxetine’s analgesic effect across diverse pain states is believed to be the inhibition of brain norepinephrine (NE) and serotonin (5-hydroxytryptamine; SHT) reuptake, as duloxetine potentially blocks transporters of both neurotransmitters, thereby increasing synaptic concentrations of analgesic neurotransmitters (Raouf et al., 2017). Both NE and SHT-containing terminals originating from brainstem areas are abundant in cortical areas, including SII/Ins, in both humans and macaques (Levitt et al., 1984; Mash et al., 2005; Varnas et al., 2004; Wilson and Molliver, 1991). An alternative mechanism of
action is suggested by the fact that duloxetine potently blocks voltage-gated sodium channels expressed on peripheral nerves, thereby blocking action potential propagation and, thus, nociceptive signaling at peripheral and central levels (Wang et al., 2010). The clinical efficacy of duloxetine in oxaliplatin-induced neuropathic pain could be a result of a combination of mechanisms, which ultimately lead to reduced SII/Ins activation (Smith et al., 2013). Interestingly, venlafaxine, another NE/SHT reuptake inhibitor, failed to demonstrate clinical efficacy on oxaliplatin-induced peripheral neuropathy even though it showed efficacy in some types of clinical neuropathic pain and demonstrated marked efficacy in rodent oxaliplatin-induced peripheral neuropathy models (Finnerup et al., 2015; Hache et al., 2015; Ling et al., 2007; Zimmerman et al., 2016). (However, see (Durand et al., 2012).) A future study could confirm whether venlafaxine, in contrast to duloxetine, does not affect cold-evoked activation in the current model.

4.2. Pregabalin mechanism of action

The putative molecular target of the gabapentinoids, including pregabalin, is the α2δ subunit of the voltage-gated calcium channel (Taylor et al., 2007). In rodents, there is dense immuno-labelling of the α2δ subunit in CNS areas involved in pain processing, including the spinal dorsal horn, sensory cortex and insula (Taylor and Garrido, 2008). In the spinal dorsal horn, α2δ expression is primarily on pre-synaptic, central terminals of primary afferent nociceptors, and block of calcium channels containing the α2δ subunit inhibits nociceptive transmission from the periphery to the CNS. Pregabalin may similarly block nociceptive transmission at subcortical and cortical levels as well. Thus, the effect of pregabalin on neuropathic pain could be at any point along the "pain neuraxis".

When administered to uninjured rodents, pregabalin does not significantly affect responding to acute noxious stimuli. However, in rodent nerve injury-induced neuropathic pain models, including oxaliplatin-induced neuropathy, an upregulation of the α2δ subunit in DRG neurons and spinal dorsal horn neurons is observed, indicating that expression of the α2δ subunit above basal levels is required for gabapentinoid antinociception (Di Cesare Mannelli et al., 2017; Gauchan et al., 2009; Luo et al., 2002; Shidahara et al., 2016; Yamamoto et al., 2016). Whether the α2δ subunit was upregulated in other pain-related brain areas in rats has not been reported.

However, in a phase III clinical study, pregabalin administered prior to the start of oxaliplatin treatment and during the course of chemotherapy did not significantly reduce neuropathic pain (de Andrade et al., 2017). (Hypersensitivity to cold was not specifically assessed.) The dose of pregabalin used in the clinical study is analgesic in other types of neuropathic pain (Finnerup et al., 2015). Pregabalin in the current study did not reduce SII/Ins activation and, previously, did not alleviate hypersensitivity to cold (Shidahara et al., 2016). The current negative finding with pregabalin supports the contention that SII/Ins inactivation in oxaliplatin-treated macaques is necessary for antinociception. Interestingly, in neuropathic pain patients, pregabalin (one-week titration with 2.5 mg/kg on the day of fMRI) reduced brush-inactivation in oxaliplatin-treated macaques (Shidahara et al., 2016). As described earlier, SHT and NE transporters have been demonstrated in SII/Ins in the macaque. While the SHT and NE transporters are important in tramadol's antinociceptive effect in rodents, their contribution to an antinociceptive effect in nonhuman primates or humans is not entirely clear. It should also be noted that while tramadol's SHT and NE transporter inhibition are in the low μM range, they are not as potent compared to duloxetine (Raouf et al., 2017). The opioid buprenorphine has been shown to activate Ins in healthy macaques and humans, thus, confirming the presence of functional opioid receptors in this area (Leppa et al., 2006; Seah et al., 2014). However, the functional status of opioid receptors in the CIPN state is not known and will need further elaboration via, for example, either invasive or noninvasive methods.

4.3. Tramadol mechanism of action

Tramadol is recommended as a second-line analgesic for the management of neuropathic pain (Finnerup et al., 2015). The main metabolite of tramadol is a µ-opioid receptor ligand and the parent drug blocks 5HT and NE reuptake. As tramadol antinociception is not entirely attenuated by the opioid receptor antagonist naloxone, it is likely that its efficacy results from a combination of inhibition of NE and SHT transporters and opioid receptor activation (Raffa et al., 1992). Tramadol’s multiple mechanisms of action suggest that it should be more efficacious than first-line drugs such as duloxetine or pregabalin. Findings from rodent models of neuropathic pain, including oxaliplatin-induced neuropathic pain, appear to support this contention (Le Cudennec and Castagne, 2014; Shidahara et al., 2016; Zhao et al., 2014). Furthermore, in a placebo-controlled, randomized clinical trial, tramadol was more effective than pregabalin in reducing pain in peripheral nerve-injured neuropathic pain patients (Wanigasekera et al., 2018). One non-randomized, non-placebo controlled study in oxaliplatin-treated patients showed reduced self-reported pain intensity following treatment with Ultracet®*, an acetaminophen and tramadol combination (Liu and Wang, 2012). Thus far, there have been no reports of a randomized clinical trial that tested efficacy of tramadol against placebo for oxaliplatin-induced neuropathic pain.

In tandem with the lack of tramadol efficacy in the macaques as reported earlier, the current study showed a lack of change in SII/Ins activity in oxaliplatin-treated macaques (Shidahara et al., 2016). As described earlier, SHT and NE transporters have been demonstrated in SII/Ins in the macaque. While the SHT and NE transporters are important in tramadol’s antinociceptive effect in rodents, their contribution to an antinociceptive effect in nonhuman primates or humans is not entirely clear. It should also be noted that while tramadol’s SHT and NE transporter inhibition are in the low μM range, they are not as potent compared to duloxetine (Raouf et al., 2017). The opioid buprenorphine has been shown to activate Ins in healthy macaques and humans, thus, confirming the presence of functional opioid receptors in this area (Leppa et al., 2006; Seah et al., 2014). However, the functional status of opioid receptors in the CIPN state is not known and will need further elaboration via, for example, either invasive or noninvasive methods.

4.4. Possible effect of anesthesia on brain activation

Macaque in the current study were treated with the sedative-hypnotic propofol, in order to limit movement during fMRI scans and cutaneous stimulation. Anesthetics could suppress stimulus-evoked brain activation, particularly in pain-associated areas. A previous study examined brain activation in capsaicin-treated macaques, which were anesthetized with ~1% isoflurane to prevent movement (Asad et al., 2016). Despite the use of isoflurane, significant brain activation in a number of pain processing areas in capsaicin-treated macaques was observed following non-noxious heat stimulation, including SII/Ins. Thus, central sensitization can be observed in anesthetized macaques. However, the authors went on to state that the use of anesthetics may confound pharmacological studies and the development of new pain therapeutics since "many" anesthetics have "analgesic effects". In fact, in their previous study, activation areas observed in awake macaques following buprenorphine treatment were not observed in macaques anesthetized with 2% isoflurane (Seah et al., 2014).

Propofol sedation, however, did not prevent observation of brain activation during non-noxious cold stimulation in areas such as the cerebellum and somatosensory cortex (Nagasaka et al., 2017). In the current study, the dose of propofol was sufficient to retard movement but not render the animal unconscious. In humans, sedating doses of propofol do not appear to markedly suppress brain activity and cerebral blood flow (Frolich et al., 2017; Veselis et al., 2005) and brain activation in response to noxious and non-noxious stimulation is generally intact with anesthetic doses of propofol (Grounds et al., 1987;
Steinbacher, 2001). Anesthetic doses of propofol in humans reduces noxious stimulation-evoked brain activation in some brain areas, but activation persists in other areas, including SII/insula (Hobbauer et al., 2004).

It is possible, nonetheless, that drug interaction with anesthetics could lead to decreased or completely suppressed brain activation, potentially outweighing the effects of drugs on brain activation. As noted earlier with buprenorphine and isoflurane, significant pharmacological interactions have been observed between opioids and isoflurane (Steffey et al., 1994; Valverde et al., 2000). A similar pharmacological interaction between pregabalin and propofol has not observed (Moreau-Bussiere et al., 2013). It is possible that there was an interaction between duloxetine and propofol, but such an interaction has yet to be reported. The potential for interaction between centrally-acting therapeutics and brain activation in propofol-sedated macaques should be considered in interpreting drug-mediated inactivation of brain areas, particularly those involved in pain processing.

5. Conclusion

To date, there are few treatments that effectively manage oxaliplatin-induced neuropathic pain. One of these is duloxetine, which was previously shown to ameliorate hypersensitivity to cold and reduce cold-evoked activation of SII/Ins. By contrast, pregabalin, a drug that did not ameliorate oxaliplatin-induced neuropathic pain in a phase III clinical study, did not reduce cold-evoked activation of SII/Ins. The current study suggests that activation of SII/Ins could be used as a physiological indicator of drug efficacy. Thus, the lack of an effect of tramadol on SII/Ins in the current study suggests that tramadol will not show efficacy if it is tested in a phase III clinical study in oxaliplatin-treated patients. In addition to potential use as a “proof of concept” tool in the development of novel therapeutics, the current macaque model system, of behavior and stimulus-evoked brain activation, could be utilized to elaborate mechanism of action of existing drugs as well. In the case of pregabalin, the currently observed lack of efficacy could be due to a number of reasons, such as a lack of expression of the molecular target that mediates pregabalin’s effect. The current macaque model system also raises the issue that molecular targets could greatly differ across species. The current model could be used to elaborate the interspecies differences in the expression or functioning of molecular targets that are under consideration for the treatment of pain. Uncovering and acknowledging interspecies differences in biological processes could help to attenuate the potential for failure in clinical trials.

Conflicts of interest


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