Antinociceptive properties of neurosteroids IV: pilot study demonstrating the analgesic effects of alphadolone administered orally to humans

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Fourteen patients scheduled for orthopaedic knee reconstruction surgery were enrolled in a prospective, double-blind, randomized study in which they received alphadolone (25–500 mg, n=9) or placebo (lactose, n=5) given orally 1 h after operation. All the subjects received a standardized general anaesthetic and the same type of surgery followed by physiotherapy using a continuous passive movement machine. Morphine was administered intravenously after operation by patient-controlled analgesia. Verbal rating and visual analogue scores assessed pain experiences for 6 h. Orally administered alphadolone up to 500 mg caused no increase in sedation, respiratory depression, nausea or vomiting. The experiences of these side-effects were all rated as none, mild or moderate. Orally administered alphadolone caused statistically significant reductions in morphine use and simultaneous highly significant reductions in pain scores. We conclude that alphadolone is a useful analgesic in humans when given by the oral route.

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Gamma aminobutyric acid (GABA) is involved with sensory processing throughout the central nervous system, including processing of nociceptive information at the level of the spinal cord. Two major subdivisions of GABA receptors exist, A and B. The GABA_A receptor is a pentameric ligand-gated chloride ion channel with binding sites for GABA and separate modulatory binding sites for barbiturates, benzodiazepines, propofol and neurosteroids. Heterogeneity of steroid binding sites on GABA_A receptors has also been suggested. GABA_A receptors are present within the dorsal horn of the spinal cord. Some of these may be targeted by intrathecal injection of benzodiazepines to produce spinally mediated antinociception in rats and pain relief in man. There is also some evidence that spinal GABA_A receptors may be targeted by inhalational general anaesthetics.

Few articles have been published describing the effect of neuroactive steroids on the spinal cord. One article reported inhibition of spontaneous and evoked electrical activity in spinal cord neurones after intravenous injection of Saffan, a veterinary steroid combination anaesthetic containing alphaxalone 9 mg ml⁻¹ and alphadolone 3 mg ml⁻¹ dissolved in Cremophor EL. These observations of effects on neuronal activity were not specific for any particular sensory modality. Intrathecal injection of water-soluble amino-steroid anaesthetics has been shown to cause antinociception in rats, which was a result of interaction of the drugs with spinal cord GABA_A receptors. Subanaesthetic doses of Saffan causes powerful antinociception in rats. Further investigations showed that all the sedative and anaesthetic properties of the mixture were due to the alphaxalone content and all of the antinociceptive properties were due to the alphadolone content. Alphadolone caused spinally mediated antinociception by an interaction with spinal cord GABA_A receptors when the drug was given intraperitoneally or intragastrically to rats without any signs of sedation, even when it was given at a very high dose. Therefore, we performed a pilot study to provide initial information on the tolerability and safety of the drug and initial data on its efficacy.

Materials and methods

The local research ethics committee approved the study. This was a small pilot study of dose escalation and proof of concept design, and incorporated some randomization and
placebo control because of the nature of the measurements, i.e. pain. The study was randomized, double-blind and placebo-controlled.

The study consisted of two parts, as shown in Fig. 1. At any time during both parts, if the patient was randomized to receive placebo, then a capsule containing lactose 250 mg was administered orally at the time of medication. If the subject was randomized to receive alphadolone, then the first patient so randomized received alphadolone acetate 25 mg orally. The patient was monitored for efficacy of the medication and side-effects. If there were no unacceptable side-effects, such as respiratory depression, severe sedation or disorientation, then the next patient randomized to receive the active treatment received a higher dose. The dose escalation planned was 25, 50, 100, 250, 500 and 1000 mg. The trial switched from the dose escalation phase to the comparison phase when there was clear evidence of efficacy. The senior author (CSG) assessed this and the study switched to part 2 without the observer’s knowledge. Patient and observer (AR) remained unaware of the nature of the treatment, whether placebo or alphadolone and what dose.

The randomization led to patients 1, 4, 9, 11 and 12 receiving placebo medication. Patient 2 received alphadolone 25 mg, patient 3 alphadolone 50 mg, patient 5 alphadolone 100 mg, patient 6 alphadolone 250 mg and patient 7 alphadolone 500 mg (Fig. 1).

There seemed to be efficacy with the 100, 250 and 500 mg doses in these patients. Thus, in the second part of the study, a double-blind comparison of placebo with alphadolone 250 mg was carried out. Patients 8, 10, 13 and 14 received alphadolone 250 mg and patients 9, 11 and 12 received placebo.

The protocol below was carried out in all patients in both parts of the study and the data were analysed for the efficacy and side-effect profile of alphadolone compared with placebo.

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**Protocol**

Patients ($n=14$) scheduled for knee reconstruction surgery gave written informed consent to be included in the study after inclusion and exclusion criteria had been met. Inclusion criteria were: ASA 1–2; age 18–70 yr; not using regular analgesics apart from paracetamol and non-steroidal anti-inflammatory drugs. All analgesic medications were withheld before surgery on the day of surgery. Subjects who had renal, hepatic or respiratory disease (other than mild asthma) and pregnant women were excluded from the study. No epidural, spinal or knee-joint local anaesthetic or opioid injections were allowed. After the patients had given informed consent, they were given an opportunity to familiarize themselves with the patient-controlled analgesia (PCA) machine and to practice completing the visual analogue scale (VAS) and verbal rating scale used for postoperative assessments.

No premedication was given. The patients were taken to the operating theatre and given a standardized anaesthetic that consisted of propofol induction followed by propofol infusion and the muscle relaxant of the anaesthetist’s preference. The trachea was intubated and the lungs were ventilated with a mixture of nitrous oxide and oxygen. Incremental doses of morphine (1–2 mg i.v.) were given as indicated by the assessment of the depth of anaesthesia by normal monitoring techniques. The amount of morphine used in the operating theatre was recorded. After surgery, muscle relaxation was reversed with atropine and neostigmine. In the recovery ward, the patient was reintroduced to the PCA machine, which contained morphine and was set to deliver a 1 mg dose with a lockout time of 5 min. The patient was discharged to the ward 30 min later.

Placebo or alphadolone was given orally with 100 ml of water 1 h after return to the ward. The following observations and measurements were made 1 h before, 30 min before and just before giving the test medication, and were repeated every 30 min thereafter until 6 h after the capsule.
had been given: sedation, on a four-point scale (none, mild, moderate, severe); nausea (none, mild, moderate, severe vomiting); morphine consumption during the last 30 min; 10 cm VAS for pain (no pain, most intense pain imaginable); respiratory depression score (none=respiratory rate greater than 10 b.p.m., oxygen saturation greater than 95%; mild=respiratory rate greater than 6 and less than 10 b.p.m., and oxygen saturation greater than 95%; moderate=respiratory rate less than 6 b.p.m. but oxygen saturation greater than 95%; severe=respiratory rate less than 6 b.p.m. and/or oxygen saturation less than 95%); light-headedness (none=no dizziness; mild=light dizziness but no nystagmus; moderate=feels dizzy and has nystagmus; severe=very dizzy with nausea); slurred speech (none, mild, moderate, severe); presence of confusion or disorientation; gaze-evoked nystagmus (none; mild=1–2 jerks; moderate=constant slow jerks; severe=fast jerks and feeling dizzy); lack of co-ordination, with two tests: (i) close eyes, touch nose with finger; (ii) touch fingers 1, 2, 3 and 4 in turn with thumb (classified as none=both tests accomplished; mild=both tests accomplished but slowly; moderate=one test achieved after two attempts; severe=failed both tests after two attempts at each).

One hour after the capsule had been given, all patients commenced physiotherapy with a continuous passive movement (CPM) machine. This machine forcibly moves the knee through an angle of 30° to 45°. Thus, after capsule administration the pain measurements were on movement, whereas before this they were at rest.

All patients had 10 ml of blood taken from a vein immediately before capsule administration and 30 min and 1, 1.5, 2, 3, 4 and 6 h after capsule administration. Blood was placed in heparinized tubes and centrifuged and the plasma was decanted for later analysis of blood alphadolone concentration by the Key Centre for Applied and Nutritional Toxicology, Royal Melbourne Institute of Technology.

**Table 1** Amounts of morphine administered intravenously to placebo- and alphadolone-treated patients as part of the anaesthetic and postoperative PCA before capsule administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Morphine received before capsule (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Placebo (n=5)</td>
<td>16.2</td>
</tr>
<tr>
<td>Alphadolone (n=7)</td>
<td>17.22</td>
</tr>
</tbody>
</table>

Fig 2 PCA morphine consumption by five placebo-treated patients and the patients who received alphadolone on an escalating scale (25, 50, 100, 250 and 500 mg).

\(\chi^2\) test was also used to compare the groups with respect to all other scores except the VAS for pain intensity. The latter measurements at each 30 min period were transformed by dividing them by the mean of the three readings taken before capsule administration. All of the values for the alphadolone-treated group were compared with those for placebo treatment using the Mann–Whitney U-test. In all statistical tests, a P value less than 0.05 was considered to be statistically significant.

**Results**

No patient in the study experienced more than mild light-headedness, or any respiratory depression, slurred speech, confusion, disorientation, gaze-evoked nystagmus or lack of co-ordination. In the control group of five patients, there were four males and one female. In the alphadolone group there were nine patients, five males and four females. The ages of the placebo-treated patients were 18, 23, 24, 26, 28, 29 and 33 yr. The ages of the alphadolone group were 18, 19, 23, 24, 24, 26, 28, 29 and 33 yr.

The doses of morphine received by the patients in the operating theatre, recovery ward and ward before capsule administration were not significantly different between placebo- and alphadolone-treated patients (Table 1).

**Dose escalation (part 1 of the study)**

Morphine consumption from the PCA machine for the first five alphadolone-treated patients (patients 2, 3, 5, 6 and 7) in the 6 h after capsule administration compared with all placebo treated patients is shown in Fig. 2.

The total range of morphine consumption of the five placebo-treated patients is shown at alphadolone dose 0. During the 6 h after capsule administration there was a progressive reduction in morphine use as the alphadolone dose increased from 25 to 100 mg. This was used as a guide to decide the dose of alphadolone to be used in part 2 of the study. Doses of alphadolone 100 mg and above were all associated with PCA morphine use, which was lower than the lowest use by placebo-treated patients.
Table 2 (A) Nausea scores. Patients’ experiences were rated on a four-point scale as none, mild, moderate or severe. There were no differences between the treatment groups. There were no cases of severe nausea and vomiting or heavy sedation. The addition of alphadolone to the postoperative medication did not lead to greater sedation, nausea or vomiting. No scores of 3 or 4. P=0.9459 ($\chi^2$ test) (B) Sedation scores. Details as for Table 2A. No scores of 3 or 4. P=0.8661 ($\chi^2$ test)

<table>
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<tr>
<th>Treatment</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>51</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Alphadolone</td>
<td>72</td>
<td>10</td>
<td>1</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
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</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>14</td>
<td>33</td>
<td>13</td>
</tr>
<tr>
<td>Alphadolone</td>
<td>20</td>
<td>48</td>
<td>15</td>
</tr>
</tbody>
</table>

On this basis, it was decided to switch the rest of the trial (part 2) to a double-blind comparison of alphadolone 250 mg with placebo in the remaining patients. Data from placebo-treated patients ($n=5$) and alphadolone-treated patients (100, 250 and 500 mg, $n=7$) were combined for statistical comparisons.

**Sedation and nausea scores**

These data were entered into a contingency table (Table 2).

A $\chi^2$ test for nausea score (Table 2A) and sedation score (Table 2B) showed that there were no significant differences between placebo and alphadolone treatment. There were no scores of 3 or 4 for either symptom, and the large majority of patients had either no nausea or sedation or only mild symptoms.

**Verbal rating scale for pain**

Verbal rating scale scores were entered into a 4×2 contingency table (Table 3). A $\chi^2$ test revealed that the alphadolone-treated patients recorded significantly lower pain scores.

**Visual analogue scale**

Scores were entered into a spreadsheet and then transformed by dividing by the mean of the three VAS scores measured at the time of capsule administration and 30 min and 1 h before that (Table 4). Thus, each VAS score after capsule administration was expressed as a ratio of the pretreatment readings. The transformed data were then collected for all placebo-treated ($n=5$) and alphadolone-treated ($n=7$) patients. These two data sets were placed in two columns and subjected to a Mann–Whitney $U$-test. There was a significant difference ($P=0.0117$) between the two groups, the alphadolone patients having significantly lower standardized VAS scores than placebo-treated patients.

**Morphine consumption**

The morphine dosage was in increments of 1 mg, i.e. one press of the button always delivered 1 mg. All of the 30 min measurements of morphine consumption were 0, 1, 2, 3 or 4 mg. The frequency of occurrence of each of these values were entered into a contingency table (Table 5).

It can be seen that the $\chi^2$ test revealed a statistically significant difference ($P=0.0153$). However, the $\chi^2$ test should not be applied to these data as they stand because the number of observations in the cells relating to morphine 4 mg is so low. If the numbers in the cells relating to morphine 4 mg are added to the 3 mg cells (this only applies to one observation in the placebo group), then the $\chi^2$ test is valid for the data and computation revealed $\chi^2=12.24997$ and $P=0.00651$.

**CPM duration**

Each patient was put on the CPM machine 1 h after capsule administration. The patient could be taken off this machine at the discretion of the nursing staff. Thus, little can be made of these data except to rule out the possibility that the alphadolone patients were subjected to knee movements for shorter periods of time.

Table 6 shows that this was not the case.

**Alphadolone blood concentrations**

Analysis of the blood samples revealed no detectable alphadolone. The lower limit of detection was 50 ng ml$^{-1}$. However, treatment of the plasma samples with the enzyme gluconoridase revealed that the alphadolone had been absorbed and conjugated to a glucuronide group. Alphadolone metabolite was found in μg ml$^{-1}$ concentrations in the plasma in all the patients treated with alphadolone that were analysed (250 mg, five patients; 500 mg, one patient). In four of these patients the metabolite was detected in the first sample taken after the alphadolone capsule had been given. In all cases the metabolite was still present, although at a lower concentration, at the 6 h sampling time point.

**Discussion**

Hans Selye showed almost 60 yr ago that steroid hormones given parenterally and orally could produce sedation and anaesthesia. Furthermore, he was able to show that it was possible to produce steroid molecules that were anaesthetics but devoid of hormonal action. The speed of onset of the sedative effects of these compounds was the first observ-
ation that suggested a non-genomic mechanism. The positive modulation of GABAA receptors as the basis for the sedative, anticonvulsant and hypnotic properties of the neuroactive steroids is now well established.17±21 Neurosteroid anaesthetic preparations were made following the pioneering work of Selye. These were attractive clinically because of good overall safety, low toxicity and rapid metabolism by the liver, which limited the duration of anaesthetic action.22±25 They have largely fallen into disuse after the withdrawal of Althesin because of anaphylactoid reactions to the Cremophor EL vehicle.22 23 Some more recent attempts have been made to make water-soluble neurosteroid anaesthetics.26 27 Throughout the development of neurosteroid compounds for use in humans, researchers and developers have concentrated on the hypnotic, sedative and anticonvulsant properties of these compounds.26±33 Such work has led to the development and patenting of new neurosteroids as anticonvulsants, acting as positive modulators at GABAA receptors to be used as anti-epileptics34 35 or for the treatment of migraine.36 There have been no reports of analgesic or antinociceptive effects of neurosteroids separate from their sedative and anticonvulsant properties. For example, effects on electrically evoked spinal cord neuronal activity after anaesthetic doses of intravenous Althesin (alphaxalone/alphadolone) have been reported.37 38 Anti-nociception in animals has been reported after injection of sedative neurosteroids directly into the brain or spinal cord

| VAS readings for pain measured in mm before (Pre) and every 30 min after capsule administration. Each VAS reading taken at each 30 min period | (B) Patient, treatment | Pre 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1, placebo | 1.00 | 0.75 | 0.64 | 0.44 | 0.75 | 0.98 | 1.02 | 0.95 | 1.02 | 0.92 | 0.75 | 0.51 | 0.31 |
| 2, alphadolone 25 mg | 1.00 | 0.60 | 0.60 | 0.84 | 0.58 | 0.46 | 0.31 | 0.55 | 1.15 | 0.65 | 0.43 | 0.60 | 1.20 |
| 3, alphadolone 50 mg | 1.00 | 0.39 | 0.34 | 0.76 | 0.67 | 0.42 | 0.36 | 0.69 | 0.67 | 0.67 | 0.55 | 0.80 | 1.11 |
| 4, placebo | 1.00 | 0.46 | 0.46 | 0.52 | 0.54 | 0.50 | 0.41 | 0.41 | 0.41 | 0.56 | 0.50 | 0.41 | 0.50 |
| 5, alphadolone 100 mg | 1.00 | 0.80 | 0.50 | 0.31 | 0.63 | 0.53 | 0.66 | 0.99 | 1.07 | 1.21 | 1.24 | 1.38 |
| 6, alphadolone 250 mg | 1.00 | 1.53 | 1.66 | 1.41 | 1.16 | 0.97 | 0.31 | 0.31 | 0.09 | 0.47 | 0.78 | 0.50 | 0.94 |
| 7, alphadolone 500 mg | 1.00 | 0.59 | 0.48 | 0.69 | 0.53 | 0.45 | 0.72 | 0.40 | 0.40 | 0.38 | 0.34 | 0.40 | 0.34 |
| 8, alphadolone 250 mg | 1.00 | 0.49 | 0.46 | 0.52 | 0.57 | 0.88 | 0.33 | 0.55 | 0.54 | 0.68 | 0.62 | 0.34 | 0.33 |
| 9, placebo | 1.00 | 0.68 | 1.20 | 1.26 | 1.22 | 0.81 | 0.48 | 0.50 | 0.54 | 0.83 | 0.54 | 1.05 | 1.28 |
| 10, alphadolone 250 mg | 1.00 | 0.39 | 0.68 | 0.29 | 0.15 | 1.06 | 0.97 | 1.39 | 1.72 | 0.97 | 1.53 | 0.93 | 0.79 |
| 11, placebo | 1.00 | 0.94 | 1.00 | 0.87 | 0.81 | 0.84 | 1.23 | 1.13 | 1.42 | 1.26 | 1.10 | 1.13 | 1.16 |
| 12, placebo | 1.00 | 0.90 | 0.71 | 1.27 | 0.64 | 0.64 | 0.49 | 0.52 | 0.56 | 0.97 | 0.79 | 0.56 | 0.49 |
| 13, alphadolone 250 mg | 1.00 | 1.35 | 0.66 | 0.44 | 0.71 | 0.64 | 0.61 | 0.59 | 0.86 | 0.49 | 0.57 | 0.30 | 0.54 |
| 14, alphadolone 250 mg | 1.00 | 0.39 | 0.39 | 0.15 | 0.10 | 0.12 | 0.10 | 0.09 | 0.00 | 0.00 | 0.09 | 0.09 | 0.05 |

**Table 4** VAS readings for pain measured in mm before (Pre) and every 30 min after capsule administration. Each VAS reading taken at each 30 min period after capsule administration (A) was transformed by dividing it by the mean of the three VAS readings taken 1 h, 30 min and immediately before capsule administration. (B) Standardized VAS scores (standardized ratio of precapsule levels shown in Table 4A. For details see legend to Table 4A). *P*<0.05 (Mann–Whitney *U*-test)

<table>
<thead>
<tr>
<th>Treatment Number of requests from the PCA machine in 30 min period</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=5)</td>
<td>25</td>
<td>18</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Alphadolone (n=7)</td>
<td>39</td>
<td>29</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 5** Contingency table showing the frequency with which the alphadolone- and placebo-treated patients pressed the PCA button in each 30 min period. Alphadolone treatment led to a significant reduction in the frequency of requests for morphine from the PCA machine. *χ²=12.280738; P=0.01534*

| Placebo Alphadolone |
|---|---|
| Mean | 4.46 | 7.82 |
| Median | 5.00 | 5.00 |
| Range | 3–6 | 2.25–17 |

**Table 6** Time spent on the CPM machine (h). No significant difference
without any control measurements to exclude the effect of sedation on the observations. By contrast, in human experiments, subnarcotic doses of Althesin, like equivalent doses of thiopentone, produced a degree of analgesia during experimental pain elicited by pressure on the tibia. It was found that Althesin alone provided insufficient analgesia for major surgery. It abolished the pain of uterine contractions only during the period of unconsciousness, which was followed by hypersensitivity to pain during recovery. There have been observations of gender differences and pregnancy-associated antinociception that have been attributed to steroid hormones. All of these compounds caused hormonal effects as well as antinociception. Therefore, apart from data reported by Nadeson and Goodchild, selective antinociceptive effects of neurosteroids were not suspected.

Experiments in rats showed that spinal cord GABA<sub>A</sub> receptors are responsible for the antinociceptive effects of subanesthetic doses of propofol and Saffan (alphaxalone/alphadolone mixture). Unexpectedly, it was found that alphadolone was responsible for this antinociception, but only when intraperitoneal or intragastric routes of administration were used. This led to inactivation of the anesthetic properties of the alphadolone, even when the compound was administered at high doses. This suggested a new use for the neurosteroid as an analgesic and that the active compound might be a metabolite.

This pilot study shows for the first time a number of properties of orally administered alphadolone in humans. Alphadolone is rapidly absorbed after oral administration and metabolized to the glucuronide. It may be well tolerated up to a dose of 500 mg orally, with no observed increase in central nervous system disturbances greater than those caused by hangover of anaesthetic and postoperative medication with morphine. No free alphadolone was detected, which may explain the lack of sedation and is in accordance with the results obtained previously in rats. This was followed by a reduced morphine requirement after operation and improved pain scores even in the presence of lower morphine use. This morphine-sparing and improved pain relief occurred in the presence of movement of the joint. Thus, we conclude that orally administered alphadolone may possess useful analgesic properties in humans and that these properties are worthy of more detailed investigation.

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