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Mammary excretion of cannabidiol in rabbits after intravenous administration

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Abstract—The present study examined the distribution of cannabidiol into milk after an intravenous bolus injection (3 mg kg^{-1}) to lactating rabbits. Drug concentrations in milk and serum were measured by HPLC. Cannabidiol was excreted into milk rapidly and the drug levels in milk increased over a 4–24-h period following the maternal injection. The mean milk to serum concentration ratio was 25·9, indicating a significant accumulation of the drug in milk.

Marijuana (cannabis, hashish) is a natural substance (*Cannabis sativa*) that is used for its hallucinogenic effects. Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) constitute the major cannabinoids found in marijuana. Unlike THC, CBD possesses relatively little psychoactive and cardiovascular properties (Perez-Reyes et al 1973) but inhibits hepatic metabolism of drugs (antipyrine and barbiturates) mediated by hepatic mixed function oxidase enzymes (Paton & Pertwee 1972; Benowitz & Jones 1981). CBD appears to be effective in treating patients with Niemann-Pick disease (Burstein et al 1984) and seizure patients refractory to anti-epileptic drugs such as phenytoin and primidone (Carlini & Cunha 1981). The anticonvulsant activity of CBD has been suggested to be due to its inhibitory effects on the hepatic metabolism of concurrently administered anti-epileptic drugs (Carlini & Cunha 1981). CBD is known to inhibit hepatic microsomal cytochrome P4503A (Bornheim & Correia 1990) and testosterone oxidation at the 2α-, 16α- and 17β-positions in male rat liver (Narimatzu et al 1990).

THC has been extensively studied for its pharmacological and pharmacokinetic properties, including newborn complications, placental transfer and mammary transport (Briggs et al 1990). In man, THC crosses the placenta to the foetus and accumulates in milk (Perez-Reyes & Wall 1982; Blackyard & Tennes 1984). Although a few reports have examined the metabolism and pharmacokinetic disposition of CBD (Martin et al 1977; Siemens et al 1980; Samara et al 1988, 1990; Harvy & Brown 1991), there is no information available on either the mammary excretion of CBD during lactation or foetal exposure during pregnancy in man or animals. CBD (mol.wt 314.5) is highly lipophilic and is, therefore, likely to be excreted into milk. The purpose of the present study is to examine the distribution of CBD into milk after intravenous injection into lactating rabbits.

Materials and methods

Four pregnant New Zealand White rabbits (mean (± s.d.) body weight = 5·2 ± 0·5 kg, ~ 1 year of age) were purchased and maintained in a temperature-controlled animal facility with 12 h/12 h light/dark cycle. The animals were housed in a whelping cage with a nesting box approximately seven days before the due date (gestation = 30–32 days). The day of whelping was considered to be postpartum day 0. CBD (3 mg kg^{-1}) dissolved in 70% ethyl alcohol was injected as an intravenous bolus to the lactating rabbits via the left marginal ear vein (20–27 days postpartum). Lactating mothers were separated from their offspring overnight before dosing. At approximately −0·5, 0·5, 1, 2, 4, 8, 12, 24 and 36 h after injection, serial blood samples (0·6 mL) were taken from the lactating rabbits by venipuncture from the right ear vein and milk samples (0·3 mL) by gentle manual expression. Exact sampling times were recorded. Harvested serum and milk samples were kept at −20°C until drug analysis. CBD concentrations in serum and milk were determined by a modified HPLC method reported previously (Samara & Bialer 1987). The chromatographic system consisted of a Shimadzu LC-10AS pump, SPD-10AV UV detector, CR501 Chromatopac integrator and Rheodyne injector. Briefly, 25 μL THC (10 μg mL^{-1}, internal standard), 200 μL methanol and 300 μL of phosphate buffer (pH 7·4) were added to milk or serum (100 μL). After addition of 2 mL hexane, CBD was extracted on a vortex mixer for 1 min. Following centrifugation at 4000 rev min^{-1} for 5 min, the organic layer was transferred to a fresh tube and dried under nitrogen. The residue was reconstituted in 50 μL methanol and a portion (20 μL) was injected into the chromatograph. CBD was eluted on a Microsorb-MV C_{18} reverse phase column using a mobile phase consisting of acetonitrile : methanol : H_2O (7 : 1 : 2) and was detected at 230 nm. Drug concentrations were measured as the mean of duplicate samples.

Results

Typical chromatograms of rabbit milk and serum extracts are presented in Fig. 1. Under the assay conditions used, the retention times were 4·7 and 9·7 min for CBD and THC, respectively. Calibration curves were linear ($r^2 > 0.99$) for milk and serum extracts over the CBD concentration range of 0·05–10 μg mL^{-1}. The minimal detectable concentration for CBD was 0·05 μg mL^{-1} using 0·1 mL sample volume. Fig. 2 shows the average CBD concentration-time profiles in milk and serum following an intravenous bolus injection (3 mg kg^{-1}). Milk CBD concentrations increased over a 4–24-h period after injection. CBD concentrations in serum declined rapidly, with an average (± s.d.) apparent half-life of 1·4 ± 1·2 h. Drug concentrations in serum samples collected between 8 and 36 h were not measured as the levels quickly decreased below the detection limit. Milk to serum drug concentration ratios ranged from 4·3 to 51·9 as a function of sampling time, indicating extensive and time-dependent accumulation (Fig. 3).

Discussion

To our knowledge, the present study reports the distribution of CBD in milk for the first time. The drug accumulated in rabbit milk significantly (mean milk to serum ratio = 25·9) and was eliminated slowly. Previous reports indicate that following intravenous bolus injection, CBD plasma concentrations initially decrease rapidly and CBD is eliminated at a much slower rate (Siemens et al 1980; Samara et al 1988). The terminal elimination half-life of CBD has been reported as 10·9 h in the rat (Siemens et al 1980) and 6·8 h in the dog (Samara et al 1988). In the present study, it was not possible to characterize fully the pharmacokinetic profiles of the drug due to the limited number of samples taken during the elimination phase. The apparent serum half-life of 1·4 h determined in this

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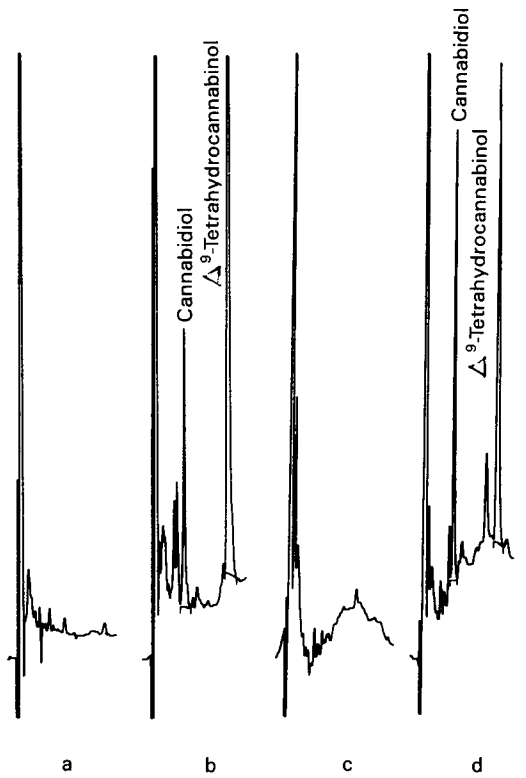

FIG. 1. Chromatograms obtained from (a) blank serum, (b) serum containing 0·5 μg mL^{-1} cannabidiol (CBD, Rt = 4·7 min) and 2·5 μg mL^{-1} Δ^9-tetrahydrocannabinol (THC, Rt = 9·7 min), (c) blank milk and (d) milk obtained 4 h after intravenous injection of cannabidiol (3 mg kg^{-1}) to a lactating rabbit.

study may be an underestimate and appears comparable with an initial distribution half-life (1·3 h) found in the rat.

A structurally related cannabinoid, THC, has been extensively studied for its pharmacological activities, placental transfer, newborn complications and mammary transport (Burstein 1973; Briggs et al 1990). Perez-Reyes & Wall (1982) reported an

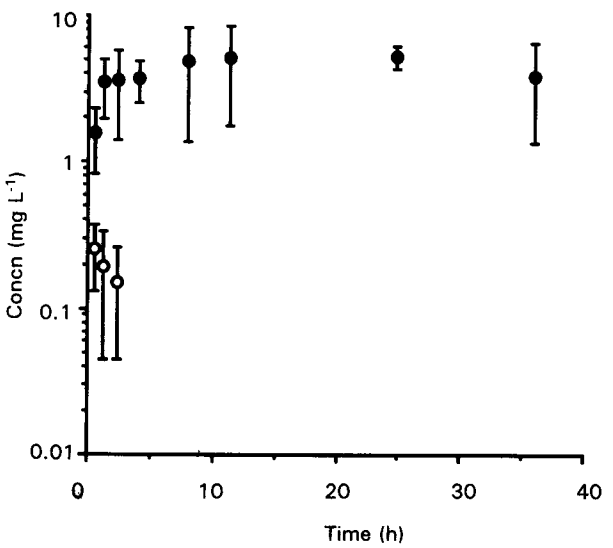

FIG. 2. Average (± s.d.) cannabidiol serum (○) and milk (●) concentration–time profiles obtained after an intravenous bolus injection (3 mg kg^{-1}) to lactating rabbits (n = 4).

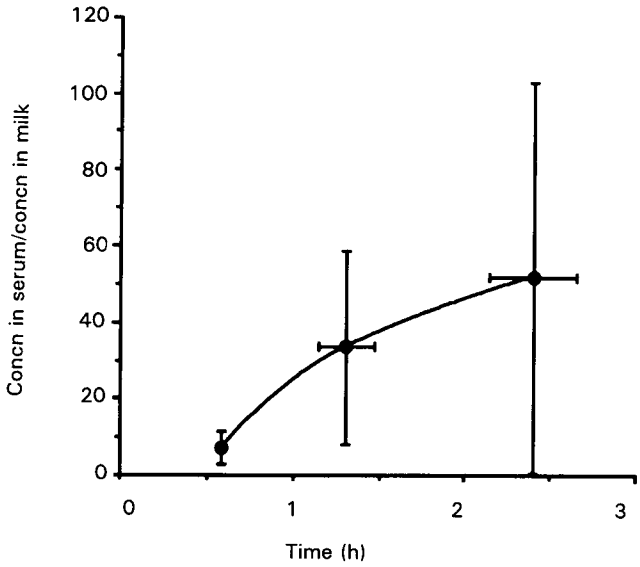

FIG. 3. Plot of the average (± s.d.) milk to serum cannabidiol concentration ratios as a function of time after intravenous injection of a 3 mg kg^{-1} bolus dose to four lactating rabbits.

8·4-fold accumulation of Δ^9-THC in human breast milk in comparison with the level in plasma 1 h after marijuana smoking. There are other reports on the excretion of THC in milk in rats and monkeys (Jakubovic et al 1973; Chao et al 1976). Although no comparisons were made between milk and plasma levels, milk drug levels appeared to reach a plateau at ~ 2–4 h after drug administration in rats and squirrel monkeys. Both CBD and THC are highly lipophilic and highly bound to serum proteins. At present, it is unclear as to why the plateau milk levels of CBD in rabbits were obtained at ~ 4–24 h. Nonetheless, the present study clearly indicates an extensive accumulation of CBD in milk. It is possible that suckling pups, upon maternal ingestion, may be exposed to CBD via breast feeding. Assuming an average milk CBD concentration of 4 μg mL^{-1} and a daily milk yield of 200 mL in New Zealand White rabbits at 25 days of lactation (Cowie 1969), the accumulation of CBD in milk over a 24-h period after intravenous dosing may account for up to 5·1% of the maternal dose per litter. Based on an average litter size of seven, the amount of CBD ingested by a suckling rabbit pup appears to be small (0·7% of the maternal dose). However, the extent of neonatal exposure may be increased significantly after the dose is normalized for the difference in body weight. Assuming an average neonatal body weight of 0·25 kg at 25 days of age, pup dose may approach 15·2% of the maternal dose normalized to the maternal body weight (3 mg kg^{-1}). In the rabbit pup, systemic drug clearance (CL) and serum protein binding are often diminished (McNamara et al 1991, 1992). Since the steady-state drug concentration (C_{ss}) is determined by the systemic clearance (C_{ss} = dosing rate/CL), the reduced clearance in the pup may result in increased steady-state total (bound and unbound) drug concentrations. Neonatal drug exposure may be further increased when comparisons are made between mother and neonate for the unbound, pharmacologically active drug concentrations, due to the altered binding in the pup. Therefore, in assessing the neonatal pharmacodynamics of CBD via breast feeding, one should consider not only the absolute neonatal dose, but also possible alterations in systemic drug clearance and serum protein binding.

In summary, the present study reports a significant and time-dependent accumulation of CBD in milk of lactating rabbits after intravenous administration.

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Comparison of distribution of brush-border exo- and endopeptidases in rat and rabbit intestine

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Abstract—The distribution of brush-border endopeptidase-2, aminopeptidase W, carboxypeptidase P, and aminopeptidase P along the rat and rabbit intestine was examined. In both species, aminopeptidases P and W increased distally and reached the highest in the ileum; their activities in the ileo-caecal junction were the lowest. Endopeptidase-2 had a uniform intestinal distribution in both species with the highest activity in the ileum and little activity in the ileo-caecal junction or caecum. With a distribution similar to that of endopeptidase-2, carboxypeptidase P also had high activity in the ileum in rats and rabbits.

From the point of view of drug delivery, brush-border membrane peptidases metabolically limit intestinal absorption of peptide and peptidiomimetic drugs; release drugs from prodrugs by cleaving the amide bond between the drug and progroup; and digest controlled-release systems, of which polymeric materials are polypeptides, to release drugs. Therefore, a knowledge of longitudinal distribution of brush-border peptidases is important for the rational delivery of drugs and peptide drugs.

There are several endo- and exopeptidases in the intestine; the distribution of some of them in rabbits, rats and man has been reported (Auricchio et al 1978; Skovbjerg 1981; Sterchi 1981; Miura et al 1983; Bai 1993a). However, the distribution of aminopeptidase P, aminopeptidase W, endopeptidase-2, and carboxypeptidase P in rat and rabbit intestine has not been compared. In this study, the distribution of these brush-border enzymes along the rat and rabbit intestine is compared and their activities in the caecum or ileo-caecal junction are also examined.

Materials and methods

Materials. Benzyloxycarbonyl-Pro-Ala, benzyloxycarbonyl-Pro, Ala, Glu-Trp, insulin B-chain, 1,10-phenanthroline, phosphoramidon, $MnCl_2$, Tris, Tyr-D-Ala-Gly, and pentobarbitone were obtained from Sigma Chemical Co. (St Louis, MO). Arg-Pro-Pro was from Bachem Bioscience Inc. (Philadelphia, PA). Cilastatin was a gift from Dr Helmut Krop (Merck Sharp and Dohme Research Laboratories, Rahway, NJ). Bovine γ-globulin and dye reagent for the protein assay were obtained from Bio-Rad Laboratories (Richmond, CA). All other chemical reagents