The 5-HT₃ blocker granisetron reduces muscle pain induced by hypertonic saline

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Abstract

Objectives: Intramuscular injections with hypertonic saline are frequently used as induction of experimental pain that mimics chronic muscle pain (myalgia). 5-HT, a neurotransmitter, participates in the pain mediation via the 5-HT₃ receptor. Granisetron, a selective 5-HT₃-receptor antagonist, has previously been reported to increase the pressure pain threshold (PPT) in healthy muscles and to reduce hyperalgesia in patients with local myalgia. The aim of this study was to investigate whether the specific 5-HT₃ receptor antagonist granisetron influences the pain induced by an intramuscular injection of hypertonic saline in the masseter muscle on healthy females.

Material and methods: This study comprised 13 healthy females with a mean (SD) age of 26 (3.3) years. The participating subjects were undergraduate dental students at the Institute of Odontology, Karolinska Institutet. This experimental study was based on a randomized, placebo-controlled, double-blind model. All subjects received two bilateral injections in the masseter muscle with hypertonic saline and one injection of granisetron on one side and isotonic saline on the contra lateral side. The subjects were asked to score their pain intensity on a visual analogue scale (VAS) immediately after the two injections of hypertonic saline and every 15 seconds until pain subsided totally. The PPT was recorded twice at each site every 5th minute after injection of the test substances.

Results: The first injection of hypertonic saline induced pain of similar intensity and duration on both sides, while the second injection of hypertonic saline induced considerably less pain after pre-injection with granisetron, than after pre-injection with isotonic saline. The PPT did not change significantly on any side after injection of hypertonic saline and there were no significant differences between sides.

Conclusion: Granisetron clearly lowers the pain induced by hypertonic saline in healthy female subjects and the pain response tends to be shorter after injection of granisetron.

Introduction

Intramuscular injections with hypertonic saline are frequently used as experimental pain initiators. This is an exogenous muscle pain model that activates muscle nociceptors and causes a dominant sensation of deep, diffuse pain which is similar to chronic myalgia (1, 2, 3). The safety of this experimental design, without any side effects is a major reason for the use of hypertonic saline in experimental pain studies (4). Hypertonic saline has further been suggested to directly stimulate muscle nociceptors by excitation of group III and IV muscle afferents and central neurons encoding nociceptive information as well as glutamate release (1, 5).

It has been shown that bradykinin, serotonin (5-HT) and glutamate, that are inflammatory mediators and algesic substances (3, 6, 7, 8) induces plastic changes in the brain steam by central sensitization and contributes to allodynia and hyperalgesia (9, 10, 11, 12).
Muscle nociceptors are free nerve endings supplied by group III and IV afferents and can be sensitized to chemical and mechanical stimuli. By applying moderate to intense pressure on human muscle tissue, high-threshold mechanosensitive (HTM) receptors are activated (13). Most of these receptors are polymodal receptors, which mean that they normally respond to a variety of stimuli including algic chemical stimulation such as intramuscular injection of hypertonic saline (9, 10).

5-HT is a neurotransmitter and neuromodulator found in platelets, the enterochromaffin cells, and in certain regions of the brain (14). It participates in the pain mediation via the 5-HT$_3$ receptor (15, 16) on the afferent sensory and sympathetic nerves and is released from platelets due to tissue damage or ischemia (9, 17). Several 5-HT$_3$ receptor antagonists have been used in previous studies that have shown that systemic administration of 5-HT$_3$-antagonists reduce pain and hyperalgesia in patients with fibromyalgia (18, 19, 20). It has been reported that the pressure pain threshold (PPT) in healthy muscles was increased after systemic administration of the selective 5-HT$_3$-antagonist which indicates that 5-HT play a role in determining the PPT in muscle tissue (21). Furthermore, a study by Ernberg et al. (2000) showed that 5-HT induced pain and allodynia in the masseter muscle of healthy women only. The pain and allodynia induced by the injection of 5-HT was reduced after local administration of the 5-HT$_3$-antagonist granisetron. In other studies, the 5-HT$_3$-antagonist tropisetron reduced clinical pain in patients with tendinopathies, low back pain and myofascial pain (22, 23, 24).

The aim of his study was to investigate whether the specific 5-HT$_3$ receptor antagonist granisetron influences the pain induced by an intramuscular injection of hypertonic saline in the masseter muscle on healthy females.

**Material and methods**

**Subjects**

This study comprised 13 healthy females with a mean (SD) age of 26 (3.29) years. The participating subjects were undergraduate dental students at the Institute of Odontology, Karolinska Institutet. They were included if they were over 18 years of age, had good general health, had no pain from the orofacial region but minor tenderness to palpation of the masticatory muscles. Use of any analgesic medication during the day of study and/or a history of allergic reactions to granisetron led to exclusion.

The study followed the principles of the Declaration of Helsinki and was approved by the local ethics committee (KI/Syd) at Karolinska University Hospital, Karolinska Institutet, Huddinge, Sweden (233/03) and by the Medical Products Agency in Uppsala, Sweden. All subjects received both verbal and written information and gave their written consent.

**Methods**

**General procedure**

This experimental study is based on a randomized, placebo-controlled, double-blind model. One of the authors, not participating in data collection, performed the randomization by a computer (www.randomization.com). The subjects were first informed about the study protocol and a written consent was obtained. The subjects were seated in a relaxed position in a conventional dental chair. They were first subjected to a clinical examination which consisted of recording the palpatory tenderness over the superficial masseter muscles. A four-graded scale was used where 0 = no tenderness, 1 = mild tenderness, 2 = moderate tenderness with a pain reflex e.g. grimace, and 3 = marked tenderness with a defensive withdrawal reflex e.g. flinch. Passive maximum mouth opening (MVO) was measured (mm) three times between the incisors in the upper and lower jaw. This was followed by registrations of the PPT, 3 times with an interval of 2 minutes, bilaterally...
Assessment of pain
The subjects were asked to score their pain intensity on a 100-mm Visual Analogue Scale (VAS) with anchors marked with “no pain” and “worst pain ever experienced” immediately after injection of hypertonic saline and every 15 seconds until pain subsided totally. When the pain decreased after 5 min, the subjects were asked to make a drawing of their maximum pain distribution after injection of hypertonic saline. The drawing was made on a pre-sketched model of the head.

Assessment of pressure pain threshold
An electronic algometer (Somedic Sales AB, Hörby, Sweden) with a blunt rubber tip of 1 cm² was used to measure the PPT (kPa). The algometer was held perpendicular to the skin surface over the most prominent region of the masseter muscle during contraction. The pressure was increased with a rate of 50 kPa/s. The subjects were instructed to press a button as soon as the sensation of pressure changed into pain. Recordings of PPT were made over the injection site of the masseter muscle as well as measurement of the reference point over the nail of the left index finger. The PPT was recorded twice at each site 5, 10, 15, 20, 25 and 30 minutes after injection of the test substances.

Injection of test substances
The injection sites on the masseter were marked with a felt pen on a standardized point (most prominent point of the muscle during contraction). The surrounding region of the injection site was cleaned thoroughly with alcohol before injection. All subjects received two bilateral injections of the masseter muscle with 0.2 mL of sterile hypertonic saline (58.5 mg/mL, Karolinska University Hospital Pharmacy) and one injection with 0.5 mL of granisetron (Kytril®, 1 mg/mL, Roche, Stockholm, Sweden) on one side and 0.5 mL of isotonic saline (Natriumklorid, 9mg/mL, Fresenius Kabi, Uppsala, Sweden) on the contra lateral side. The injections were made perpendicular to the skin surface with a 19 mm long needle (diameter 0.4 mm) at a depth of approximately 15 mm. The needles were connected to an infusion pump (Harvard Infusion Pump 22, Harvard Apparatus, Great Britain) to ensure that the bilateral infusion occurred simultaneously. Hypertonic saline 0.2 mL was first injected bilaterally into the masseter muscle during 10 seconds (infusion rate1200 µL/min) to ensure that pain of similar intensity was induced on both sides. 30 min after the first injection, 0.5 mL of granisetron on one side and 0.5 ml of placebo on the other side were injected during 25 seconds, in a randomized and a double-blind manner. Two minutes later 0.2 mL of hypertonic saline was again injected, bilaterally during 10 seconds.

Preparation and blinding of syringes were made by a non participating author shortly before the injections, but in time to allow the solution to become room-tempered. The syringes were marked A for the hypertonic saline and BR (right side) and BL (left side) for the test substances (granisetron and isotonic saline).

Statistics
Data in tables and figures are presented as mean and standard deviation (SD) unless other is stated. Baseline pressure pain thresholds were analyzed for statistical differences with paired t-test. The VAS values after injection 2 were normalized to the values after injection 1, i.e. the difference in values between injection 1 and 2 was calculated at each time point and used in the statistical analyses. The PPT values after injections were normalized (%) to baseline. Two-way analysis of variance (ANOVA) for repeated measures with side and injection as the independent factor and time as the repeated factor was used to test the statistical differences in VAS and PPT for each injection separately. The significance of the difference between sides
in VAS peak was tested with Wilcoxon signed rank test, which test also was used for pain duration, since it was non-normally distributed. Two-way repeated measures ANOVA with injection as the independent factor and time as the repeated factor was used to test the significance of the difference in MVO after injection. One-way repeated measures ANOVA was used to test the significance of the difference in PPT over the reference point. For all tests the level of significance was set at $P < 0.05$.

**Results**

**Baseline**
The PPT did not differ between sides at baseline (Table 1)

**Table 1.**
The mean (SD) pressure pain threshold (PPT; kPa) of the masseter muscles assessed at baseline 1 and baseline 2 in 13 healthy female subjects.

<table>
<thead>
<tr>
<th>Side</th>
<th>Granisetron</th>
<th>Isotonic saline</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 1</td>
<td>233.9 (58.8)</td>
<td>241.9 (78.1)</td>
<td>0.528</td>
</tr>
<tr>
<td>Baseline 2</td>
<td>234.4 (97.8)</td>
<td>241.0 (110.7)</td>
<td>0.566</td>
</tr>
</tbody>
</table>

SD = standard deviation and $P = P$-value.

**Pain intensity after the first injection of hypertonic saline**
Injection of hypertonic saline induced pain of similar intensity and duration on both sides (Fig 1a). As could be expected the ANOVA showed a significant time effect ($F = 23.329; P < 0.001$), but there were no significant differences between sides ($F = 0.909; P = 0.359$) and no interaction between time and side ($F = 1.135; P = 0.312$). The peak pain and pain duration did not differ between sides (Table 2).

**Table 2**
The median (IQR) and the mean (SEM) pain duration after injection 1 and injection 2 in 13 healthy female subjects.

<table>
<thead>
<tr>
<th>Side</th>
<th>Granisetron</th>
<th>Isotonic saline</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td>225 (82.5)</td>
<td>210 (71.25)</td>
<td>0.5</td>
</tr>
<tr>
<td>Injection 2</td>
<td>120 (153.75)</td>
<td>195 (183.75)</td>
<td>0.078</td>
</tr>
<tr>
<td>Injection 1</td>
<td>207.7 (17.4)</td>
<td>201.9 (18.3)</td>
<td>0.821</td>
</tr>
<tr>
<td>Injection 2</td>
<td>140.8 (27.2)</td>
<td>190.4 (25.7)</td>
<td>0.192</td>
</tr>
</tbody>
</table>

IQR = interquartile range (75 percentile minus 25 percentile), SEM = standard error of the mean and $P = P$-value.
**Pain intensity after the second injection of hypertonic saline**

Although the pain intensity after the second injection of hypertonic saline was slightly lower than after the first injection on the saline treated side, injection of hypertonic saline after pre-injection with granisetron induced considerably less pain than after pre-injection with isotonic saline (Fig 1b). The ANOVA showed a significant main effect for sides ($F = 8.279; P = 0.014$) and time ($F = 3.731; P < 0.01$). There was also a significant interaction between side and time ($F = 4.543; P < 0.001$). The post hoc test showed that the difference between sides was significant 15-165 s after injection ($P < 0.05$). The peak pain was significantly lower after pretreatment with granisetron ($P = 0.002$). The pain duration on the granisetron treated side tended to be shorter, but this difference did not quite reach statistical significance ($P = 0.078$; shown in table 2).

![Figure 1A and 1B](image1.png)

**Figure 1A and 1B:** Figure A shows the induced pain intensity on both sides. The pain intensity was similar and there were no significant differences regarding the pain duration and the peak of pain. Figure B shows the pain intensity after the second injection of hypertonic saline, i.e. after pretreatment with granisetron/isotonic saline. The pain intensity was significantly lower after pre-treatment with granisetron and the pain duration also tended to be shorter on the granisetron side.

**Pressure pain threshold**

The PPT did not change significantly on any side after injection of hypertonic saline ($F = 0.571; P = 0.752$) and there were no significant differences between sides ($F = 0.508; P = 0.490$) and no interaction between time and side ($F = 0.353; P = 0.906$; Fig 2a). Neither was there any significant difference in the PPT with time ($F = 0.320; P = 0.582$) or between sides ($F = 0.684; P = 0.663$) and no interaction between side and time ($F = 0.349; P = 0.908$) after the second injections of hypertonic saline (Fig 2b).

The PPT over the reference point did not change significantly with time ($F = 0.532; P = 0.891$).
Figure 2A and 2B: Figure 2A shows the PPT change with time after the first injection of hypertonic saline. There were no significant differences not even after the second injection of hypertonic saline which is shown in figure 2B.

Maximum voluntarily mouth opening
There was a slight increase of MVO with time, both after injection 1 and 2 ($F = 2.451; P = 0.033$), but no difference between the injections ($F = 2.378; P = 0.149$) and no interaction between time and injection ($F = 0.353; P = 0.906$).

Discussion
The major finding of this study is that granisetron clearly lowers the pain induced by hypertonic saline in healthy female subjects and the pain response tendered to be shorter after injection of granisetron. These findings suggest that granisetron inhibits the stimulation hypertonic saline has on muscle nociceptors by the excitation of group III and IV muscle afferents and central neurons encoding nociceptive information (1, 5) and thereby suppresses the pain response. Moreover, granisetron probably blocks the 5-HT$_3$ receptors and thereby suppresses the 5-HT binding and also the pain response.

Pain intensity
As expected, hypertonic saline induced pain with similar intensity and duration on both sides; this is not surprising and is in agreement with several earlier studies (25, 26, 27). After the second injection the pain intensity was slightly lower on the saline side. This can be explained by the subjects’ expectations after the first injection which also was their first experience on muscle pain. But on the other hand after pre-injection with granisetron the pain induced after the second injection of hypertonic saline was much lower than the side pre-injected with saline. The lower pain intensity on the granisetron side suggests that hypertonic saline activates the HTM receptors and that granisetron inhibits the pain mediation via the 5-HT$_3$ receptor (15, 16). 5-HT$_3$ receptor antagonists are potent and highly selective competitive inhibitors of the 5-HT$_3$ receptors with negligible affinity for other receptors. They are rapidly absorbed and penetrate the blood-brain barrier easily. Metabolites are excreted mainly in urine. Clinical efficacy was first established in chemotherapy induced emesis. In this indication, 5-HT$_3$ receptor antagonists set a new standard regarding efficacy and tolerability.
Further established indications are radiotherapy-induced and post-operative emesis. Antiemetic efficacy results from a simultaneous action at peripheral and central 5-HT3 receptors. Other peripheral actions include reduction of experimentally induced pain. In migraine, 5-HT3 receptor antagonist show moderate efficacy as well. Several studies have demonstrated that 5-HT3 receptor antagonists have an effect on patients suffering from fibromyalgia but further studies are needed. 5-HT3 receptor antagonist diminishes serotonin-induced release of substance P from C-fibers and prevent unmasking of NK2 receptors in the presence of serotonin. These observations possibly provide an approach for the causal explanation of favorable treatment results with 5-HT3 receptor antagonist in fibromyalgia (28).

Granisetron pre-treatment may be used to reduce the incidence of pain on injection of propofol, which is an induction agent used to alleviate pain. This is an advantage added to the useful prevention of post operative nausea and vomiting (29).

Granisetron has also been used as an intra-articular administration on temporomandibular joint (TMJ) in patients with chronic polyarthritis with an indication of stronger short-term analgesic effect on TMJ movement pain (30). In other studies the use of other substances such as ketamine, morphine, lidocaine for reducing experimental muscle pain was found (26).

**Pressure pain threshold**
The injections of hypertonic saline did not affect the pressure pain threshold on any of the sides examined. This might be explained by that the dose of granisetron injected was too low to increase the PPT and that hypertonic saline does not alter the PPT. Previous findings indicate that intramuscular injection of granisetron increases the PPT over the masseter muscles of healthy subjects (31). However, it is reported that injection of hypertonic saline produces a short lasting decrease of PPT that cannot be detected when pain after injection declines (32).

**Maximum voluntary mouth opening**
The slight increase of MVO might be explained from the stretching that occurs after opening the mouth at several points.

**Confounders**
During this study some participants showed some difficulties defining the administered pain. The site of injection could also be a misleading source of pain resulting in diffuse perception of pain distribution after injection of granisetron.

**Conclusion**
Granisetron clearly lowers the pain induced by hypertonic saline in healthy female subjects and the pain response tenders to be shorter after injection of granisetron.

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References


