Pharmacokinetics of Florfenicol in Cerebrospinal Fluid and Plasma of Calves

B. A. DE CRAENE,1 P. DEPREZ,2 E. D’HAЕSE,1 H. J. NELIS,1* W. VAN DEN BOSSCHE,3 AND A. P. DE LEENHEER1

Laboratorium voor Farmaceutische Microbiologie,1 Kliniek voor Inwendige Ziekten van Grote Huisdieren,2 Laboratorium voor Ontleding van Geneesmiddelen,3 and Laboratorium voor Medische Biochemie en voor Klinische Analyse, Universiteit Gent, Ghent, Belgium4

Received 30 December 1996/Returned for modification 26 April 1997/Accepted 30 June 1997

Florfenicol, a fluorinated analog of thiamphenicol, is of great value in veterinary infectious diseases that formerly responded favorably to chloramphenicol. In view of the treatment of meningitis in calves, we studied its pharmacokinetics in the cerebrospinal fluid (CSF) and plasma of six animals. To this end, a new high-performance liquid chromatography method was developed which, unlike previous ones, uses solid-phase instead of double-phase extraction to isolate the drug. After a single intravenous dose of 20 mg/kg of body weight, a maximum concentration in CSF of 4.67 ± 1.51 μg/ml (n = 6) was reached, with a mean residence time of 8.7 h. The decline of florfenicol in both CSF and plasma fitted a biexponential model with elimination half-lives of 13.4 and 3.2 h, respectively. Florfenicol penetrated well into CSF, as evidenced from an availability of 46% ± 3% relative to plasma. The levels remained above the MIC for Haemophilus somnus over a 20-h period. Our results provide evidence indicating the effectiveness of florfenicol in the treatment of bacterial meningitis of calves.

Florfenicol is a fluorinated analog of thiamphenicol, which has been used as an alternative to chloramphenicol in veterinary medicine due to its lower toxicity and reduced development of resistance. However, its use has been restricted by the potential for bacterial inactivation and the risk of allergic reactions.

The present study investigates the pharmacokinetics of florfenicol in the cerebrospinal fluid of calves. The authors report a maximum concentration in CSF of 4.67 ± 1.51 μg/ml after a single intravenous dose of 20 mg/kg. The drug exhibits a biexponential decline in both CSF and plasma, with elimination half-lives of 13.4 and 3.2 h, respectively. Florfenicol was found to penetrate well into the CSF, indicating its potential for the treatment of bacterial meningitis.

The results suggest that florfenicol is an effective alternative to chloramphenicol in the treatment of bacterial meningitis in calves, offering a better safety profile and lower risk of resistance development.
Animals. Six healthy male Holstein-Friesian calves weighing between 110 and 290 kg were used for the pharmacokinetic study. The calves were kept in individual pens and were fed a diet of hay and a commercial nonmedicated concentrate for growing calves. Water was available ad libitum.

The day before the trial was started, all calves were weighed and deeply sedated with xylazine i.v. (4 ml/100 kg; Rompun 2%; Bayer, Sint-Truiden, Belgium), the skin from the poll to the atlas was prepared for surgery, analgesia was obtained by infiltrating 5 ml of 2% lidocaine (2% Xylocaine; Astra Pharmaceutica, Brussels, Belgium) in the dorsal midline over the atlanto-occipital joint, and a catheter for peridural anesthesia (Catheter RXC 19G; Vygon Steriel N.V., Frameries, Belgium) was fitted in the subarachnoid space. The catheter was inserted through a trochar (Tuohy 17-gauge needle; Vygon Steriel N.V.), and between 5 to 10 cm of the catheter was left in the subarachnoid space. After removal of the trochar, the exposed catheter was saturated with the skin and covered with gauze and tape, leaving only the proximal plugged opening visible.

After termination of the trial, the catheters were removed, the calves received an additional dose of florfenicol and one dose of a nonsteroidal anti-inflammatory drug (6 ml/100 kg i.v.; Tomanol; Bayer) and recovered without problems.

Drug administration. Florfenicol (Nuflof; Schering-Plough) was administered to all calves via the left jugular vein at a dose of 20 mg/kg over a period of 30 s.

Samples. Blood samples were collected from the right jugular vein before (pretreatment) and at 300, 560, 900, 1,800, 3,600, 5,400, 7,200, 10,800, 21,600, 28,800, 32,400, 43,200, 54,000, 57,600, 72,000, 86,400, 108,000, 129,600, 151,200, and 172,800 after drug administration. At the same time, a 1-ml CSF sample was obtained.

The blood samples were collected in 10-ml evacuated glass tubes containing lithium heparin (Venoject; Terumo Europe N.V., Leuven, Belgium); they were stored at -20°C until analysis.

The CSF samples were collected in glass tubes without anticoagulant and stored at -20°C until analysis.

Apparatus and chromatographic conditions. The liquid chromatographic system consisted of a Kontron 325 ternary pump (Kontron Instruments, Everett, Mass.), a Rheodyne 7725i injector (Rheodyne, Cotati, Calif.), a Kontron 440 diode array detector set at 224 nm for monitoring of the signal and at 200 to 400 nm for spectral information (bunching, 2 nm; band width, 20 nm), and a Kontron Kromasystem 2000 PC integration package. A 5-µm HyperSil octadecyl silane (ODS) column (25×0.46 cm; Shandon, Runcorn, United Kingdom) was eluted with aqueous 0.05 M ammonium acetate–acetonitrile (78:22 [vol/vol]). The flow rate was 1.5 ml/min, and the temperature was 40°C (obtained by submerging the column in a thermostated glycerol bath).

Sample preparation. To 250 µl of bovine CSF or plasma were added 750 µl of distilled water and 40 µl of the internal standard solution (400- or 8 µg/ml chloramphenicol, depending on the expected florfenicol concentration). After vortex mixing, the mixture was applied on top of a C18 extraction cartridge which had been preconditioned successively with 2 ml of methanol and 2 ml of water. The cartridge was washed with 2 ml of water-acetonitrile (85:15 [vol/vol]) and 3 ml of hexane. Elution was carried out with 3 ml of acetonitrile. The eluate was transferred to a conical tube and evaporated to dryness by applying vacuum (membrane pump) under continuous vortexing of the tubes (Rotary Evapo-Mix; Büchler Instruments, Fort Lee, N.J.). The residue was redissolved in 500 µl of water-acetonitrile (78:22 [vol/vol]) and filtered over an Acrodisc 0.45-µm pore-size polysulfone dithiofloure pyrrole filter (Gelman, Ann Arbor, Mich.). A 100-µl aliquot was injected.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated with a program of the pharmacokinetics laboratory (14), according to a biexponential pharmacokinetic model. Model discrimination was based on the correlation coefficient of the curve fit, and the absolute error was independent of the concentration (weighted regression). The parameters evaluated for plasma were determined after direct injection without extraction, of water samples containing equivalent concentrations. The percent difference between the two sets of data was a measure of the bias of the method.

Method validation. (i) Linearity. The slopes, intercepts, and correlation coefficients of the calibration curves were calculated by linear regression analysis.

(ii) Precision. Sample pools with high and low concentrations of florfenicol and chloramphenicol were analyzed 10 times on the same day to determine within-run precision. The fluctuation of the slopes of the calibration curves was an indication of the day-to-day precision.

(iii) Recovery. The recovery was determined by repetitively analyzing blank CSF or plasma supplemented with 600 µg/ml of florfenicol, and the internal standard (expected florfenicol concentration) and were analyzed as the unknown samples. Calibration curves were constructed by plotting peak area or peak height ratios (florfenicol to chloramphenicol) versus the corresponding florfenicol concentrations. The florfenicol concentrations in the unknown samples were calculated by extrapolation from the calibration curves.

(iv) Specificity. In each run, a blank sample of CSF or plasma of the same animal(s) as the unknown was analyzed to note the absence of interferences in the elution positions of florfenicol and chloramphenicol.

(v) Accuracy. For lack of certified samples or reference methods, accuracy could be only indirectly evaluated. To this end, blank CSF or plasma supplemented with known amounts of florfenicol (0.38 and 3.82 µg/ml for CSF and 0.19, 1.53, and 38.20 µg/ml for plasma), but with the addition of the internal standard at the same ratio, after reconstitution of the residue with water-acetonitrile (78:22 [vol/vol]). Extracts of CSF or plasma to which both florfenicol and chloramphenicol had been added just before the injection were used to construct a calibration curve, corresponding to 100% recovery. Likewise, for the determination of the recovery of chloramphenicol, florfenicol was added as the internal standard at the end of the procedure.

(vi) Stability of florfenicol in extracts. The peak areas of florfenicol and chloramphenicol in the internal standard extracts were determined at time zero and after 24 h of storage at room temperature.

(vii) Detection limit and quantification limit. The detection limit and quantification limit were estimated from the size of the florfenicol peak in spiked samples of blank CSF or blank plasma. They were defined as the concentrations that resulted in a detectable peak of approximately 4 and 10 times the noise level, respectively.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated with WinNonlin, a part of the MW/Pharm software package (14), according to a biexponential pharmacokinetic model. Model discrimination was based on the correlation coefficient of the curve fit, and the absolute error was independent of the concentration (weighted regression). The parameters evaluated for plasma were area under the concentration-time curve from 0 to infinity (AUC0–∞), clearance (CL), volume of distribution (Vd), half-life (t1/2), and mean residence time (MRT). For CSF the same parameters were evaluated, as well as the absorbed fraction (F), speed of absorption (t1/2 absorption), maximum concentration (Cmax), time to maximum concentration (Tmax), and absorption t1/2.

RESULTS

Chromatography and profiles of CSF and plasma. Representative chromatograms of postdose CSF and plasma are given in Fig. 2A and B. The retention times of florfenicol and chloramphenicol (internal standard) were 6.00 min (capacity factor k' = 4.56) and 7.50 min (k' = 5.94), respectively. No significant endogenous peaks coeluted with both compounds, as shown in the chromatograms of blank CSF and plasma (Fig. 2C and D).

Method validation. A linear relationship existed between peak area or peak height ratios (florfenicol versus chloramphenicol) and the concentrations of florfenicol in both CSF and plasma in the ranges of 0.03 to 1 µg/ml (low) and 1 to 90 µg/ml (high). Linear regression analysis of calibration curves always afforded correlation coefficients exceeding 0.9999. The day-to-day relative standard deviations on the slopes for CSF were 0.01% (low range) and 0.51% (high range). The corresponding...
values for plasma were 5.08 and 2.03%, respectively. Data on recovery and precision are summarized in Table 1. Chloramphenicol yielded a recovery from plasma ranging from 84.4% (low [n = 10]) to 92.0% (high [n = 10]). The method bias at low concentration (0.38 μg/ml) was −1.9% (CSF) and −4.8% (plasma). At medium concentration (3.1 to 3.8 μg/ml), the values were 4.7 and 1.9%, respectively. For a plasma level of 38.2 μg/ml, a bias of 3.9% was calculated. After storage of extracts for 24 h, the peak areas of florfenicol and chloramphenicol were 99.64 and 100.33% of the initial values, respectively. The detection limit was 0.020 μg/ml, and the quantification limit was 0.030 μg/ml.

**Application.** The method was used in a pharmacokinetic study of florfenicol in the CSF and plasma of six calves. Dosing was done by i.v. injection in the left jugular vein at 20 mg of florfenicol/kg. Mean CSF-florfenicol versus time and plasma-florfenicol versus time plots are given in Fig. 3. Tables 2 and 3 list the calculated pharmacokinetic parameters for CSF and plasma, respectively.

**DISCUSSION**

Although at the onset of this study several HPLC methods for florfenicol had been reported (1, 2, 6, 8, 11, 25), our aim was to develop a new procedure that would be more in line with current trends in sample preparation. Specifically, the common double-phase extraction with ethyl acetate (1, 2, 6, 8, 11, 25) was replaced by solid-phase extraction on C18 cartridges. Solid-phase extraction is easier to perform in series, lends itself better to automation, and results in lower volumes of organic solvent waste. Owing to its high solubility in polar nonaqueous solvents (15), florfenicol is not strongly retained on reverse phase, so that the cartridge could only be washed with weak solvent mixtures, such as water-acetonitrile (85:15 [vol/vol]). The additional washing with hexane did not displace florfenicol and was included to remove nonpolar late-eluting compounds. However, it was observed that hexane and possibly also acetonitrile liberated some polymeric substances from the plastic cartridge, resulting in a visible film on the analytical column and its progressive clogging. This adverse effect could be avoided by working at elevated temperature, which apparently kept the polymers in solution (12a). Chloramphenicol was used as an internal standard to compensate for analytical variability. Although thiamphenicol is structurally more related to florfenicol, it proved less useful because of insufficient retention, both on the solid-phase cartridge and the HPLC column (k′ = 1.72).

The performance of the present method compares favorably with that of Lobell et al. (8), which is thus far the most thoroughly validated existing method for the determination of florfenicol in plasma. They reported a quantification limit of 0.025 μg/ml, recoveries ranging from 98 to 100%, and a within-run precision of 1.5 to 5.0%. However, the linearity of our method extends over a wider range: 0.03 to 1 mg/ml and 1 to 90 mg/ml versus 0.15 to 16 μg/ml and 15 to 50 μg/ml in the study by Lobell et al. (8).

This new method was applied to a pharmacokinetic study of florfenicol in the CSF and plasma of calves. The experimental data best fitted a biexponential model, as indicated by a maximum correlation coefficient, although other authors favored a triexponential model (2, 8) or even a noncompartmental model (21). To the best of our knowledge, the present paper is the

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**TABLE 1. Method validation**

<table>
<thead>
<tr>
<th>Conc (μg/ml)</th>
<th>Mean ± SD (%)</th>
<th>RSD (%)</th>
<th>No. of samples</th>
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<tr>
<td>Recovery CSF</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.38</td>
<td>87.92 ± 0.77</td>
<td>4.96</td>
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</tr>
<tr>
<td>3.82</td>
<td>96.80 ± 1.98</td>
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<td>5</td>
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<tr>
<td>Recovery plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.19</td>
<td>97.38 ± 6.74</td>
<td>6.74</td>
<td>10</td>
</tr>
<tr>
<td>1.53</td>
<td>100.03 ± 5.05</td>
<td>5.05</td>
<td>10</td>
</tr>
<tr>
<td>38.2</td>
<td>88.22 ± 2.07</td>
<td>2.07</td>
<td>10</td>
</tr>
<tr>
<td>Within-run precision CSF</td>
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<td></td>
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<tr>
<td>0.3</td>
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<td>3.88</td>
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<tr>
<td>4.97</td>
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<tr>
<td>Within-run precision plasma</td>
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<td></td>
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<td>0.27</td>
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<tr>
<td>4.79</td>
<td>1.22</td>
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* RSD, relative standard deviation.
first to report levels of florfenicol in CSF of calves obtained after single i.v. dosing (20 mg/kg) and over a prolonged period of time (48 h). A maximum concentration of 4.67 μg/ml was found in CSF at 2 h postdose, which is remarkably similar to the value of 4.5 μg/ml obtained with chloramphenicol under the same conditions of dosage and sampling time (20). Previously, Adams et al. (1) found concentrations in CSF to be 1/4 of the plasma. Here, our study the concentrations in CSF were 1/2.5 to 1/1.29 of the plasma. In our study, the AUC\textsubscript{0–12 h} after multiple oral dosing of 11 mg/kg, whereas in our study the concentrations in CSF were 1/25.5 to 1/12 of the concentrations in plasma at 4, 8, and 12 h after multiple oral dosing of 11 mg/kg, whereas in our study the concentrations in CSF were 1/2.5 to 1/1.29 of the concentrations in plasma at 4, 8, and 12 h postdose. The AUC\textsubscript{0–12 h} for florfenicol was 44.35 h \cdot μg/ml.

To allow a meaningful interpretation of the pharmacokinetic data of florfenicol in CSF, a similar study was conducted for plasma. Here, a comparison with earlier work (8, 21, 26) was possible. In our hands, the mean level in plasma declined from 45.74 μg/ml at 5 min to 1.13 μg/ml after 720 min, whereas Lobell et al. (8) and Varma et al. (26) found 44.6 and 0.9 μg/ml at the same dose level, respectively. The total body CL and steady-state V were 0.22 liter/kg/h and 0.82 liter/kg versus 0.23 to 0.24 liter/kg/kg and 0.77 liter/kg in previous studies (8, 26), respectively. Our terminal t\textsubscript{1/2} of 3.17 h also closely approaches values in the published literature (2.65 to 2.77 h) (8, 26). Soback et al. reported a terminal t\textsubscript{1/2} of 2.93 h, a V at steady state of 0.35 liter/kg, and a total CL of 0.162 liter/kg by a noncompartmental calculation method (21). A particularly important observation was that the ratio between the AUC\textsubscript{0–12 h} in CSF and plasma amounted to as much as 0.46, with a standard deviation of only 0.03 (n = 6). This result proves the excellent penetration of florfenicol in the CSF of calves, which appears to come close to that of chloramphenicol. The levels of florfenicol in CSF remained above the MIC at which 90% of the isolates are inhibited (MIC\textsubscript{90}) (0.25 μg/ml) for H. somnus over a period of 20 h, which suggests the therapeutic efficacy against bacterial meningitis caused by this important pathogen. Other gram-negative bacteria, such as Escherichia coli and Salmonella typhimurium, can also cause bacterial meningitis in calves. In a study by Neu and Fu (12), it was shown that for chloramphenicol-resistant strains of E. coli and S. typhimurium, MIC\textsubscript{90}s for florfenicol were 3.1 μg/ml. Since concentrations of as much as 4.67 μg/ml in CSF were obtained and higher concentrations in inflamed meninges may be expected, due to an impairment of the blood-brain barrier, activity against these strains could also be anticipated. However, it has been postulated that optimal antimicrobial activity in CSF can be expected only at levels that exceed the MIC\textsubscript{90} by at least 10-fold (13, 16, 23).

For example, the recommended dosage for penicillin G sodium in the treatment of general infections is 10,000 to 20,000 IU/kg, but in the treatment of bacterial meningitis doses of as much as 240,000 IU/kg are given four times a day (4). M. A. Sande stated in 1981 that the pharmacokinetic parameters influencing the efficacy of an antibiotic in the treatment of bacterial meningitis are not fully understood (16). Tauber et al., who conducted a study of the postantibiotic effect in the treatment of experimental Streptococcus pneumoniae meningitis in rabbits (23) and who recommended that therapeutically effective concentrations should exceed the MBC by at least 10-fold, admitted that their results are valid only for one particular drug (ampicillin) and one specific organism (S. pneumoniae). Although the definitive test of efficacy is a clinical trial, results of clinical studies with chloramphenicol in bovine meningitis are inconsistent; some investigators found 100% animal recovery, while others found 100% mortality (17, 19). Since florfenicol exhibits a low level of mammalian toxicity (18), it might be possible to increase the dosage of the product in the treatment of meningitis caused by E. coli or S. typhimurium; however, this still has to be experimentally verified. Further studies other than pharmacokinetic ones are needed to determine a possible application of florfenicol in these cases.
ACKNOWLEDGMENTS

We thank K. J. Varma for the donation of the reference substance and for helpful advice and Vanessa Van Hoorick for outstanding technical assistance. E. D’Haese is a research assistant of the Fund for Scientific Research Flanders (Brussels, Belgium).

REFERENCES