Oxycodone concentrations in the central nervous system and cerebrospinal fluid after epidural administration to the pregnant ewe

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Abstract

The main sites of the analgesic action of oxycodone are the brain and spinal cord. The present study describes the concentrations of oxycodone and its metabolites in the brain and spinal cord after epidural administration to the ewe. Twenty pregnant ewes undergoing laparotomy were randomised into two groups to receive epidural oxycodone; infusion group (n=10, 0.1 $mg \cdot kg^{-1}$ bolus followed by continuous infusion of 0.05 $mg \cdot kg^{-1} \cdot h^{-1}$ for five days) or repeated boluses group (n=10, 0.2+ 2x0.1 $mg \cdot kg^{-1}$ bolus followed by a 0.2 $mg \cdot kg^{-1}$ bolus every 12 hours for five days). After five days of oxycodone administration, arterial blood samples were collected, the sheep were euthanized, and a CSF sample and tissue samples from the cortex, thalamus, cerebellum and spinal cord were obtained for the quantification of oxycodone and its main metabolites. The median plasma and CSF concentration of oxycodone were 9.0 and 14.2 $ng \cdot mL^{-1}$ after infusion and 0.4 and 1.1 $ng \cdot mL^{-1}$ after repeated boluses. In the infusion group, the cortex, thalamus and cerebellum oxycodone concentration were 4-8-fold higher and in the spinal cord 1310-fold higher than in plasma. In the repeated boluses group, brain tissue concentrations were similar in the three areas, and in the spinal cord were 720-fold higher than in plasma. Oxymorphone was the main metabolite detected, which accumulated in the brain and spinal cord tissue. In conclusion, first, accumulation of oxycodone and oxymorphone in the CNS was observed, and second, high spinal cord concentrations suggest that epidural oxycodone may provide segmental analgesia.

Bullet point summary:

What is already known

- Oxycodone appears to actively penetrate the blood-brain barrier to the CNS after systemic administration.

What this study adds

- Oxycodone concentrations are excessive in the spinal cord after epidural administration.
- Oxymorphone, an active metabolite, accumulates in the brain and spinal cord after epidural oxycodone administration.

Clinical significance

- Epidural oxycodone could provide a highly effective segmental analgesia in the spinal cord level.
- The CSF concentration of oxycodone does not predict tissue concentrations in the CNS.

Abbreviations

BBB: blood-brain barrier

CNS: central nervous system

CSF: cerebrospinal fluid

CYP: cytochrome P450

LLoQ: lower limit of quantification

Introduction and background

Oxycodone is a semisynthetic opioid agonist and is commonly used to treat moderate to severe pain (1). The analgesic effects of oxycodone are mediated via opioid receptors, which are widely distributed in the central nervous system (CNS), mainly in the cortex, thalamus, hippocampus, locus coeruleus and dorsal horn of the spinal cord (2,3). Drug access to the CNS depends on several factors, such as drug lipophilicity, molecular weight, electric charge, blood concentration, plasma protein binding, cerebral blood flow and affinity to active transport systems at the blood-brain/blood-cerebrospinal fluid barriers (4,5).

The penetration of the CNS by oxycodone and its metabolites has been poorly evaluated. In humans, cerebrospinal fluid (CSF) concentrations are used as surrogate markers of CNS penetration. Recent data indicate that the area under the concentration curve is similar in CSF and plasma after intravenous oxycodone administration, but after epidural administration, it is 100-fold higher in lumbar CSF compared to that in plasma (6,7). In experimental studies, ample penetration of oxycodone into the CNS has been demonstrated with rats indicating active transport across the blood-brain barrier (BBB) (5,8).

Oxycodone is metabolised mainly by hepatic cytochrome P450 (CYP) enzymes. CYP3A catalyses the N-demethylation to noroxycodone, the primary metabolite in humans, and CYP2D6 catalyses the O-demethylation to oxymorphone. These metabolites are further metabolised to noroxymorphone (9). The role of these metabolites in the analgesic response of oxycodone is not entirely established. Oxymorphone has high μ-opioid receptor affinity, and it is in clinical use in some countries (9). Noroxymorphone seems to have an analgesic effect when administered intrathecally but not after systemic administration,

which indicates possible negligible permeability through the BBB (10). Noroxycodone has no or only weak analgesic properties (11).

The aim of this experimental study was to evaluate the tissue concentrations of oxycodone and its major metabolites in cortical, thalamic, cerebellar and spinal cord tissues and in CSF after epidural administration to the pregnant ewe and to compare tissue concentrations to those in plasma. We hypothesised that, first, oxycodone concentrations in brain and spinal cord tissue samples would exceed those in plasma, and, second, CSF concentrations would predict CNS tissue concentrations after epidural administration.

Materials and methods

The study protocol was reviewed and approved by the National Animal Experiment Board of Finland (ESAVI/1007/04.10.07/2014, http://www.avi.fi/web/avi/elainkoelautakunta-ella). The animal transport, housing, care and experimental procedures were conducted according to the national legislation (12,13) and the EU Directive 2010/63/EU (14). The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (15). The study was conducted in two sessions, between 18 March and 26 March 2014, and between 8 March and 18 March 2015.

Twenty 2-8-year-old Åland landrace pregnant ewes (Lammastila Sikka Talu, University of Turku, Rymättylä, Finland) were included in this experimental study at the Department of Experimental Surgery, Laboratory Animal Centre, University of Oulu and Oulu University Hospital, Oulu, Finland. This animal study was a part of foetal asphyxia research, and the species was selected primarily on the strength of obstetrical research (16). Moreover, in

sheep, epidural administration of a local anaesthetic produces regional anaesthesia similar to that observed in humans (17). The sheep were pregnant after time-mating with either one or two foetuses at 122-128 gestational days (term 145 days) and weighed 42-73 kg (median 52).

Two weeks before the experiments, the sheep were transported from the breeder to the Laboratory Animal Centre. The sheep were group-housed in pens with straw bedding and in individual pens after the operations, always having contact with each other through the window between the pen walls. No individual sheep was left alone in the animal room.

Animals were monitored daily by veterinarians, animal technicians and investigators for signs of pain, distress, injury or disease. Intramuscular fentanyl was administered as required for rescue analgesia during recovery.

Surgical interventions included ewe's laparotomy, hysterotomy and foetal cannulation under standardised general anaesthesia followed by a five-day recovery period. Thereafter, similar general anaesthesia was induced for experiments (see (16)).

Transdermal fentanyl patches (2 $\mu g \cdot kg^{-1} \cdot h^{-1}$) were attached to the antebrachium 24 hours before surgery. The ewes were premedicated with intramuscular racemic ketamine (2 $mg \cdot kg^{-1}$) and midazolam (0.2 $mg \cdot kg^{-1}$) 30 minutes before surgery. Standardised endotracheal anaesthesia was induced with intravenous propofol 4-5 $mg \cdot kg^{-1}$ and maintained with sevoflurane 1.5–2.5% in an oxygen–air mixture with positive pressure ventilation via an endotracheal tube. Maternal heart rate, arterial blood pressure, end tidal partial pressure of carbon dioxide ($ETCO_2$) and peripheral capillary oxygen saturation (SpO_2) were monitored constantly during anaesthesia (AS3 Patient Monitor, Datex-Ohmeda, Helsinki, Finland).

After the induction of anaesthesia, an epidural catheter was placed at the presacral or one to two interlaminar spaces cranially and the tunnelled catheter was secured with stiches.

A laparotomy was performed to access the foetal upper body, and catheters were positioned into the foetal internal jugular vein and carotid artery. This surgical procedure has been described in detail previously (16).

The sheep were randomised into two groups, the infusion group (n=10) or repeated boluses group (n=10), to receive epidural oxycodone hydrochloride trihydrate (Oxycodone, Takeda Oy, Helsinki, Finland). Randomisation was computer-generated (www.randomization.com). Blinding was considered not feasible since all ewes received the study infusion/injections into the epidural catheter.

In the infusion group, the sheep received a 0.1 $mg \cdot kg^{-1}$ epidural oxycodone hydrochloride trihydrate bolus at the beginning of surgery, followed by a 0.1 $mg \cdot kg^{-1} \cdot h^{-1}$ continuous infusion during surgery. In the repeated boluses group, the sheep received an initial bolus of 0.2 $mg \cdot kg^{-1}$ oxycodone, followed by a 0.1 $mg \cdot kg^{-1}$ bolus every 60 minutes during the surgery. During the five-day recovery period, 0.05 $mg \cdot kg \cdot h^{-1}$ epidural oxycodone (1.2 $mg \cdot kg^{-1} \cdot 24\ h^{-1}$) was administered to the infusion group with an elastomeric pump (Autofuser 100 mL, 2 $ml \cdot h^{-1}$, Acemedical, Gyenonggi-Do, Korea) and the repeated boluses group was given a 0.2 $mg \cdot kg^{-1}$ epidural oxycodone bolus every 12 hours (0.4 $mg \cdot kg^{-1} \cdot 24\ h^{-1}$). An oxycodone hydrochloride trihydrate dose of 0.1 $mg \cdot kg^{-1}$ corresponds to 0.087 $mg \cdot kg^{-1}$ oxycodone hydrochloride and 0.078 mg kg^{-1} oxycodone base. Function of the elastomeric pumps in the infusion group were ensured daily at 8 a.m. and 8 p.m.

After the recovery period, on the day of experiment, epidural oxycodone administration was continued similarly to the recovery phase, i.e., epidural infusion was continued in the infusion group and the last epidural bolus was given at 8 a.m. in the repeated boluses group. The sheep underwent an experiment, which included both a maternal and foetal hypoxaemic period of 150 minutes. When the hypoxaemia phase was complete, normoxaemia was restored with inhaled oxygen for 60 minutes.

After the experiment, the ewes were prepared under the same anaesthesia by cannulating the left internal carotid artery and internal jugular vein. A loose ligature was positioned around these vessels. The foetus and ewe were euthanized using an overdose of intravenous phenobarbital. The loose ligature around the carotid artery was tightened proximal to the cannulation following euthanasia and the left hemisphere was perfused with 1000 mL of 0.9% saline through the cannula. The ligature in the internal jugular vein was tightened, and the vein was cut to allow the outflow of blood and perfusate from the brain.

An arterial blood sample was obtained just before euthanasia and a CSF sample was obtained by a cisternal puncture immediately after euthanasia. Three tissue samples were collected from the perfused side of the ewe's brain: the frontal cortex, thalamus and cerebellum (18). After ensuring the location of the epidural catheter tip, a fourth tissue sample was taken from the spinal cord, a 3 cm long segment 2-5 cm above the epidural catheter tip site. The blood and CSF samples were centrifuged at 2500 x *g* for 10 minutes, and the separated plasma and CSF were collected in Eppendorf tubes. The tissue samples were frozen using liquid nitrogen. The samples were stored at -70°C until analysis at Admescope Ltd., Oulu, Finland.

The plasma and CSF samples were treated as described earlier (6). The cortex, cerebellum, thalamus and spinal cord samples were first homogenized with 4-fold volume of 150 mM phosphate-buffered saline (pH 7.4) using Omni Bead Ruptor 24 homogenizer (Omni International, Kennesaw, GA, USA) and extracted with a two-fold volume of acetonitrile: methanol (1:1) containing internal standards for 20 minutes under ultrasonicator. The extracts were centrifuged for 20 minutes at 2272 x *g*, and supernatants were transferred to Waters Sirocco precipitation plate (Waters Corporation, Milford, MA, USA) diluted 1:1 150 mM phosphate-buffered saline (pH 7.4), shaken and centrifuged for 20 minutes at 2272 x *g*. Standard and quality control samples were prepared by spiking 1/100 blank brain homogenate with external standard solution in methanol, and further processed as the samples. The samples were collected on Waters UPLC 1 ml 96-well plate for analysis as described earlier (6).

The concentrations of oxycodone and its metabolites oxymorphone, noroxymorphone and noroxycodone were analysed with an ultra-performance liquid chromatography mass spectrometry system in two patches (6). The lower limits of quantification (LLoQ, $ng \cdot mL^{-1}$ or $ng \cdot g^{-1}$) were 0.05 for oxycodone, and 0.1 for oxymorphone, noroxymorphone and noroxycodone. The linear calibration ranges ($ng \cdot mL^{-1}$ or $ng \cdot g^{-1}$) were fitted as follows: oxycodone 0.05-1000, oxymorphone 0.1-500, noroxymorphone 0.1-1000, and noroxycodone 0.1-100. Accuracies were between 101-124% at the LLoQ and 85-112% above the LLoQ. The precisions were 0.9-14% over the entire range of calibration. All concentrations of oxycodone and metabolites are reported as their corresponding hydrochlorides. Plasma has a density of 1.03 $g \cdot mL^{-1}$, and this coefficient was used when the ratios between the tissue and plasma concentrations were calculated.

No formal sample size calculation was performed, but the sample of 20 ewes available was considered to provide pertinent data on the epidural oxycodone evaluation. The data were analysed using the Statistical Package for Social Sciences software (IBM SPSS Statistics 23, IBM Corporation, Armonk, NY, USA). Differences in the concentrations between the infusion and repeated boluses groups were analysed with the Mann-Whitney U-test. The Pearson correlation coefficients with two-tailed significance testing were used for correlation evaluation. A p-value of less than 0.05 was considered statistically significant. The results are presented as the median along with the minimum and maximum.

Results

There were a few protocol deviations unlikely to affect the results of this study. In the infusion group, one sheep (sheep 3) was euthanized on the day of surgery (had paraplegia and opisthotonos after anaesthesia), and two sheep (sheep 2 and 5) on the second postoperative day (intrauterine foetal death). In the repeated boluses group, one sheep was euthanized on the fourth (sheep 12) and two on the fifth postoperative day (sheep 13 and 19) (intrauterine foetal death). These six sheep did not undergo the hypoxemic period. One bolus dose was omitted due to vomiting (sheep 14) and one bolus dose was omitted due to suspected opioid overdose (sheep 13). Two epidural catheters in the repeated boluses group were in the paravertebral space (sheep 16 and 20).

Tissue samples were available for nine sheep in both groups, and CSF samples for 19 sheep. Weight of the tissue samples was between 1.5 and 4.8 g (median 2.4) for the cerebellum

samples, between 1.5 and 5.6 g (3.1) for the cortex samples, between 1.1 and 4.1 g (2.1) for the thalamus samples and between 0.8 and 2.7 g (1.8) for the spinal cord samples.

The oxycodone concentrations in plasma, cortex, cerebellum, thalamus, spinal cord and CSF are presented in Table 1.

In the infusion group, the median oxycodone concentration in the cortex was 43 $ng \cdot g^{-1}$ (range 1.2-95), in the thalamus 53 $ng \cdot g^{-1}$ (3.0-352) and in the cerebellum 43 $ng \cdot g^{-1}$ (1.9-114), compared to 6.9 $ng \cdot g^{-1}$ (1.4-178, p=0.49), 4.0 $ng \cdot g^{-1}$ (<LLoQ-171, p=0.14) and 7.4 $ng \cdot g^{-1}$ (0.6-252, p=0.094), respectively, in the repeated boluses group. The median oxycodone concentration in the spinal cord of 27,200 $ng \cdot g^{-1}$ (22-63,600) and in the CSF of 14 $ng \cdot mL^{-1}$ (0.3-32) were higher in the continuous infusion group than in the repeated boluses group, 51 $ng \cdot g^{-1}$ (1.8-79,900, p=0.04) and 1.1 $ng \cdot mL^{-1}$ (0.07-109, p=0.028), respectively. The two lowest spinal cord concentrations of oxycodone were in the sheep with unintentional paravertebral epidural catheters.

The median plasma oxycodone concentration was 9.0 $ng \cdot mL^{-1}$ (1.2-23) in the infusion group. In the repeated boluses group, the plasma oxycodone concentrations were below the LLoQ in five sheep and were low in the five that could be quantified, with a median of 0.4 $ng \cdot mL^{-1}$ (0.4-3.8, p=0.008 compared to the infusion group). Cerebral accumulation of oxycodone occurred; in the infusion group the median cortex/plasma-ratio was 4.3 (0.07-9.5). In the repeated boluses group the cortex/plasma-ratio could be calculated for four sheep with a median of 96 (0.4-424).

In the repeated boluses group, the time interval between the last oxycodone bolus and sample collection varied between 1.3 and 22 hours (median 7.3). There was a moderate

negative correlation between the time from last bolus to oxycodone concentration in the cortex, r = -0.41 (p=0.28), thalamus, r = -0.38 (p=0.32), cerebellum, r = -0.47 (p=0.2), spinal cord, r = -0.51 (p=0.17), CSF, r= -0.48 (p=0.17) and plasma, r = -0.34 (p=0.33). CSF oxycodone had a strong, positive correlation with the oxycodone concentration in the cortex, r = 0.8 (p<0.001), cerebellum, r = 0.88 (p<0.001) and spinal cord, r = 0.77 (p<0.001), and moderate correlation to oxycodone in the thalamus, r = 0.37 (p=0.15).

Oxymorphone was the main metabolite detected. Oxymorphone could be quantified from all tissue and CSF samples. The tissue concentrations were 4-fold higher in the infusion group than in the repeated boluses group (Table 2).

Oxymorphone accumulated in the CNS. In the infusion group, the cortex/plasma ratio of oxymorphone was 6.4 (0.12-12), and in the repeated boluses group this ratio was 10 (5.5-108), the spinal cord/plasma-ratio was 35 (2.5-109) and 57 (5.5-548), and the CSF/plasma-ratio was 2.6 (0.29-19) and 2.9 (1.6-56), respectively.

In the repeated boluses group, there was a strong negative correlation between the time since the last oxycodone bolus and the oxymorphone CSF concentration (r=-0.79, p=0.011). There were no correlations between the plasma and tissue concentrations. In contrast, CSF oxymorphone had a moderate-strong positive correlation with the concentration in the cortex, r = 0.56 (p=0.018), cerebellum, r = 0.47 (p=0.056) and spinal cord, r = 0.77 (p<0.001).

The concentrations of noroxycodone were mostly low or below the LLoQ (Table 2). In the infusion group, two thalamic (165 and 248 $ng \cdot g^{-1}$) and four spinal cord (56-256 $ng \cdot g^{-1}$) samples had noroxycodone concentrations that were meaningful but still lower than those

of oxymorphone. In the repeated boluses group, there were two meaningful spinal cord concentrations of noroxycodone, 97 and 230 $ng \cdot g^{-1}$.

Noroxymorphone concentrations were low, ≤3.4 $ng \cdot g^{-1}$ or below the LLoQ.

Discussion

In the present study, we determined the oxycodone and its metabolite concentrations in the cortex, cerebellum, thalamus, spinal cord, CSF and plasma after a 5-day epidural oxycodone infusion or repeated boluses every 12 hours to pregnant ewes. The novel findings of this study were, first, that oxymorphone, an active metabolite, was the main metabolite after epidural administration, and second, that oxymoprhone accumulated in the brain, spinal cord tissue and CSF. The highest oxymorphone concentrations were in the spinal cord, which were 35-fold higher than in plasma in the infusion group and 57-fold higher than in plasma in the repeated boluses group. Our data are consistent with a previous experimental study. Sadiq *et al.* (19) gave rats one hour intravenous oxymorphone infusion and observed that CNS concentrations were significantly higher compared to those in plasma 15 minutes after the infusion, indicating oxymorphone accumulation in the brain.

Plasma oxymorphone concentrations were clinically meaningful, with median value of 4 $ng \cdot mL^{-1}$ after infusion and 0.5 $ng \cdot mL^{-1}$ after repeated boluses. These concentrations are within the therapeutic range reported for humans for immediate release oxymorphone tablets at doses of 5–20 mg where peak plasma concentrations range between 1 and 7 $ng \cdot mL^{-1}$, and for extended release doses of 5–40 mg with peak plasma concentrations between 0.3 and 4 $ng \cdot mL^{-1}$ (20,21). A single sheep had a toxic plasma oxymorphone concentration of 44 $ng \cdot mL^{-1}$ after 350 mg of epidural oxycodone in 120 hours. However, this

sheep did not have any clinical signs or symptoms of opioid overdose. The concentrations in human toxicology specimens have ranged between 11 and 590 $ng \cdot mL^{-1}$ (22).

To the best of our knowledge, there are human data of epidural oxycodone only after a single bolus dose administration. However, these data are consistent with the present study. In two human studies in which patients were given a single 0.1 $mg \cdot kg^{-1}$ bolus of oxycodone, oxymorphone could be detected in all CSF samples after epidural administration with a median concentration of 0.4 $ng \cdot mL^{-1}$ but was only detected in a few CSF samples and in significantly lower concentrations after intravenous administration (6,7). After oxycodone administration by mouth, plasma oxymorphone concentrations have been negligible (9,23), and after repeated intravenous doses, plasma oxymorphone concentrations have been relatively low with a noroxycodone/oxymorphone ratio of over ten (24,25). Taken together, these data suggest that the route of oxycodone administration may contribute to the metabolism of oxycodone. Further human data on repeated or continuous epidural administration may clarify this issue.

Consistent with our first hypothesis, compared to the plasma concentrations, the spinal cord oxycodone concentration was 1310- and 720-fold higher for the infusion and repeated boluses groups, respectively. For the parent compound, the novel finding was that the oxycodone concentrations in spinal cord tissue were excessive, as the median was 23,400 $ng \cdot g^{-1}$ after epidural infusion. The highest spinal cord oxycodone concentration of 79,900 $ng \cdot g^{-1}$ was observed 1.3 hours after an epidural bolus of 0.2 $mg \cdot kg^{-1}$. In the infusion group, the spinal cord oxycodone concentrations were 154-405-fold higher than in the brain, and in the repeated boluses group, the spinal cord oxycodone concentrations were 8-28-fold higher. These results indicate that epidural oxycodone may provide direct segmental spinal

analgesic efficacy. Interestingly, the median spinal cord oxycodone concentration was 460-fold higher in the infusion group than in the repeated boluses group, although the daily dose was only three-fold higher (1.2 $mg \cdot kg^{-1} \cdot 24\ h^{-1}$ vs. 0.4 $mg \cdot kg^{-1} \cdot 24\ h^{-1}$). Moreover, there was a moderate negative correlation between spinal cord oxycodone concentration and the time since last epidural bolus. These findings may indicate a relatively rapid clearance of oxycodone from the spinal cord.

As we hypothesised, oxycodone accumulation in the brain was observed also. The cortex/plasma-ratio of oxycodone was 4.3 after epidural infusion. After epidural boluses, the median cortical oxycodone concentration was 6.9 $ng \cdot g^{-1}$, while plasma concentrations were 2.3 $ng \cdot mL^{-1}$ or lower. These results of total oxycodone concentrations are consistent with previous experimental data in rats, where brain concentrations of oxycodone, measured with microdialysis, were three-fold higher than those in plasma after a single intravenous infusion (8,26). Recently, this result has been shown to be the case after six days of subcutaneous/intraperitoneal oxycodone administration in mice also. Consistent with our results, brain oxycodone concentrations were three-fold higher than those in plasma with a therapeutic plasma concentration of 80 $ng \cdot mL^{-1}$ (27).

On the contrary to our second hypothesis, CSF oxycodone concentration did not predict the tissue concentrations. Since it is not ethical to measure actual drug concentrations in the human brain and spinal cord during treatment, CSF concentrations are used as surrogate markers of CNS exposure (6,7,28). However, in the present study, CSF oxycodone had a strong, positive correlation with oxycodone concentrations in the cortex and spinal cord, and a moderate correlation to oxycodone in the thalamus. The median cortex/CSF -ratio of oxycodone concentration was 3 in the infusion group and 6 in the repeated boluses group,

although the range was extensive in the latter group, between 0.6 and 393. Moreover, the spinal cord concentrations were between 15 and 4845 times higher compared to CSF oxycodone in the infusion group and between 0.5 and 3240 times higher in the repeated boluses group. In humans, a single epidural bolus of oxycodone resulted in a 100-350-fold higher peak concentration of oxycodone in CSF compared to plasma (6,7). In the present study, CSF samples were collected from the cisterna magna and were mainly similar to plasma concentrations. This difference may be explained by an assumption that CSF is more dilute when the sample is collected far from the epidural injection site (6,7).

Noroxymorphone is another active metabolite of oxycodone. Noroxymorphone is assumed to penetrate the blood-brain/blood-cerebrospinal fluid barriers remotely (5,9,10). Very low concentrations of noroxymorphone were detected in nine of the 72 CNS tissue samples and nine of the 19 CSF samples, and in only one plasma sample. Thus, it is unlikely that noroxycodone or oxymorphone would have been metabolised to noroxymorphone in any significant amount in the CNS. This result is contradictory to what was proposed by McMillan and Tyndale as they extrapolated data with codeine and tramadol to cover the CNS metabolism of oxycodone via CYP2D6 enzymes (29).

Few high noroxycodone concentrations, up to 250 $ng \cdot g^{-1}$, were observed in the thalamic and spinal cord samples, but the concentrations in plasma and CSF were very low. An explanation for these high tissue and low CSF concentrations remains unclear. Recently, it was shown that CYP3A4 is expressed by BBB endothelial cells and by neurons in the brain (30). Oxycodone is metabolised to noroxycodone via CYP3A4, and thus, we assume that BBB and CNS tissue CYP3A4 expression may have contributed to noroxycodone tissue

concentrations. In humans, CSF noroxycodone concentrations have been modest after a single epidural oxycodone bolus (6,7).

Our study has some limitations. First, in humans oxycodone clearance is higher during pregnancy (31), and pharmacokinetics are likely affected during pregnancy in sheep, too. Therefore, pharmacokinetics of epidural oxycodone may differ in non-pregnant subjects. However, data on late pregnancy are essential because intrathecal opioids are commonly used in labour analgesia (31). Second, ewes were subjected to an acute hypoxaemia for 150 min with 60-min recovery period (normoxaemia). It has been shown that hypoxic hypoxia increases cerebral blood flow by 15% that may have affected the disposition of oxycodone. Cerebral blood flow returns to pre-experimental values relatively rapidly, in less than 1 min after return to normoxia. Thus, we assume that hypoxaemia challenge may have affected but not substantially on disposition of oxycodone into the CNS as the ewes were normocapnic during the whole experiment (32). Third, there could be some differences in oxycodone metabolism between sheep and humans – oxymorphone seems to be a more abundant metabolite in sheep than in humans (32). Fourth, some differences in the BBB exist. In the case of morphine, an active influx has been observed in sheep, but not in other species, including humans (33). Active transport of oxycodone across the BBB is supported by experimental data in a non-pregnant sheep model (34). One of the main limitations was that there was a lot of variance in the measured concentrations in both study groups, and this large variance was obtained both in the tissue concentrations and in the plasma and CSF concentrations. However, similar large between-subjects variance has been shown also in human studies after epidural oxycodone administration (6,7,35,36). We do not have explanation for this large variance and this warrants further studies. In summary, these data

should be considered as experimental and can be extrapolated to humans only with great caution.

Conclusions

In conclusion, the accumulation of oxycodone and oxymorphone, an active metabolite, in the CNS after epidural oxycodone administration was observed, and these concentrations were significantly higher than in plasma. These results support previous evidence of the active intake of oxycodone into the CNS. Furthermore, oxycodone and oxymorphone concentrations were relatively high in spinal cord tissue indicating that a direct spinal effect may contribute substantially to epidural analgesia with oxycodone. Contrary to our hypothesis, CSF oxycodone concentrations did not correlate to CNS tissue concentrations.

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Table 1

Oxycodone concentrations in plasma, brain, spinal cord and cerebrospinal fluid (CSF) after five days of epidural oxycodone by continuous infusion or repeated boluses.

Variable	**Total amount of oxycodone**	**Time from last bolus to sample**	**Oxycodone concentrations**					
	mg	hours	Plasma $ng \cdot mL^{-1}$	Cortex $ng \cdot g^{-1}$	Cerebellum $ng \cdot g^{-1}$	Thalamus $ng \cdot g^{-1}$	Spinal cord $ng \cdot g^{-1}$	CSF $ng \cdot mL^{-1}$
Infusion group								
Sheep 1	280		10.3	94.7	109	111	61,700	31.8
Sheep 2	160		5.2	42.6	42.7	52.9	34,200	16.9
Sheep 3	25		1.2	No sample	No sample	No sample	No sample	1.4
Sheep 4	290		10.7	57.2	35.1	61.2	52,800	10.9
Sheep 5	120		5.2	34.4	31.7	39.0	63,600	23.2
Sheep 6	320		7.7	9.5	50.3	11.7	1,090	3.1
Sheep 7	310		1.2	1.2	1.9	3.0	21.9	0.33
Sheep 8	350		19.6	1.3	6.6	10.9	278	18.5
Sheep 9	420		22.6	93.3	114	352	27,200	11.5
Sheep 10	330		18.3	59.3	74.6	156	24,000	16.9
Repeated boluses group								
Sheep 11	140	10	<LLoQ	36.5	13.1	4.9	562	0.21
Sheep 12	83	22	<LLoQ	4.0	1.7	<LLoQ	18.6	0.07
Sheep 13	110	1.3	0.42	178	252	171	79,900	109
Sheep 14	85	6.8	<LLoQ	6.9	7.7	3.2	17.3	No sample
Sheep 15	130	6.5	0.41	No sample	No sample	No sample	No sample	1.3
Sheep 16*	130	7.7	2.3	1.5	1.2	1.8	11.3	1.5
Sheep 17	130	11	0.43	82.6	68.1	119	344	0.21
Sheep 18	120	4.3	3.8	1.4	1.8	1.8	50.5	1.1
Sheep 19	120	6.3	<LLoQ	7.3	7.2	17.9	2500	0.77
Sheep 20*	100	11.8	<LLoQ	2.3	0.60	0.75	1.8	3.6

* Epidural catheter was in paravertebral space

Table 2
Concentrations of oxycodone metabolites in plasma, cortex, cerebellum, thalamus, spinal cord and CSF samples after epidural infusion or repeated boluses. Data are the median (number of cases with concentration above the lower limit of quantification) and range of those that could be quantified. Differences in the concentrations between the infusion and repeated boluses groups were analysed with the Mann-Whitney U-test.

Variable		**Plasma,** ng·mL^{-1}	**Cortex,** ng·g^{-1}	**Thalamus,** ng·g^{-1}	**Cerebellum,** ng·g^{-1}	**Spinal cord,** ng·g^{-1}	**CSF,** ng·mL^{-1}
Oxycodone	***Infusion group***						
	• Median	9.0 (n=10/10)	43 (n=9/9)	53 (n=9/9)	43 (n=9/9)	27,200 (n=9/9)	14 (n=10/10)
	• Range	1.2-23	1.2-95	(3.0-352)	1.9-114	22-63,600	0.3-32
	Repeated boluses						
	• Median	0.4 (n=5/10)	6.9 (n=9/9)	4.0 (n=8/9)	7.4 (n=9/9)	51 (n=9/9)	1.1 (n=9/9)
	• Range	0.4-3.8	1.4-178	0.75-171	0.6-252	1.8-79,900	0.07-109
p-value		0.008	0.49	0.14	0.094	0.04	0.028
Oxymorphone	***Infusion group***						
	• Median	4.0 (n=10/10)	26 (n=9/9)	19 (n=9/9)	29 (n=9/9)	110 (n=9/9)	11 (n=10/10)
	• Range	1.6-44	1.5-39	1.8-282	1.3-41	25-412	3.8-53
	Repeated boluses						
	• Median	0.48 (n=9/10)	5.0 (n=9/9)	5.4 (n=9/9)	6.2 (n=9/9)	27 (n=9/9)	2.6 (n=9/9)
	• Range	0.11-2.0	1.9-40	1.4-41	1.4-40	1.7-169	0.50-9.0
p-value		0.001	0.077	0.031	0.077	0.063	0.001
Noroxymorphone	***Infusion group***						
	• Median	0.22 (n=1/10)	- (n=0/9)	2.0 (n=2/9)	0.28 (n=1/9)	3.0 (n=3/9)	0.14 (n=7/10)
	• Range	-	-	1.7-2.3	-	0.97-3.4	0.12-0.32
	Repeated boluses						
	• Median	- (n=0/10)	0.82 (n=1/9)	0.44 (n=1/9)	- (n=0/9)	0.5 (n=1/9)	0.16 (n=2/9)
	• Range	-	-	-	-	-	0.10-0.21
Noroxycodone	***Infusion group***						
	• Median	0.20 (n=5/10)	- (n=0/9)	165 (n=3/9)	- (n=0/9)	56 (n=7/9)	0.24 (n=7/10)
	• Range	0.11-0.43	-	2.6-248	-	4.0-156	0.13-0.49
	Repeated boluses						
	• Median	- (n=0/10)	- (n=0/9)	- (n=0/9)	- (n=0/9)	18 (n=5/9)	0.22 (n=2/9)
	• Range	-	-	-	-	2.7-230	0.10-0.33

Figure legends

Figure 1. Flowchart. DOS = day of surgery; POD = postoperative day; CSF = cerebrospinal fluid; CNS = central nervous system