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Research Paper

Comparison of the Pharmacokinetics of Oxycodone and Noroxycodone in Male Dark Agouti and Sprague–Dawley Rats: Influence of Streptozotocin-Induced Diabetes

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Purpose. The aims of this study are to evaluate whether cytochrome P450 (CYP)2D1/2D2-deficient dark agouti (DA) rats and/or CYP2D1/2D2-replete Sprague–Dawley (SD) rats are suitable preclinical models of the human, with respect to mirroring the very low plasma concentrations of metabolically derived oxymorphone seen in humans following oxycodone administration, and to examine the effects of streptozotocin-induced diabetes on the pharmacokinetics of oxycodone and its metabolites, noroxycodone and oxymorphone, in both rodent strains.
Methods. High-performance liquid chromatography–electrospray ionization–tandem mass spectrometry was used to quantify the serum concentrations of oxycodone, noroxycodone, and oxymorphone following subcutaneous administration of bolus doses of oxycodone (2 mg/kg) to groups of nondiabetic and diabetic rats.
Results. The mean (±SEM) areas under the serum concentration *vs.* time curves for oxycodone and noroxycodone were significantly higher in DA relative to SD rats (diabetic, $p < 0.05$; nondiabetic, $p < 0.005$). Serum concentrations of oxymorphone were very low (<6.9 nM).
Conclusions. Both DA and SD rats are suitable rodent models to study oxycodone's pharmacology, as their systemic exposure to metabolically derived oxymorphone (potent μ-opioid agonist) is very low, mirroring that seen in humans following oxycodone administration. Systemic exposure to oxycodone and noroxycodone was consistently higher for DA than for SD rats showing that strain differences predominated over diabetes status.

KEY WORDS: diabetes; metabolism; oxycodone; pharmacokinetics; rat.

INTRODUCTION

Oxycodone produces dose-dependent analgesic and antinociceptive effects in humans and rats, respectively (1–5). Based on clinical studies in patients with postoperative and cancer-related pain, the analgesic potency of oxycodone is ~1.5 times higher than that of morphine (6–8). Because oxycodone's *O*-demethylated metabolite, oxymorphone, is a potent μ-opioid receptor agonist (9) that is ~10-fold more potent than morphine for the relief of chronic cancer-related pain (10), it was proposed that oxycodone may act as a prodrug for oxymorphone (1,9). However, more recent studies in humans have discounted this hypothesis because the plasma concentrations of metabolically derived oxymorphone are very low (<2.7 nM) (11–14), and oxycodone's pharmacodynamic actions in humans are correlated with the plasma concentrations of oxycodone rather than its metabolite, oxymorphone (11,12).

Confirming that oxycodone has intrinsic pain-relieving effects, intracerebroventricular administration of oxycodone was found to produce dose-dependent naloxone-sensitive antinociception in Sprague–Dawley and dark agouti (DA) rats (3,4), via a putative κ-opioid receptor mechanism (15), with a potency ~50% of that of intracerebroventricularly administered morphine (3,4). As serum oxymorphone concentrations in humans are very low, when oxycodone is given via oral, intramuscular, or intravenous routes of administration (11–14), it is important that this also occurs in rodent models used to study oxycodone's pharmacology. For this reason, studies of oxycodone antinociception, following systemic routes of administration, have often been undertaken in DA rats (4,16,17), as this strain is genetically deficient in cytochrome P450 (CYP)2D1/2D2 (18), the rat homologue

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ABBREVIATIONS: AUC, area under the serum concentration *vs.* time curve for parent drug or metabolite; AUC_{0-t}, area under the serum concentration *vs.* time 0, until the time of the last quantifiable serum drug/metabolite concentration; $AUC_{t-\infty}$, area in the tail of the serum concentration *vs.* time curve for parent drug or metabolite; β, terminal elimination rate constant; C_{last}, last quantifiable serum drug/metabolite concentration; C_{max}, maximum serum drug/metabolite concentration; CNS, central nervous system; CYP, cytochrome P450; DA, dark agouti; HPLC–ESI–MS–MS, high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry; SD, Sprague–Dawley; SEM, standard error of the mean; STZ, streptozotocin; $t_{1/2\ abs}$, absorption half-life; $t_{1/2\ elim}$, terminal elimination half-life; T_{max}, time of maximum serum drug/metabolite concentration.

of the human CYP2D6 isozyme, which catalyzes the *O*-demethylation of oxycodone to oxymorphone (19).

Studies using recombinant human CYPs as well as human liver microsomes have confirmed that oxycodone is metabolized principally by oxidative *N*-demethylation to noroxycodone, and that this pathway is catalyzed by both CYP3A4 and CYP3A5 (19). In contrast, *O*-demethylation of oxycodone to oxymorphone by human liver microsomes represented an 8-fold lesser pathway (19), consistent with its low plasma concentrations (<2.7 nM) in humans after oral, intramuscular, or intravenous oxycodone administration (11–14). After oral oxycodone dosing (0.22 mg/kg) in humans, the peak plasma concentrations of oxycodone and noroxycodone were 121 and 48 nM, respectively, and ~8% of the dose was eliminated as either unconjugated (~6%) or conjugated (~2%) oxycodone (14). Additionally, unconjugated noroxycodone was found to be the predominant urinary metabolite, with oxymorphone eliminated principally as conjugated species that accounted for <5% of the oxycodone dose (14).

Although the CYP2D1/2D2-deficient DA rat seems to be a suitable rodent model for studies of oxycodone antinociception following systemic routes of administration, there are no pharmacokinetic studies that have evaluated the validity of this assumption. Thus, the present investigation was undertaken to examine this issue. Because SD rats are frequently used in preclinical studies to investigate the antinociceptive effects of opioid drugs, the pharmacokinetics of oxycodone and its oxidative metabolites were also investigated in SD rats for comparative purposes.

Laboratory rodents treated with streptozotocin (STZ) to induce diabetes become hyporesponsive to the antinociceptive effects of μ-opioid agonists (20–24), but seem to remain normally responsive to δ- and κ-opioid agonists (20). Extending previous findings in diabetic rodents, recent work by our group has shown that the efficacy of morphine is abolished by 12 weeks post-STZ administration (24). By contrast, the antinociceptive efficacy of the putative κ-opioid agonist, oxycodone, was maintained in DA rats over a 24-week post-STZ study period (24), in a manner similar to the findings of two recent clinical trials showing that orally administered oxycodone was significantly more effective than placebo for the relief of painful diabetic neuropathy (25,26). Although oxycodone maintained full antinociceptive efficacy in diabetic rats, there was an ~2-fold decrease in oxycodone's potency by 3 weeks after STZ administration and an additional ~2-fold decrease in potency by 24 weeks (24). Hence, the present study also investigated whether this loss of potency reflects diabetes-induced changes in the pharmacokinetics and/or metabolism of oxycodone in DA rats, 3 weeks after the induction of diabetes with STZ. Additionally, for comparative purposes, the pharmacokinetics and metabolism of oxycodone were also investigated in 3-week post-STZ diabetic SD rats.

MATERIALS AND METHODS

Materials

Oxycodone hydrochloride used in the pharmacokinetic investigations was a generous gift from Tasmanian Alkaloids Pty. Ltd. (Westbury, Australia). Oxycodone hydrochloride United States Pharmacopeia (Macfarlan Smith Ltd., Edinburgh, UK), noroxycodone (Du Pont Merck, Wilmington, DE, USA), and oxymorphone (Sigma-Aldrich, Sydney, Australia) were used for the preparation of analytical standards. Deuterated (d_3) analogs of oxycodone, noroxycodone, and oxymorphone (Cambridge Isotope Laboratories, Andover, MS, USA) were used as internal standards. Xylazine hydrochloride (Xylazil-20™), tiletamine HCl/zolazepam HCl combined (Zoletil 100®), and topical antibiotic powder (neomycin sulfate 2.5 mg, sulfacetamide sodium 100 mg, nitrofurazone 2 mg, phenylmercuric nitrate 0.05 mg, and benzocaine 5 mg, in 50-g soluble powder) were purchased from Provet Qld Pty Ltd (Brisbane, Australia). Sodium benzylpenicillin (Benpen™) was purchased from the Royal Brisbane Hospital Pharmacy (Brisbane, Australia). Isoflurane (Isoflo™) was purchased from Abbott Australasia (Sydney, Australia), whereas medical grade CO_2 and O_2 were purchased from BOC Gases Ltd. (Brisbane, Australia). Streptozotocin, citric acid, and trisodium citrate were purchased from Sigma-Aldrich. Blood glucose meters (Precision Q.I.D.™) and glucose testing electrodes (Precision Plus™) were purchased from the Campus Pharmacy at The University of Queensland (Brisbane, Australia). High-performance liquid chromatography grade acetonitrile and methanol, AR grade formic acid and hydrochloric acid, and heparinized saline (Astra Zenica, Sydney, Australia) were obtained from The University of Queensland's Biological and Chemical Sciences Store (Brisbane, Australia).

Animals

Adult male DA and SD rats were purchased from the Central Animal Breeding House and the Herston Medical Research Centre, respectively, both located at The University of Queensland. Rats were housed in a temperature-controlled environment (21 ± 2°C) with a 12/12-h light/dark cycle; food and water were available *ad libitum*. Rats were given at least 3 days to acclimatize before any surgery or experimentation was commenced. Ethical approval was obtained from The University of Queensland's Animal Experimentation Ethics Committee.

Induction of Diabetes

Pancreatic beta cells were selectively destroyed by intravenous administration of STZ. Initially, rats were lightly anesthetized with a mixture (50:50%) of carbon dioxide/oxygen, before the combined intraperitoneal administration of the anesthetic agents Zoletil 100® (0.09 mL/200 g) and Xylazil-20™ (0.1 mL/200 g). A polypropylene cannula (0.9 mm O.D. × 0.5 mm I.D.), prefilled with heparinized saline (50 IU/mL), was subsequently inserted ~1.5 cm into the jugular vein at the bifurcation of the internal and external branches. After the correct placement of the cannula was confirmed, it was secured into position with surgical silk. Streptozotocin dissolved in 0.3 mL of ice-cold sodium citrate buffer (20 mM, pH 4.5) was then administered via the cannula at 80 mg/kg in DA rats and 75 mg/kg in SD rats. Topical antibiotic powder and subcutaneous benzylpenicillin (60 mg) were administered to provide postoperative antimicrobial cover.

The presence of diabetes was confirmed if water intake was found to exceed 100 mL/day and blood glucose concentrations were ≥15 mM by day 10 and at 3 weeks following STZ administration. Rats not meeting these criteria were euthanized.

Femoral Artery Cannulation

Rats were lightly anesthetized with a 50:50% mixture of carbon dioxide/oxygen to facilitate application of an anesthetic mask. Anesthesia was subsequently maintained using 3% isoflurane in 97% oxygen, delivered via a calibrated Trilene™ vaporizer. Using blunt dissection, the femoral artery was exposed and carefully separated from the femoral vein and the sciatic nerve. A polypropylene cannula (0.9 mm O.D. × 0.5 mm I.D.), prefilled with heparinized saline (50 IU/mL), was then inserted ~1 cm into the femoral artery, at the bifurcation of the internal and external iliac arteries. A loop of tubing was tied into a subcutaneous pocket at the incisional site to allow adequate flexion during normal movement. The femoral artery cannula was subsequently exteriorized between the scapulae and protected by a stainless-steel spring sutured into a subcutaneous pocket. Topical antibiotic powder and subcutaneous benzylpenicillin (60 mg) were administered postoperatively for antimicrobial cover. Rats were monitored postoperatively and housed singly in metabolic cages; food and water were available *ad libitum*. Heparinized saline (50 IU/mL) was infused through the cannula overnight at 375 μL/h to maintain patency.

Drug Administration, Blood Sample Collection, and Handling

Oxycodone hydrochloride was dissolved in deionized water. On the day following femoral artery cannulation, each rat received a 2-mg/kg bolus dose of oxycodone in a volume of 200 μL by subcutaneous injection into the back of the neck. This dose was the approximate ED_{50} for the relief of tactile allodynia determined in a previous pharmacodynamic study undertaken in similar diabetic rats at 3 weeks post-STZ administration by our laboratory (24). Blood samples (0.2 mL) were collected into 1.5-mL polypropylene tubes from the indwelling arterial cannula, immediately predose, and at 0.083, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 h following oxycodone administration. Blood samples were left sitting on ice until the conclusion of the study, when they were centrifuged at 4,000 × *g* for 10 min. Serum was subsequently transferred to fresh polypropylene tubes and stored frozen (−20°C) until analysis.

HPLC–ESI–MS–MS Instrumentation and Chromatography Conditions

The HPLC system configuration for delivery of mobile phase and sample injection consisted of Dual Shimadzu LC-10AT pumps, with an SCL-10A system controller, and an Agilent series 1100 autoinjector. Chromatographic separations were performed on an Agilent Zorbax SB-C18 (5 μm, 2.1 mm I.D. × 50 mm) column, with Phenomenex C18 (4.0 × 2.0 mm) security-guard cartridges acting as the precolumn; the sample injection volume was 50 μL. The mobile phase consisted of two components and was delivered in a stepwise gradient fashion at 0.15 mL/min. Component A was a 0.1 % (v/v) solution of formic acid, whereas component B consisted of 90% (v/v) methanol/water containing 0.1% (v/v) formic acid. Initially, component A was delivered for 0.5 min, then components B/A (11:89) were delivered for 1.6 min, followed by components B/A (50:50) for 2.6 min; component A was subsequently delivered for an additional 3.4 min; the total run time was 8.1 min. The column effluent was split, so that only 10% of the flow was introduced into the MS system. At the conclusion of an assay, the column was washed with 100% acetonitrile at 0.2 mL/min for ~6–8 h. The guard column was replaced prior to commencing a new assay.

A PE Sciex API 3000 triple quadrupole mass spectrometer, operating in electrospray mode with a standard nebulizer ion-spray source, was used for sample analysis. The sensitivity was optimized by infusing a solution containing oxycodone (2.85 μM), noroxycodone (3.32 μM), and oxymorphone (3.47 μM) in mobile phase components B/A (11:89) directly into the electrospray source at 20 μL/min using a Harvard 11 syringe pump. The nebulizer gas (nitrogen) flow rate was 10 L/min, and the ion spray source voltage was 4.6 kV. Orifice and ring voltages were set at 60 and 200 V, respectively. Nitrogen was used as the collision gas, and the collision cell energy was 40 V. Deuterated (d_3) analogs of each compound were used as the internal standards. The following ion transitions for each compound and its deuterated analog were monitored with a dwell time of 200 ms: oxycodone, m/z = 316.2/241.1; d_3-oxycodone, m/z = 319.2/244.2; noroxycodone, m/z = 302.2/227.2, d_3-noroxycodone, m/z = 305.2/230.2; oxymorphone, m/z = 302.2/227.2, d_3-oxymorphone, m/z = 305.2/230.2. The retention times for oxycodone, noroxycodone, and oxymorphone were 5.5, 5.6, and 5.1 min, respectively.

Drug and Metabolite Analysis

All of the standard solutions and their serial dilutions were prepared in 18.0 MΩ deionized water. Stock solutions of oxycodone hydrochloride, noroxycodone, and oxymorphone were prepared in cetrimide-treated volumetric flasks and stored at 4°C. Oxycodone hydrochloride was dissolved in deionized water, whereas noroxycodone and oxymorphone were dissolved in 0.1 M HCl. On the day of each assay, serial dilutions of each stock solution were prepared in 1.5-mL polypropylene tubes. A stock assay standard solution was prepared containing oxycodone (2.85 μM), noroxycodone (3.32 μM), and oxymorphone (3.47 μM). Serial dilutions were subsequently performed to prepare the working assay standards (oxycodone, 5.7–569.0 nM; noroxycodone, 6.6–664.4 nM; oxymorphone, 6.9–694.4 nM). The stock internal standard solution was prepared in a 10-mL cetrimide-treated volumetric flask and contained 3.14 μM d_3-oxycodone, 3.29 μM d_3-noroxycodone, and 3.29 μM d_3-oxymorphone. The working internal standard solution was subsequently prepared by a one in ten dilution of the stock internal standard solution, in a 15-mL polypropylene tube. Both stock and working internal standard solutions were stored at 4°C.

Assay and quality control standards (triplicate replicates) were prepared by adding appropriate aliquots (50 μL) of the working assay standards to 1.5-mL polypropylene

tubes. Aliquots of the working internal standard (50 μL) solution and rat serum (50 μL) were then added to each tube, and the tubes were vortex-mixed. Serum samples were allowed to thaw at room temperature, briefly vortex-mixed, and centrifuged at 10,280 × *g* for 5 min. Serum aliquots (50 μL) were then transferred to 1.5-mL polypropylene tubes, internal standard (50 μL) and deionized water (50 μL) were added, and the tubes briefly vortex-mixed. Acetonitrile (300 μL) was subsequently added to all the tubes. The tubes were then briefly vortex-mixed, allowed to stand for ~30–60 min at 4°C to facilitate protein precipitation, and then centrifuged at 20,160 × *g* for 5 min. The supernatants were transferred to fresh 1.5-mL polypropylene tubes, which were placed in a Savant rotatory evaporative centrifuge until the volume was reduced to ~90 μL. Formic acid (1% v/v, 10 μL) was subsequently added to each tube, and the tubes were briefly vortex-mixed, before being centrifuged again at 20, 160 × *g* for 5 min. The supernatants were then transferred to 250-μL polypropylene inserts for analysis.

Chromatograms were integrated using PE Sciex software (Mac Quan version 1.6). Linear regression of the peak area ratios for each analyte relative to its deuterated analog *vs.* analyte concentration was performed to produce standard curves; inverse prediction was used to calculate sample analyte concentrations. Between-day accuracy and precision were determined for quality control standards at the lower, middle, and upper limits of quantitation. Accuracy was expressed as the percent deviation (% Dev) of the mean analyte concentrations from their nominal concentrations in each quality control standard. Precision was expressed as the percent coefficient of variation (% CV) for each quality control standard.

Data Analysis

The maximum values for serum drug and metabolite concentrations (C_{max}) as well as the time to reach the maximum concentration (T_{max}) were determined for each concentration-time data set by Microsoft Excel Function Arguments. The terminal elimination half-life ($t_{1/2\ elim}$) was calculated using the equation:

$$t_{1/2\ elim} = \ln 2/\beta \quad (1)$$

where β is the terminal elimination rate constant, estimated by linear regression of the terminal linear portion of the ln-transformed serum concentration *vs.* time data set from each rat (27). The absorption half-life ($t_{1/2\ abs}$) was calculated from the absorption rate constant (k_a) as shown below.

$$t_{1/2\ abs} = \ln 2/k_a \quad (2)$$

Values of k_a were calculated by solving the equation shown below, describing T_{max} in a linear one-compartment model, for k_a using an iterative nonlinear regression technique.

$$T_{max} = \ln (k_a/\beta)/(k_a - \beta) \quad (3)$$

The area under the serum concentration *vs.* time curve (AUC), from time zero to the time of the last quantifiable serum drug or metabolite concentration (C_{last}), was estimated using trapezoidal integration (AUC_{0-t}) (27). The area in the tail of the curve ($AUC_{t-\infty}$) was estimated from C_{last} to infinity by the equation:

$$AUC_{t-\infty} = C_{last}/\beta(27) \quad (4)$$

The total area under the curve, $AUC_{0-\infty}$, was determined by addition of the AUC_{0-t} and $AUC_{t-\infty}$ values. Serum concentrations and pharmacokinetic parameter estimates were reported as the mean ± standard error of the mean (SEM) values. The level of statistical significance for between-group comparisons of pharmacokinetic parameter estimates was determined by the Mann–Whitney *U*-test (GraphPad™ Prism version 3).

RESULTS

Assay Performance

Assay standard calibration curves were highly linear over the standard concentration ranges used for each analyte (oxycodone, 5.7–284.5 nM, $R^2 \geq 0.9995$; noroxycodone, 6.6–332.2 nM, $R^2 \geq 0.9998$; oxymorphone, 6.9–347.2 nM, $R^2 \geq 0.9996$). Between-day accuracy and precision were ≤6.8 and 18.0% respectively, for the assay quality control standards at the lower limit of quantitation. For the assay quality control standards at the upper limit of quantitation, between-day accuracy and precision were ≤1.8 and 3.4%, respectively (Table I).

Serum Concentrations of Oxycodone, Noroxycodone, and Oxymorphone

The mean (±SEM) serum oxycodone and noroxycodone concentrations determined in groups of nondiabetic DA and SD rats are shown in Fig. 1, whereas the mean (±SEM) serum oxycodone and noroxycodone concentrations for groups of diabetic DA and SD rats are shown in Fig. 2. Irrespective of rat strain or diabetes status, the serum concentrations of metabolically derived oxymorphone were below the lower limit of quantification (<6.9 nM) over the 6-h period that followed subcutaneous oxycodone administration.

Nondiabetic Dark Agouti *vs.* Nondiabetic Sprague–Dawley Rats

Mean (±SEM) values for the body weights of nondiabetic DA and SD rats were 237 (±6) and 263 (±10) g, respectively. The mean (± SEM) pharmacokinetic parameter estimates for nondiabetic DA and SD rats are shown in Table II. Values of C_{max} for oxycodone were higher in nondiabetic DA rats (908 ± 222 nM) compared with nondiabetic SD rats (577 ± 100 nM), but this difference did not reach statistical significance ($p > 0.05$) owing to the small sample size. However, the mean value of T_{max} for oxycodone was significantly longer ($p < 0.02$) in nondiabetic DA rats (0.83 ± 0.05 h) compared with nondiabetic SD rats (0.55 ± 0.05 h). Similarly, the mean value of $t_{1/2\ elim}$ for oxycodone in nondiabetic DA rats (1.05 ± 0.10 h) was significantly longer ($p < 0.005$) than for nondiabetic SD rats (0.52 ± 0.04 h),

Table I. Between-Day Accuracy and Precision Data for Oxycodone, Noroxycodone, and Oxymorphone from Quality Control Standards Assayed in Triplicate Within Each Assay

	Oxycodone (nM)			Noroxycodone (nM)			Oxymorphone (nM)		
Group	5.7	57.7	284.5	6.6	66.4	332.2	6.9	69.4	347.2
Nondiabetic dark agouti (DA) rats (n = 12)									
Accuracy	2.6	2.1	1.8	6.8	1.3	0.2	−1.8	0.4	0.0
Precision	8.5	2.7	3.4	18.0	2.9	1.5	11.9	3.1	3.4
Nondiabetic Sprague–Dawley (SD) rats (n = 12)									
Accuracy	−3.3	0.4	−0.1	−0.4	0.7	0.2	−0.9	1.1	−0.1
Precision	14.7	4.2	1.3	9.4	2.7	1.7	6.5	1.2	1.0
Diabetic DA rats (n = 9)									
Accuracy	0.6	0.0	−0.8	4.5	1.5	0.3	2.1	−0.7	−0.8
Precision	8.7	2.7	1.9	6.3	1.8	0.8	8.5	2.4	1.6
Diabetic SD rats (n = 9)									
Accuracy	3.9	1.5	0.5	4.8	1.1	0.2	−2.5	−0.6	−1.0
Precision	5.4	2.4	1.4	12.3	1.3	1.2	4.7	3.1	1.3

Accuracy was expressed as the percent deviation (% Dev) of the mean analyte concentrations from their nominal concentrations in each quality control standard. Precision was expressed as the percent coefficient of variation (% CV) for each quality control standard.

but there was no significant difference ($p > 0.05$) in the mean values for $t_{1/2\ abs}$ between nondiabetic DA and SD rats. Mean values of AUC_{0-t} and $AUC_{0-\infty}$ for oxycodone were 2.3-fold larger ($p < 0.005$) in nondiabetic DA rats than in nondiabetic SD rats.

The mean value of C_{max} for noroxycodone in nondiabetic DA rats (276 ± 76 nM) was 3.5-fold higher ($p < 0.005$) compared with nondiabetic SD rats (78 ± 14 nM). Likewise, the corresponding mean value of T_{max} for noroxycodone in nondiabetic DA rats (1.50 ± 0.13 h) was significantly longer

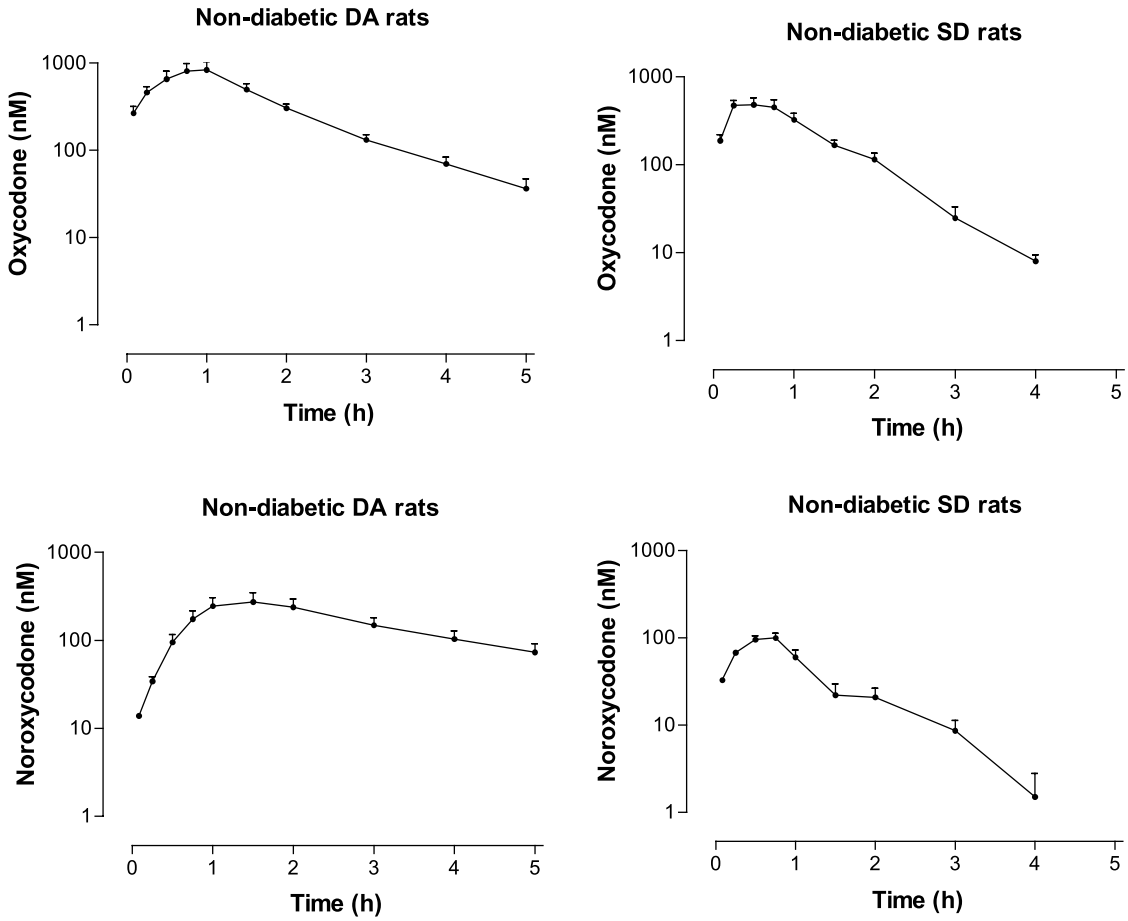

Fig. 1. Mean (±SEM) serum concentrations of oxycodone and noroxycodone in nondiabetic Dark Agouti (left panels, n = 6) and Sprague–Dawley (right panels, n = 5) rats following the administration of single bolus subcutaneous doses of oxycodone (2 mg/kg).

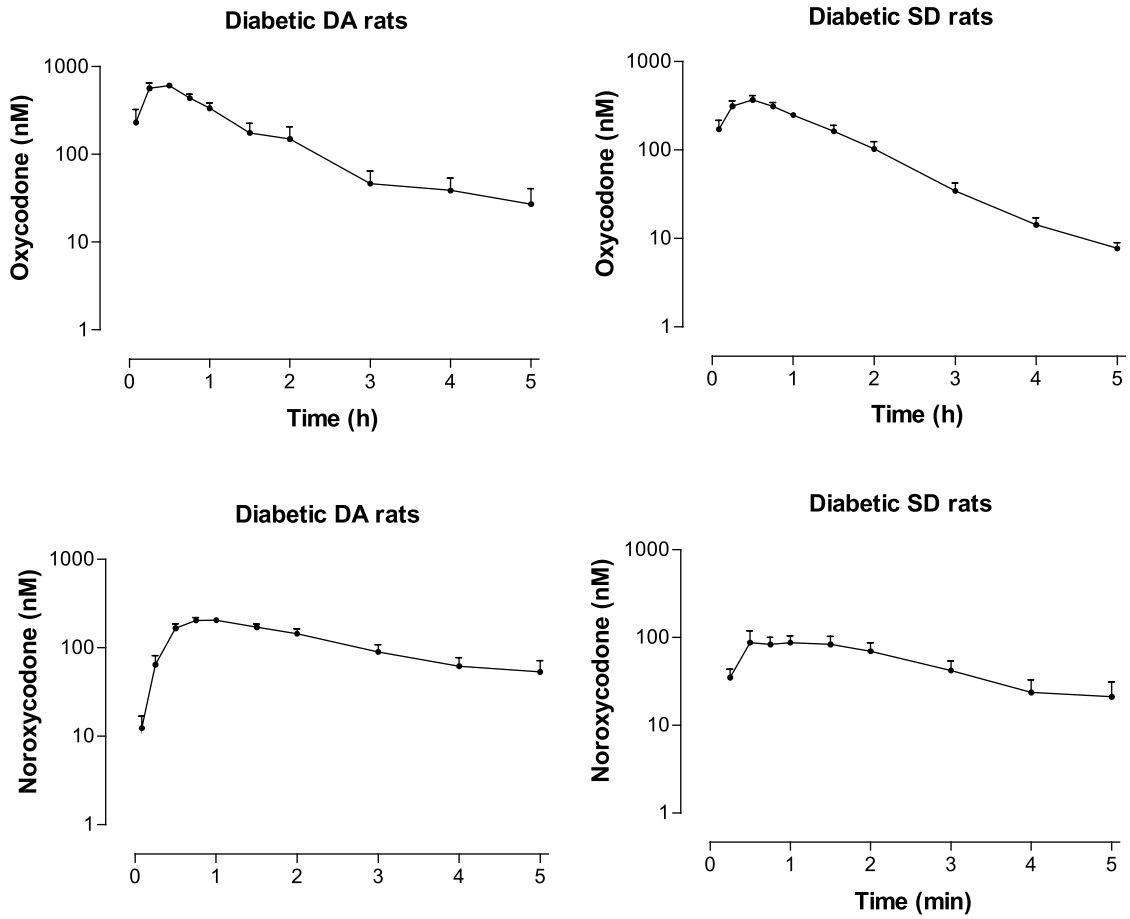

Fig. 2. Mean (±SEM) serum concentrations of oxycodone and noroxycodone in 3-week post-STZ diabetic dark agouti (left panels, $n = 5$) and Sprague–Dawley (right panels, $n = 6$) rats following the administration of single bolus subcutaneous doses of oxycodone (2 mg/kg).

($p < 0.01$) than that for nondiabetic SD rats (0.85 ± 0.06 h). Similarly, the mean value of $t_{1/2\ elim}$ for noroxycodone was significantly longer ($p < 0.02$) in nondiabetic DA rats (1.92 ± 0.23 h) compared with nondiabetic SD rats (1.06 ± 0.14 h). Mean values of AUC_{0-t} and $AUC_{0-\infty}$ for noroxycodone were ~6-fold larger ($p < 0.005$) in nondiabetic DA rats compared with nondiabetic SD rats. The mean value of the noroxycodone/oxycodone $AUC_{0-\infty}$ ratio was significantly larger (2.6-fold, $p < 0.005$) in nondiabetic DA rats (0.58 ± 0.08) compared with nondiabetic SD rats (0.22 ± 0.02). Finally, the observation that the mean T_{max} values for noroxycodone were 1.8-fold larger than for oxycodone, in both nondiabetic DA ($p < 0.005$) and SD rats ($p < 0.02$), suggests that the rate of formation of noroxycodone from oxycodone may be the rate-limiting step with respect to its elimination in both DA and SD rats. However, future studies documenting the pharmacokinetics of systemically administered noroxycodone itself are required to distinguish between this notion and the possibility that the elimination of noroxycodone is the rate-limiting step with respect to the clearance of oxycodone from the systemic circulation.

Diabetic Dark Agouti *vs.* Diabetic Sprague–Dawley Rats

Mean (±SEM) values for the body weights of diabetic DA and SD rats were 200 (±10) and 320 (±22) g, respectively. The mean value of $t_{1/2\ abs}$ for oxycodone was longer in diabetic SD rats (0.30 ± 0.11) compared with diabetic DA rats (0.17 ± 0.03), but the values were not significantly different ($p > 0.05$). The mean (±SEM) pharmacokinetic parameter estimates for diabetic DA and SD rats are shown in Table II. Despite the fact that the absolute dosages (mg) of oxycodone administered to diabetic DA rats were only ~60% of those administered to diabetic SD rats because of the heavier body weight of the latter group, the mean value of C_{max} for oxycodone was 1.7-fold higher ($p < 0.02$) in diabetic DA rats (656 ± 66 nM) compared with diabetic SD rats (386 ± 42 nM). Mean values of AUC_{0-t} and $AUC_{0-\infty}$ for oxycodone were also ~1.5-fold larger ($p < 0.05$) in diabetic DA rats compared with diabetic SD rats.

The mean value of C_{max} for noroxycodone in diabetic DA rats (214 ± 11 nM) was approximately twice that for diabetic SD rats (103 ± 28 nM), but this difference did not reach statistical significance ($p > 0.05$) possibly because of the small sample size. However, the mean value of $AUC_{0-\infty}$ for noroxycodone was significantly larger (2.8-fold, $p < 0.05$) in diabetic DA rats compared with diabetic SD rats. The mean value of the noroxycodone/oxycodone $AUC_{0-\infty}$ ratio for diabetic DA rats was 1.7-fold higher than that for diabetic SD rats, but similarly, it did not reach statistical significance ($p > 0.05$).

Table II. Mean (±SEM) Pharmacokinetic Parameter Estimates for Oxycodone and Its Principal Oxidative Metabolite, Noroxycodone, in Groups of Nondiabetic and 3-Week Post-STZ Diabetic DA and SD Rats, Following the Administration of Single Bolus Subcutaneous Doses of Oxycodone (2 mg/kg)

Pharmacokinetic parameter	Nondiabetic DA (n = 6)	Diabetic DA (n = 5)	Nondiabetic SD (n = 5)	Diabetic SD (n = 6)
Oxycodone				
C_{max} (nM)	908 ± 222	656 ± 66	577 ± 100	386 ± 42[h]
T_{max} (h)	0.83 ± 0.05[b,c]	0.45 ± 0.05	0.55 ± 0.05	0.54 ± 0.12
$t_{1/2\ abs}$ (h)	0.37 ± 0.05[a]	0.17 ± 0.03	0.35 ± 0.07	0.30 ± 0.11
$t_{1/2\ elim}$ (h)	1.05 ± 0.10[e]	0.89 ± 0.26	0.52 ± 0.04	0.69 ± 0.08
AUC_{0-t} (nmol h/L)	1546 ± 218[e]	849 ± 130	668 ± 71	556 ± 38[g]
$AUC_{0-\infty}$ (nmol h/L)	1581 ± 224[e]	896 ± 155	675 ± 70	563 ± 39[g]
Noroxycodone				
C_{max} (nM)	276 ± 76[e]	214 ± 11	78 ± 14	103 ± 28
T_{max} (h)	1.50 ± 0.13[a,d]	0.90 ± 0.06	0.85 ± 0.06	1.00 ± 0.17
$t_{1/2\ elim}$ (h)	1.92 ± 0.23[c]	2.03 ± 0.27	1.06 ± 0.14	1.24 ± 0.17
AUC_{0-t} (nmol h/L)	821 ± 192[e]	605 ± 81	143 ± 24	259 ± 74
$AUC_{0-\infty}$ (nmol h/L)	995 ± 245[e]	796 ± 192	156 ± 26	289 ± 88[g]
Noroxycodone/oxycodone				
$AUC_{0-\infty}$ ratios	0.58 ± 0.08[e]	0.85 ± 0.10	0.22 ± 0.02	0.51 ± 0.17[f]

Nondiabetic DA *vs.* diabetic DA: [a] $p < 0.01$; [b] $p < 0.005$.
Nondiabetic DA *vs.* nondiabetic SD: [c] $p < 0.02$; [d] $p < 0.01$; [e] $p < 0.005$.
Diabetic SD *vs.* nondiabetic SD: [f] $p < 0.005$.
Diabetic SD *vs.* diabetic DA: [g] $p < 0.05$; [h] $p < 0.02$.

Nondiabetic *vs.* Diabetic Dark Agouti Rats

The mean value of T_{max} was significantly longer in nondiabetic compared with diabetic DA rats, for both oxycodone (1.8-fold, $p < 0.005$) and noroxycodone (1.7-fold, $p < 0.01$). Similarly, the mean value for $t_{1/2\ abs}$ of oxycodone was also significantly longer ($p < 0.01$) in nondiabetic DA rats compared with diabetic DA rats. However, although the mean value of the noroxycodone/oxycodone $AUC_{0-\infty}$ ratio was 1.5-fold higher in diabetic DA rats relative to nondiabetic DA rats, this trend did not reach statistical significance ($p > 0.05$).

Nondiabetic *vs.* Diabetic Sprague–Dawley Rats

The mean noroxycodone/oxycodone $AUC_{0-\infty}$ ratio was significantly larger (2.3-fold, $p < 0.005$) in diabetic SD rats compared with nondiabetic SD rats.

DISCUSSION

To the best of our knowledge, this is the first study to show that noroxycodone is the principal circulating metabolite of oxycodone in the rat, which is consistent with findings from previous studies in dogs (28), as well as several earlier studies in humans (11–14). Interestingly, there were significant quantitative strain-related differences in the pharmacokinetics of oxycodone and noroxycodone between DA and SD rats. Hence, it is plausible that strain-related differences in opioid pharmacokinetics may contribute, at least in part, to the often-marked between-strain differences in the pharmacodynamic effects of opioid analgesics in rats and mice (29–32).

In the present study, the systemic exposure (AUC values) to oxycodone and noroxycodone was significantly higher for DA rats compared with SD rats, irrespective of their diabetes status. The $t_{1/2\ elim}$ values were also significantly longer for both oxycodone and noroxycodone in nondiabetic DA rats compared with nondiabetic SD rats, and a similar trend was also apparent between the respective diabetic groups. However, the values of $t_{1/2\ abs}$ for oxycodone did not differ significantly in nondiabetic DA and SD rats, or diabetic DA and SD rats. Thus, strain-related differences in the absorption of oxycodone following subcutaneous administration were not apparent.

The increased systemic exposure to oxycodone and noroxycodone found herein in DA rats is consistent with their well-documented genetic CYP2D1/2D2 deficiency, which prevents significant *O*-demethylation of oxycodone to oxymorphone occurring in this strain (18). Furthermore, the ~3-fold higher serum noroxycodone/oxycodone AUC ratio values for nondiabetic DA rats compared with nondiabetic SD rats suggests that CYP2D1/2D2 deficiency in the DA rat is associated with a compensatory increase in the *N*-demethylation of oxycodone to noroxycodone. This notion is supported by the findings of a human study, in which quinidine-induced impairment of CYP2D6 (homologue of rat CYP2D1/2D2) metabolism, in CYP2D6-extensive metabolizers, resulted in a compensatory marked increase in plasma noroxycodone concentrations (12). In the CYP2D1/2D2-replete SD rat, however, concurrent rapid clearance of any metabolically derived oxymorphone may have occurred, and this possibly may have also contributed to the lower systemic exposure to oxycodone seen in SD rats compared with DA rats.

Overall, in the present study, between-strain differences in the pharmacokinetics of oxycodone and its major metabolite, noroxycodone, in DA and SD rats were quantitatively more important than the influence of diabetes by 3 weeks

after diabetes induction with STZ. Importantly, between strain differences in $t_{1/2\ abs}$ values for oxycodone were not apparent. The significantly lower value of $t_{1/2\ abs}$ for oxycodone, in diabetic compared with nondiabetic DA rats, is consistent with the lower body weights of diabetic DA rats. However, $t_{1/2\ abs}$ values for oxycodone did not differ significantly between diabetic and nondiabetic SD rats, presumably because of the larger and more variable body weights in diabetic SD rats, compared with nondiabetic SD rats. Dark agouti and Sprague–Dawley rats differ in their sensitivity to STZ, and for this reason, a lower dose of STZ was administered to SD rats. As a consequence, the diabetic state was less severe in SD rats than in DA rats, and SD rats gained some weight over the 3-week post-STZ period, whereas DA rats did not.

Although there was a trend for higher exposure to oxycodone and noroxycodone in nondiabetic than diabetic rats, this difference did not reach statistical significance. By contrast, a similar study comparing the pharmacokinetics of morphine, in nondiabetic and diabetic SD rats, found that the clearance and volume of distribution of morphine were increased significantly by 4 weeks after the induction of diabetes with STZ (22). Interestingly, in the present study, the mean noroxycodone/oxycodone $AUC_{0-\infty}$ ratio was significantly higher ($p < 0.005$) in diabetic compared with nondiabetic SD rats, and a similar trend was also apparent in DA rats. This suggests that by 3 weeks after STZ-diabetes induction, there may have been an increase in the metabolism of oxycodone to noroxycodone in STZ-diabetic rats, relative to their nondiabetic counterparts.

Irrespective of rat strain or diabetes status, the serum concentrations of metabolically derived oxymorphone were very low (<6.9 nM) in all rats over the 6-h period that followed subcutaneous oxycodone administration, in a manner analogous to the low serum concentrations of metabolically derived oxymorphone (<0.7 nM) produced by single bolus doses of oral oxycodone in human CYP2D6-extensive metabolizers (12). In DA rats, this finding is consistent with their genetic CYP2D1/2D2 deficiency (18), whereas for SD rats, it is plausible that concurrent rapid conjugation of any metabolically derived oxymorphone may have also contributed (33). Thus, the low systemic exposure to metabolically derived oxymorphone, found in both DA and SD rats herein, is analogous to that seen in the plasma of humans administered with oxycodone via oral, intramuscular, and intravenous routes (11–14). Furthermore, it is also consistent with the findings of recent *in vitro* human liver microsomal studies, showing that the oxidative metabolism of oxycodone to oxymorphone is an 8-fold lesser pathway than the *N*-demethylation of oxycodone to noroxycodone (19).

In healthy human volunteer CYP2D6-extensive metabolizers, quinidine-induced inhibition of CYP2D6 metabolism did not significantly alter the subjective pharmacodynamic effects of oxycodone (12), thus providing indirect evidence that any circulating metabolically derived oxymorphone makes an insignificant contribution to oxycodone's analgesic effects. This notion is supported by the findings of an earlier study showing that the levels of analgesia experienced by patients after oral oxycodone administration were highly correlated with the plasma oxycodone concentrations, but not with the corresponding (very low) oxymorphone concentrations (11). In previous work, pretreatment of CYP2D1/2D2 genetically deficient DA rats with quinine or quinidine did not significantly alter oxycodone's antinociceptive effects, relative to untreated DA rats (16,17), confirming that oxymorphone does not contribute to the antinociceptive effects of oxycodone in the DA rat.

When the above findings are considered in association with the very low serum oxymorphone concentrations (<6.9 nM) that were found in DA rats in the present study, they corroborate the validity of the DA rat as a suitable model of the human for the study of oxycodone's pharmacology. Similarly, the very low serum concentrations of metabolically derived oxymorphone (<6.9 nM) found in SD rats herein indicate that from the pharmacokinetic perspective, the SD rat also seems to be a suitable rodent model of the human in which to study oxycodone's pharmacology.

In diabetic rats, multiple mechanisms seem to be responsible for the development of μ-opioid agonist hyporesponsiveness. These include a reduction in the number of functional μ-opioid receptors in the dorsal horn of the spinal cord (34) and, in the case of morphine, an increase in its clearance and volume of distribution (22). Although changes in μ-opioid agonist binding affinity and density have not been seen in the brain or spinal cord of diabetic rats by 4 weeks after the induction of diabetes with STZ (22,35), additional studies in rats with more advanced diabetes are still required to more fully examine this issue. In a recent study, impaired G-protein coupling to μ-opioid receptors (34) was shown in STZ-diabetic rats, which is highly likely to contribute to the well-documented morphine hyposensitivity seen in these animals (20–22,24). The concurrent increases in the clearance and volume of distribution of morphine in STZ-diabetic rats (22) are also likely to contribute to the observed hyporesponsiveness to morphine (20–22,24) by reducing the amount of morphine available to reach the central nervous system (CNS).

In a recent study by our laboratory, the antinociceptive potency of oxycodone administered intracerebroventricularly was not diminished in DA rats by 3 weeks after the induction of diabetes with STZ relative to nondiabetic animals (36). This finding contrasts with the ~2-fold decrease in the antinociceptive potency of subcutaneous oxycodone in similar diabetic animals (24), thereby implicating pharmacokinetic factors as being responsible for this latter observation. Thus, the ~2-fold decrease in systemic exposure to oxycodone, determined at 3 weeks post-STZ administration in DA rats herein, provides a plausible explanation for this ~2-fold decrease in the antinociceptive potency of subcutaneous oxycodone seen in STZ-diabetic DA rats by 3 weeks after STZ administration in our previous study (24).

The significantly higher noroxycodone/oxycodone $AUC_{0-\infty}$ ratios observed in diabetic compared with nondiabetic SD rats in the present study suggest that the formation of noroxycodone was increased as a consequence of STZ-diabetes induction. A similar trend was also apparent between diabetic and nondiabetic DA rats, but it did not reach statistical significance, possibly because the *N*-demethylation of oxycodone to noroxycodone is already higher in DA rats because of their genetic CYP2D1/D2 deficiency (15).

Noroxycodone is a secondary amine with a high pK_a (~11), and consequently, it is fully ionized under physiolog-

ical conditions. This would be expected to restrict noroxycodone's ability to passively diffuse into the CNS. Additionally, the intrinsic antinociceptive potency of noroxycodone is only 0.39 compared with oxycodone (3). Thus, although systemic exposure to metabolically derived noroxycodone increased after subcutaneous oxycodone administration to STZ-diabetic DA rats, owing to noroxycodone's low antinociceptive potency and poor ability to enter the CNS, it was unable to compensate for the ~2-fold reduction in antinociception (24) that followed the ~2-fold decrease in systemic exposure to oxycodone, found at 3 weeks post-STZ administration in diabetic DA rats herein.

CONCLUSIONS

The present investigation found significant quantitative strain-related differences in the pharmacokinetics of oxycodone and its *N*-demethylated metabolite, noroxycodone, following subcutaneous administration of single bolus doses of oxycodone to groups of DA and SD rats. In particular, systemic exposure to oxycodone and noroxycodone was higher in DA than in SD rats, consistent with the well-documented CYP2D1/D2 deficiency of DA rats. The serum concentrations of oxymorphone were very low (<6.9 nM) in both the DA and the SD rat strains irrespective of diabetes status. Because both strains exhibit very low circulating concentrations of metabolically derived oxymorphone in a manner analogous to that seen in humans after systemic oxycodone administration, both the DA and the SD rat strains would seem to provide suitable rodent models from the pharmacokinetic perspective, for conducting preclinical studies of oxycodone's pharmacology. By 3 weeks after the induction of diabetes with STZ, there was an ~2-fold decrease in the systemic exposure to oxycodone in STZ-diabetic DA rats compared with nondiabetic DA rats. Thus, altered oxycodone pharmacokinetics seem to explain the ~2-fold decrease in the potency of subcutaneous bolus doses of oxycodone in DA rats, seen by 3 weeks after the induction of diabetes with STZ in a previous study by our group (24).

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REFERENCES

1. R. Pöyhia and E. A. Kalso. Antinociceptive effects and central nervous system depression caused by oxycodone and morphine in rats. *Pharmacol. Toxicol.* **70**:125–130 (1992).
2. R. Pöyhia, A. Vainio, and E. Kalso. A review of oxycodone's clinical pharmacokinetics and pharmacodynamics. *J. Pain Symptom Manage.* **8**:63–67 (1993).
3. K. P. Leow and M. T. Smith. The antinociceptive potencies of oxycodone, noroxycodone and morphine after intracerebroventricular administration to rats. *Life Sci.* **54**:1229–1236 (1994).
4. C. K. Nielsen, F. B. Ross, and M. T. Smith. Incomplete, asymmetric and route-dependent cross-tolerance between oxycodone and morphine in the dark agouti rat. *J. Pharmacol. Exp. Ther.* **295**:91–99 (2000).
5. M. H. Levy. Advancement of opioid analgesia with controlled-release oxycodone. *Eur. J. Pain* **5**(Suppl A):113–116 (2001).
6. E. Kalso, R. Poyhia, P. Onnela, K. Linko, I. Tigerstedt, and T. Tammisto. Intravenous morphine and oxycodone for pain after abdominal surgery. *Acta Anaesthesiol. Scand.* **35**:642–646 (1991).
7. T. Heiskanen and E. Kalso. Controlled-release oxycodone and morphine in cancer related pain. *Pain* **73**:37–45 (1997).
8. E. Bruera, M. Belzile, E. Pituskin, R. Fainsinger, A. Darke, Z. Harsanyi, N. Babul, and I. Ford. Randomized, double-blind, cross-over trial comparing safety and efficacy of oral controlled-release oxycodone with controlled-release morphine in patients with cancer pain. *J. Clin. Oncol.* **16**:3222–3229 (1998).
9. Z. R. Chen, R. J. Irvine, A. A. Somogyi, and F. Bochner. Mu receptor binding of some commonly used opioids and their metabolites. *Life Sci.* **48**:2165–2171 (1991).
10. W. T. Beaver, S. L. Wallenstein, R. W. Houde, and A. Rogers. Comparisons of the analgesic effects of oral and intramuscular oxymorphone and of intramuscular oxymorphone and morphine in patients with cancer. *J. Clin. Pharmacol.* **17**:186–198 (1977).
11. R. F. Kaiko, D. P. Benziger, R. D. Fitzmartin, B. E. Burke, R. F. Reder, and P. D. Goldenheim. Pharmacokinetic–pharmacodynamic relationships of controlled-release oxycodone. *Clin. Pharmacol. Ther.* **59**:52–61 (1996).
12. T. Heiskanen, K. T. Olkkola, and E. Kalso. Effects of blocking CYP2D6 on the pharmacokinetics and pharmacodynamics of oxycodone. *Clin. Pharmacol. Ther.* **64**:603–611 (1998).
13. R. Pöyhia, K. T. Olkkola, T. Seppala, and E. Kalso. The pharmacokinetics of oxycodone after intravenous injection in adults. *Br. J. Clin. Pharmacol.* **32**:516–518 (1991).
14. R. Pöyhia, T. Seppala, K. T. Olkkola, and E. Kalso. The pharmacokinetics and metabolism of oxycodone after intramuscular and oral administration to healthy subjects. *Br. J. Clin. Pharmacol.* **33**:617–621 (1992).
15. F. B. Ross and M. T. Smith. The intrinsic antinociceptive effects of oxycodone appear to be κ-opioid receptor mediated. *Pain* **73**:151–157 (1997).
16. J. Cleary, G. Mikus, A. Somogyi, and F. Bochner. The influence of pharmacogenetics on opioid analgesia: Studies with codeine and oxycodone in the Sprague–Dawley/dark agouti rat model. *J. Pharmacol. Exp. Ther.* **271**:1528–1534 (1994).
17. F. B. Ross, S. C. Wallis, and M. T. Smith. Co-administration of sub-antinociceptive doses of oxycodone and morphine produces marked antinociceptive synergy with reduced CNS side-effects in rats. *Pain* **84**:421–428 (2000).
18. Y. Yamamoto, T. Tasaki, A. Nakamura, H. Iwata, A. Kazusaka, F. J. Gonzal, and S. Fujita. Molecular basis of the dark agouti rat drug oxidation polymorphism: importance of CYP2D1 and CYP2D2. *Pharmacogenetics* **8**:73–82 (1998).
19. B. Lalovic, B. Phillips, L. L. Risle, W. Howald, and D. D. Shen. Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. *Drug Metab. Dispos.* **32**:447–454 (2004).
20. J. Kamei, Y. Ohhashi, T. Aoki, N. Kawasima, and Y. Kasuya. Streptozotocin-induced diabetes selectively alters the potency of analgesia produced by mu-opioid agonists, but not by δ- and κ-opioid agonists. *Brain Res.* **571**:199–203 (1992).
21. C. Courteix, M. Bardin, C. Chantelauze, J. Lavarnne, and A. Eschalier. Study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. *Pain* **57**:153–160 (1994).
22. C. Courteix, P. Bourget, F. Caussade, M. Bardin, F. Coudore, J. Fialip, and A. Eschalier. Is the reduced efficacy of morphine in diabetic rats caused by alterations of opiate receptors or of morphine pharmacokinetics? *J. Pharmacol. Exp. Ther.* **285**:63–70 (1998).
23. J. R. Zurek, R. Nadeson, and C. S. Goodchild. Spinal and supraspinal components of opioid antinociception in streptozotocin induced diabetic neuropathy in rats. *Pain* **90**:57–63 (2001).
24. S. Lotfipour, F. B. Ross, K. Saini, and M. T. Smith. Oxycodone, but not morphine, is efficacious for the relief of tactile allodynia in diabetic rats at the three and six months after the induction of diabetes with streptozotocin. *Abstracts—10th World Congress on*

Pain, IASP Press, Seattle IASP Press, Seattle, 2002, p. 1131 (1531-P79), 2002.
25. J. S. Gimbel, P. Richards, and R. K. Portenoy. Controlled-release oxycodone for pain in diabetic neuropathy: A randomized controlled trial. *Neurology* **60**:927–934 (2003).
26. C. P. N. Watson, D. Moulin, J. W. Watt-Watson, A. Gordon, and J. Eisenhoffer. Controlled-release oxycodone relieves neuropathic pain: a randomized controlled trial in painful diabetic neuropathy. *Pain* **105**:71–78 (2003).
27. M. Gibaldi. *Biopharmaceutics and Clinical Pharmacokinetics*, Lea & Febiger, Philadelphia, 1984.
28. S. H. Weinstein and J. C. Gaylord. Determination of oxycodone in plasma and identification of a major metabolite. *J. Pharm. Sci.* **68**:527–528 (1979).
29. B. Kest, E. Hopkins, C. A. Palmese, M. Adler, and J. S. Mogil. Genetic variation in morphine analgesic tolerance: a survey of 11 inbred mouse strains. *Pharmacol. Biochem. Behav.* **73**:821–828 (2002).
30. B. Kest, C. A. Palmese, E. Hopkins, M. Adler, A. Juni, and J. S. Mogil. Naloxone-precipitated withdrawal jumping in 11 inbred mouse strains: Evidence for common genetic mechanisms in acute and chronic morphine physical dependence. *Neuroscience* **115**:463–469 (2002).
31. J. S. Mogil, E. J. Chesler, S. G. Wilson, J. M. Juraska, and W. F. Sternberg. Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. *Neurosci. Biobehav. Rev.* **24**:375–389 (2000).
32. S. G. Wilson, S. B. Smith, E. J. Chesler, K. A. Melton, J. J. Haas, B. Mitton, K. Strasburg, L. Hubert, S. L. Rodriguez-Zas, and J. S. Mogil. The heritability of antinociception: common pharmacogenetic mediation of five neurochemically distinct analgesics. *J. Pharmacol. Exp. Ther.* **304**:547–559 (2003).
33. A. B. Wey and W. Thormann. Capillary electrophoresis and capillary electrophoresis–ion trap multiple-stage mass spectrometry for the differentiation and identification of oxycodone and its major metabolites in human urine. *J. Chromatogr. B* **770**:191–205 (2002).
34. S. R. Chen, K. L. Sweigart, J. M. Lakoski, and H. L. Pan. Functional mu opioid receptors are reduced in the spinal cord dorsal horn of diabetic rats. *Anesthesiology* **97**:1602–1608 (2002).
35. S. R. Chen and H. L. Pan. Antinociceptive effect of morphine, but not mu opioid receptor number, is attenuated in the spinal cord of diabetic rats. *Anesthesiology* **99**:1409–1414 (2003).
36. M. Patterson, F. B. Ross, and M. T. Smith. Differential changes in the potency of supraspinally administered oxycodone relative to morphine for the alleviation of tactile allodynia in adult male dark agouti rats with streptozotocin-induced diabetes. (Abstract) In W. C. Hodgson and R. E. Loiacono (eds.), *Proc. Aust. Soc. Clin. Exp. Pharmacol. Toxicol.*, ASCEPT, 2001, p. 80.