In Vivo Disposition of Dermorphin Analog (DALDA) in Nonpregnant and Pregnant Sheep

HAZEL H. SZETO, JAMES F. CLAPP, DOMINIC M. DESIDERIO, PETER W. SCHILLER, OLGA O. GRIGORIANTS, YI SOONG, DUNLI WU, NICULINA OLARIU, JIH-LIE TSENG and ROBERT BECKLIN

Department of Pharmacology, Cornell University Medical College, New York, New York (H.H.S., V.S., D.W.); Department of Reproductive Biology and Obstetrics and Gynecology, Case Western Reserve University and MetroHealth Medical Center, Cleveland, Ohio (J.F.C., N.O.); The Charles B. Stout Neuroscience Mass Spectrometry Laboratory (D.M.D., O.G., J.L.T., R.B.) and Departments of Neurology and Biochemistry (D.M.D.), University of Tennessee, Memphis, Tennessee; and Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Quebec, Canada (P.W.S.)

Accepted for publication September 9, 1997 This paper is available online at http://www.jpet.org

ABSTRACT

Although synthetic opioid peptide analogs have been used extensively to study the functional roles of opioid receptors, little is known about their in vivo disposition. Our goal was to develop novel opioid drugs with limited transfer across the placenta. DALDA (Tyr-D-Arg-Phe-Lys-NH2) is a potent and highly selective mu agonist that is quite polar because of its 3-charge at physiological pH. It can therefore be expected that the distribution of DALDA across the placenta would be highly restricted. In this study, we determined the pharmacokinetics and placental transfer of DALDA after systemic administration in sheep. DALDA was infused intravenously to four nonpregnant and four pregnant sheep at a dose of 0.6 mg/kg/hr for 4 hr. Steady state plasma levels of DALDA were 5436 ± 464 ng/ml in nonpregnant sheep and 5214 ± 661 ng/ml in pregnant sheep. A one-compartment open model provided an excellent fit for nonpregnant and pregnant plasma data. The apparent volume of distribution was estimated to be 45.6 ± 4.4 and 59.2 ± 7.3 ml/kg in nonpregnant and pregnant animals, respectively. There was no difference in the elimination half-life of DALDA in nonpregnant (1.4 ± 0.1 hr) and pregnant (1.7 ± 0.2 hr) animals, and clearance was also similar in nonpregnant (23.1 ± 1.1 ml/kg/hr) and pregnant (23.7 ± 1.3 ml/kg/hr) animals. These data suggest that the distribution of DALDA is restricted to plasma volume and that its disposition is not altered in pregnancy. DALDA was not detected in any of the fetal plasma samples (<50 ng/ml), indicating that fetal plasma concentration is <1% of maternal concentration. The highly restricted placental distribution of DALDA suggests that it may be a promising opioid drug for obstetrical use.

Opiate alkaloids such as meperidine and morphine are extensively used for pain relief during labor and delivery. Their use, however, is associated with a number of adverse effects in the neonate because of their rapid transfer across the placenta to the fetus. The distribution of compounds across the placenta is dependent on molecular size and lipophilicity, and all available opiate alkaloids are small and highly lipophilic. The goal of our research group has been to develop novel opioid drugs with limited transfer across the placenta.

The physicochemical characteristics of a peptide drug may be advantageous in the design of an opioid drug for obstetrical use. The incorporation of D-amino acids in endogenous opioid peptides protects them from certain peptidase activity and significantly increases their stability in vitro, but no information exists on their disposition in vivo. Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2) is an endogenous opioid peptide found in amphibian skin that naturally contains a D-amino acid and has been shown to possess many opioid properties (Broccardo et al., 1981; Montecucchi et al., 1981). In vitro degradation studies revealed that dermorphin is hydrolyzed at the Gly-Tyr bond, resulting in dermorphin(1–4) being the major metabolite (Sasaki et al., 1985; Scalia et al., 1986). A synthetic analog of dermorphin(1–4), DALDA (Tyr-D-Arg-Phe-Lys-NH2), has been found to be a potent and highly selective agonist for the mu opioid receptor (Schiller et al., 1989). In addition, DALDA is unique in that it has a 3-charge at physiological pH and is therefore very polar and has very limited distribution across the blood-brain barrier (Samii et al., 1994). It can therefore be expected that

Received for publication May 27, 1997.

1 This work was supported in part by National Institute on Drug Abuse Multicenter Consortium Grant PO1-DA08924.

ABBREVIATIONS: DALDA, Tyr-D-Arg-Phe-Lys-NH2; dermorphin, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2; DAMGO, Tyr-D-Ala-Gly-Phe(NMe)-Gly-ol; DPDPE, Tyr-D-Pen-Gly-Phe-D-Pen; DAMME, Tyr-D-Ala-Gly-Gly-Met(O)-ol; Vd, apparent volume of distribution; t1/2, elimination half-life; CL, clearance; MRT, mean residence time; AUC, area under the plasma concentration-time curve; AUMC, area under the first moment of the plasma concentration-time curve; HPLC, high-performance liquid chromatography; MS, mass spectrometry.
the distribution of DALDA across the placenta would also be highly restricted, which would make this peptide a promising candidate for use as an obstetrical analgesic.

The objective of this study was to determine the pharmacokinetics of DALDA in nonpregnant and pregnant sheep. We recently developed a specific and sensitive method for the quantification of DALDA in ovine plasma samples (Grigoriants et al., 1997). Here, we present evidence that supports the highly restricted transfer of DALDA across the placenta.

Materials and Methods

Animal preparation. Chronic indwelling catheters were surgically placed in four nonpregnant ewes and four pregnant ewes (gestational age, 115–120 days; term, ~145 days) as described by Szeto et al. (1990). One polyvinyl catheter was inserted into the femoral artery and advanced to the distal aorta for blood sampling and another was advanced into the inferior vena cava via the femoral vein for drug infusion. In pregnant animals, a fetal hindlimb was exposed via a hysterotomy incision, and chronic indwelling catheters were also placed in the fetal distal aorta and inferior vena cava. Guidelines approved by the Institution for the Care and Use of Animals at Cornell University Medical College were followed for all surgical procedures and experimental protocols.

Compounds. DALDA was prepared by solid-phase synthesis according to a protocol described earlier (Schiller et al., 1989). The synthesis of the deuterated DALDA analog [H-Tyr-d-Arg-Phe(d5)-Lys-NH2] was based on the same protocol, except Boc-Phe(d5)-OH was used in place of Boc-Phe-OH. Pentadeuterophenylalanine was purchased from C/D/N Isotope (Vaudreuil, Quebec, Canada) and converted to Boc-Phe(d5)-OH through reaction with di-tert-butyldicarbonate. The deuterated peptide was purified by semipreparative reversed-phase HPLC as described previously (Schiller et al., 1989). The purity of DALDA and d5-DALDA was verified by fast atom bombardment-MS, and the precise amino acid sequence was confirmed by tandem MS.

Experimental protocol. Experiments were performed a minimum of 5 days after surgery to ensure complete recovery from surgical stress. The ewe was placed in a mobile cart with free access to food and water. All studies commenced at 8:00 a.m. with a minimum of 2.5 hr to allow for acclimation of the animal to the study environment.
conditions. DALDA (supplied by Dr. Peter Schiller and the National Institute on Drug Abuse) was administered as a constant-rate intravenous infusion (0.6 mg/kg/hr for 4 hr) to the sheep, and blood samples were collected at 0, 1, 2, 3, 3.5, 4, 4.25, 4.5, 5, 6, 7, 12 and 24 hr. Blood samples were collected from the fetus at 0, 3, 3.5, 4, 5 and 6 hr. All blood samples were collected into chilled borosilicate glass tubes containing EDTA and centrifuged, and the plasma was stored in glass containers with Teflon-lined caps and frozen at ~80°C until they were shipped overnight to Dr. Dominic Desiderio.

Quantitative analysis of DALDA. DALDA was quantified by using reversed-phase HPLC and MS detection. Details of the quantitative method have been published (Grigorian et al., 1997) and are presented here briefly. Plasma samples were deproteinized and eluted through a solid-phase extraction cartridge (Sep-Pak C18; Milipore, Milford, MA) with CH3CN. An internal standard, the deuterated DALDA analog [Tyr-D-Arg-Phe(d5)-Lys-NH2], was added to each plasma sample before deproteinization. The filtered plasma sample was chromatographed on an RPanalytical column (Delta Pak, 5 μm, C18, 100Å, 150 × 3.9 mm; Waters, Milford, MA) at a flow rate of 1.5 ml/min, and UV absorption was monitored at 200 nm (Varian Assoc., Walnut Creek, CA). One-minute fractions were collected, and each fraction was lyophilized for MS analysis (AutoSpecQ tandem mass spectrometer; Micromass, Altrincham, UK). Continuous-flow-LSIMS was used to quantify DALDA. The (M+H)+ ion current for DALDA at m/z 612 was compared with the ion current from d5-DALDA at m/z 617. The limit of sensitivity of this method is 50 ng/ml DALDA.

Pharmacokinetic analyses. Plasma levels of DALDA during and after the 4-hr infusion of DALDA were analyzed using both model-dependent and-independent methods with nonlinear regression (WINNONLIN). A one-compartment open model with zero-order input and first-order elimination provided an excellent fit for non-pregnant and pregnant plasma data. The derived pharmacokinetic constants were used to calculate the apparent volume of Vd, MRT and CL. The plasma data were also analyzed using a model-independent approach with area analysis (Perrier and Mayersohn, 1982). Rather than ascribing the data to a specific model, the model-independent approach assumes only that all dispositional processes may be described by first-order kinetics, with elimination occurring from the rapidly equilibrating compartment. The parameters of Vd, CL and MRT are determined from the total AUC and AUMC.

Results

DALDA was infused into four nonpregnant and four pregnant sheep at a dosage of 0.6 mg/kg/hr for 4 hr. This infusion rate was selected on the basis of preliminary studies using 0.3 to 0.9 mg/kg/hr for 2 to 4 hr. From these preliminary studies, it was apparent that a 4-hr infusion was necessary for steady-state kinetics; all subsequent studies were therefore carried out using 4-hr infusions. Figures 1 and 2 illustrate the mean plasma DALDA levels in nonpregnant and pregnant sheep, respectively. Steady-state levels were achieved by the end of the 4-hr infusion, with no statistical difference between plasma DALDA levels at 3.5 and 4 hr (paired t test, P > .05). There was no significant difference between the plasma concentration at the end of infusion in nonpregnant (5436 ± 464 ng/ml) and pregnant (5214 ± 661 ng/ml) animals. Samples obtained at 24 hr did not contain measurable levels of DALDA. DALDA was not detected in any of the fetal plasma samples. Because the limit of sensitivity of the analytical method is 50 ng/ml, the ratio of fetal-to-maternal plasma concentration is <0.01.

Individual plasma data from nonpregnant and pregnant sheep were analyzed using compartmental and noncompartmental methods. Compartmental analysis revealed that a one-compartment open model provided an excellent fit for both nonpregnant and pregnant animals, with the correlation between observed and predicted values ranging from .92 to .99. Figures 3 and 4 are the observed and predicted values for a representative nonpregnant and pregnant animal, respectively. The calculated parameters for nonpregnant and pregnant animals are summarized in tables 1 and 2, respectively. The coefficient of variation was <10% for all parameter estimates. There was no significant difference in any of the pharmacokinetic parameters between nonpregnant and pregnant animals.

The results of the noncompartmental analyses are summarized in tables 3 and 4. The calculated values for Vd, MRT and CL are quite similar to those estimated from the compartmental analysis. The data also show no significant difference between the disposition of DALDA in nonpregnant and pregnant animals.

Discussion

Although synthetic opioid peptide analogs have been used extensively to study the functional roles of opioid receptor subtypes, there is no information available on the pharmacokinetics of these peptide analogs in vivo. These data represent the first attempt in understanding the in vivo disposition of synthetic opioid peptide analogs, and this is the first time that detailed pharmacokinetic analyses have been performed using a highly specific method for peptide quantitation in biofluids. The endogenous opioid peptides are rapidly degraded in plasma, and the t1/2 of met-enkephalin was reported to be 2 min in plasma in vitro (Hambrook et al., 1976). Substitution with D-amino acids significantly increases peptide stability and has been the rationale behind the synthesis of a large number of peptide analogs (D-Ala2) based on the enkephalin structure. However, pharmacokinetic data are not available for even the most widely used analogs, such as DAMGO and DPDPE. Using radioimmunoassay, the t1/2 of DAMME was determined to be 52 min in sheep (Bolton et al., 1982). The disadvantage with radioimmunoassay, however, is the lack of specificity for the parent peptide, and the antibody may cross-react with degradative fragments. Other
The results of this study suggest that the pharmacokinetics of DALDA are not significantly altered in pregnancy. Pregnancy is associated with a number of physiological changes that are expected to affect the disposition of hydrophilic and hydrophobic drugs, including an increase in plasma volume, total body water and fat mass (Reynolds and Knott, 1989). Although the apparent volume of distribution of DALDA was higher in pregnant animals based on compartmental and noncompartmental estimation, the difference did not reach statistical significance. The influence of pregnancy on pharmacokinetics may be more considerable for lipophilic drugs with more extensive distribution. Similarly, neither the $t_{1/2}$ nor CL of DALDA was altered in pregnant animals.

As we predicted, the placental transfer of DALDA was highly restricted, with no measurable levels detected in fetal plasma even after 4 hr of drug infusion to the mother. With the limit of sensitivity of our analytical method being 50 ng/ml, our data suggest that $<1\%$ of the peptide reaches the fetus. In contrast, the percentages of methadone, meperidine and morphine reaching the ovine fetus are 30%, 15% and 13%, respectively. The negligible distribution of DALDA to the fetus can readily account for the lack of effects on fetal cardiovascular and metabolic function after maternal DALDA administration (Clapp et al., in press).

The very low extent of fetal drug exposure suggests that DALDA would be a very safe drug for use in pregnancy. However, the restricted distribution of DALDA would also mean that it would have difficulty gaining access into the central nervous system. In fact, available data have shown

**TABLE 1**
Pharmacokinetic parameters derived from one-compartment model in nonpregnant sheep

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sheep</th>
<th>Mean</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>3481</td>
<td>3491</td>
<td>8654</td>
</tr>
<tr>
<td>$k_{10}$ (h⁻¹)</td>
<td>0.493</td>
<td>0.495</td>
<td>0.462</td>
</tr>
<tr>
<td>$Cl$ (ml/kg/hr)</td>
<td>18.8</td>
<td>21.6</td>
<td>28.0</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**TABLE 2**
Pharmacokinetic parameters derived from one-compartment model in pregnant sheep

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sheep</th>
<th>Mean</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>3481</td>
<td>3491</td>
<td>8654</td>
</tr>
<tr>
<td>$k_{10}$ (h⁻¹)</td>
<td>0.327</td>
<td>0.527</td>
<td>0.343</td>
</tr>
<tr>
<td>$Cl$ (ml/kg/hr)</td>
<td>26.2</td>
<td>20.0</td>
<td>22.7</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>2.1</td>
<td>1.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**TABLE 3**
Pharmacokinetic parameters derived from noncompartmental analysis in nonpregnant sheep

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sheep</th>
<th>Mean</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>3481</td>
<td>3491</td>
<td>8654</td>
</tr>
<tr>
<td>$Cl$ (ml/kg/hr)</td>
<td>18.2</td>
<td>18.9</td>
<td>26.7</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**TABLE 4**
Pharmacokinetic parameters derived from noncompartmental analysis in pregnant sheep

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sheep</th>
<th>Mean</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>3481</td>
<td>3491</td>
<td>8654</td>
</tr>
<tr>
<td>$Cl$ (ml/kg/hr)</td>
<td>28.3</td>
<td>18.9</td>
<td>21.3</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>1.8</td>
<td>1.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>
that the distribution of DALDA to the central nervous system is very slow (Samii et al., 1994), and DALDA has only a small effect in the hot-plate test 2 hr after subcutaneous administration to mice (Schiller et al., 1990). A solution to the problem would be to administer DALDA via the intrathecal route. When administered intrathecally to rats with indwelling intrathecal catheters, a single dose of DALDA was found to be highly effective in the tail-flick assay with a duration of action >4 hr. The polar character of DALDA, together with its metabolic stability, can account for the extraordinary long duration of antinociception. When administered in the subdural space, its distribution across the dura is likely to be very restricted, and DALDA can therefore remain in cerebrospinal fluid for a long time. Together, these data suggest that DALDA is a most promising drug for regional obstetrical analgesia.

References


Send reprint requests to: Hazel H. Szeto, M.D., Ph.D., Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021. E-mail: hhszeto@mail.med.cornell.edu