Toxicological, Pathological, and Teratological Studies in Animals with Cephradine

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Cephradine, a semisynthetic cephalosporin antibiotic, has a low order of oral and parenteral toxicity in animals. The oral LD₉₅ in mice and rats ranged from 5 to >8 g/kg, and the intraperitoneal LD₉₀ values in mice and rats were 0.7 to 1.5 g/kg and 4.0 g/kg, respectively. The intravenous LD₉₀ in mice ranged from 3.0 to 3.8 g/kg. In anesthetized dogs, intravenous doses of cephradine (40 and 120 mg/kg, given 45 min apart) had no effect on either the renal or cardiovascular systems. Single intramuscular injections (0.25 ml or 0.5 ml of a solution containing 125 to 235 mg of cephradine/ml) elicited no signs of either pain or local irritation in dogs, and only transient signs of slight-to-moderate irritation were observed in rabbits. In subacute toxicity studies, cephradine was administered for 4 weeks to rats (daily intraperitoneal doses of 160, 480, or 1,600 mg/kg) and dogs (daily intravenous doses of 80, 240, or 800 mg/kg); in addition, over a 2-week period, monkeys were given daily intravenous doses of 60, 180, or 600 mg/kg. No clinical, biochemical, gross, or micropathological changes due to cephradine were observed in these animals; especially notable was the absence of any signs of nephrotoxicity. In chronic toxicity studies, daily doses of cephradine were administered orally to rats (100 to 1,000 mg/kg), dogs (50 to 500 mg/kg), and monkeys (50 to 500 mg/kg) for 26, 26, and 13 weeks, respectively. Significant responses were observed only in rats, in which grossly enlarged, but histologically normal, ceca developed, a common finding in rodents dosed with antibiotics; in addition, there were increases in the relative and absolute weights of the adrenal glands. None of these effects was observed in rats that were necropsied 3 weeks after termination of dosage. In reproduction studies in mice and rats given either daily oral doses (100 or 300 mg/kg) or daily intraperitoneal doses (rats only; 80 or 320 mg/kg) of cephradine, no drug-related teratogenic changes in the offspring were observed.

The identification of cephalosporin C by Abraham and Newton (1) led to the synthesis of an entirely new series of β-lactam antibiotics. Abraham (2) found that cephalosporin C could be cleaved by acid to provide a low-yield conversion to the nucleus, 7-aminocephalosporanic acid (7-ACA). When this nucleus became available in quantity, many new derivatives (semisynthetic cephalosporins) were produced either by acylation of the amino group or by replacement of the acetoxy group, or by a combination of these processes. It was hoped that, among these many derivatives, some might possess certain desirable properties that were lacking in the parent compound. Especially to be desired were broadening of the antimicrobial spectrum, more efficient absorption after oral administration, and greater resistance to metabolic degradation.

Cephradine, a semisynthetic cephalosporin, 7-[D(-)-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]octene-2-carboxylic acid, hydrate, possesses the desirable properties noted above. This compound was synthesized in 1969 at The Squibb Institute for Medical Research (4). The antimicrobial activities in vitro of cephradine, and its chemotherapeutic efficacy in animals after oral administration, have been described recently (H. Gadebusch et al., Progr. Antimicrob. Anticancer Chemother., in press). Pharmacological studies, both in animals and in humans, will be reported elsewhere. Various papers on the evaluation of cephradine in the treatment of human disease have been published (3, 5, 6, 7, 9, 10), and reports on other clinical studies with the drug are in press.

The studies reported here are concerned with
the toxicological, pathological, and teratological responses observed in animals after either the oral administration of bulk cephradine or the parenteral administration of Cephradine for Injection, a blend of sodium carbonate and cephradine; the latter material has been formulated for parenteral administration. The cephradine component contained in both the oral and parenteral preparations was identical.

MATERIALS AND METHODS

Acute toxicity. The animals used in these studies and their weight ranges are described below: CD-1, Charles River mice, 18 to 23 g, and Charles River CD Sprague-Dawley rats, 125 to 160 g. These animals were fasted for 1 to 2 h prior to treatment. For oral and intraperitoneal administration, bulk cephradine was usually prepared at a concentration of 10 to 20% in 0.15% agar; in oral studies, this suspension was administered by gavage. For intravenous use, Cephradine for Injection was prepared as an aqueous solution; the intravenous injection rate was 0.1 ml/5 s.

In one study, an aqueous solution of Cephradine for Injection was administered in single intravenous injections at doses of 500, 1,000, 2,000, or 4,000 mg of cephradine/kg to groups of 10 to 20 male or female mice. In another study, liver or kidney damage was established in male mice by pretreatment with either oral doses of carbon tetrachloride or intravenous doses of uranyl nitrate, respectively. These mice were then given single intravenous doses of cephradine (500, 1,000, 2,000, or 4,000 mg/kg); there were 10 to 15 mice per group.

The LD₅₀ values were calculated by the method of Miller and Tainter (8), based on 8- to 11-day mortality data.

Studies were also carried out to determine the effects of intravenous doses of Cephradine for Injection on the renal and cardiovascular systems of anesthetized dogs. Two fasted young-adult male beagles (10.8 and 10.9 kg) were hydrated with water, anesthetized with intravenous sodium pentobarbital, intubated with an endotracheal tube, and maintained under anesthesia by a constant intravenous infusion of sodium pentobarbital. Urine was collected from cannuæ inserted into both ureters. Renal blood flow was determined by means of a Micron blood flowmeter, with a flow probe placed around the exposed left renal artery. Glomerular filtration rate was estimated from the clearance of exogenous creatinine. In addition, continuous recordings were made of arterial pressure, heart rate, electrocardiogram (ECG), respiration, and rectal temperature. After basal renal and cardiovascular functions had been established, a 20% aqueous solution of Cephradine for Injection was injected intravenously into both dogs during 1-min periods at doses of 40 and 120 mg of cephradine/kg; the two doses were given 45 min apart. The polygraphic records were examined for evidence of arrhythmia and for changes in ECG waveforms, arterial pressure, and heart rate.

Muscle irritation studies. Cephradine for Injection was dissolved in sterile water, and single injections were made into the thigh muscles of New Zealand white rabbits (0.25 ml; 35 to 60 mg of cephradine) and beagles (0.5 ml; 115 to 235 mg of cephradine). Some animals were sacrificed on the second day postdose, and the remainder 5 to 8 days later; the injected muscles were excised and examined for signs of local irritation.

Subacute studies in rats, dogs, and monkeys. In a 4-week study in rats and beagles, and in a 2-week study in rhesus monkeys (Macaca mulatta), sterile aqueous solutions of Cephradine for Injection were administered twice daily, 6 days a week, as follows: to rats, total daily intraperitoneal doses of 160, 480, or 1,600 mg of cephradine/kg; 12 rats per group; to dogs, total daily intravenous doses of 80, 240, or 800 mg of cephradine/kg, three dogs per group; and to monkeys, total daily intravenous doses of 60, 180, or 600 mg of cephradine or cephalexin/kg, two monkeys per group. A complete postmortem was done on all animals, 10 organs from each animal were weighed, and samples of 50 tissues were taken from each animal for histological examination.

In all the subacute and chronic toxicity studies reported here, the criteria for evaluation included survival data, changes in body weight, food and water consumptions, physical condition, behavior, the results of extensive clinical laboratory tests, and gross and micropathological examinations. The dogs and monkeys used in these studies weighed 8.5 to 11.0 kg and 3.0 to 5.5 kg, respectively.

Chronic toxicity studies in rats, dogs, and monkeys. Bulk cephradine was suspended in 0.15% agar and administered twice daily by gavage to rats, dogs, and monkeys for 26, 26, and 13 weeks, respectively. The total daily doses administered were as follows: rats (36 per group), 100, 300, and 1,000 mg of cephradine/kg; dogs (8 per group), 50, 150, and 500 mg of cephradine/kg; and monkeys (4 per group), 50, 150, and 500 mg of cephradine/kg. In each study, some animals were sacrificed during treatment, and the remainder 3 weeks after treatment had ended; all animals were submitted for gross and micropathological examinations.

Reproduction studies in mice and rats. In rats, the reproduction studies consisted of a fertility and general reproductive performance study (stage I), a teratology study (stage II), and a peri- and postnatal study (stage III). In each of these experiments, bulk cephradine was suspended in 0.15% agar or 1% carboxymethylcellulose and administered by gavage twice daily, 7 days a week, at total daily doses of 100 or 300 mg of cephradine/kg. In the stage I, II, and III studies, there were 12, 20, and 22 female rats, respectively, per group. In the stage I study, matings were made with male rats that had been dosed with cephradine for 10 to 23 weeks on a regimen similar to the one described above; at the time of mating, the females had been dosed for 2 weeks. The female rats in the teratology study were dosed from gestation day 6 until day 15 and then were killed on gestation day 21. The fetuses were removed, fixed in Bouin’s fluid, and examined for gross malformations and abnormalities; in addition, all pups were necropsied and prepared for detailed skeletal examinations. In the peri- and postnatal study, groups of pregnant rats were given the drug daily, beginning on gestation day
15, and continuing to parturition and through lactation until the pups had been weaned.

In addition to the teratology study in rats, a similar stage II study was carried out in mice (20 per group); the daily oral doses of cephradine used in the mouse study were the same as those given to the rats, i.e., either 100 or 300 mg/kg.

RESULTS

Acute toxicity. The acute toxicity of cephradine in mice and rats is shown in Table 1. For all routes of administration, the majority of deaths, in both mice and rats, occurred within 24 to 72 h after dosing. At the highest intraperitoneal and intravenous doses of cephradine (4,000 mg/kg and greater), ataxia was the only sign of toxicity. At the highest oral dose (8,000 mg/kg), there was no sign of toxicity.

In mice with hepatic or renal damage induced experimentally, the intravenous LD₅₀ was similar to that obtained in normal animals.

Intravenous doses of cephradine (40 and 120 mg/kg, given 45 min apart) had no effect on the renal or cardiovascular functions of anesthetized dogs.

Muscle irritation studies. Intramuscular injections in dogs caused no signs of either pain or irritation. In rabbits, there were signs of mild-to-moderate irritation, slight-to-marked degeneration without necrosis, a trace-to-moderate diffuse hemorrhage, and slight-to-moderate edema; there was no evidence of drug deposition.

Subacute studies in rats, dogs, and monkeys. In a 4-week study in rats, Cephradine for Injection, given intraperitoneally at total daily doses of 160, 480, or 1,600 mg of cephradine/kg, caused no drug-induced toxic signs or histological changes, with the exception of a dose-related enlargement of the cecum. Enlarged ceca are commonly seen in rodents dosed with antibiotics; hence, this effect should not be interpreted as evidence of toxicity.

Dogs given Cephradine for Injection intravenously for 4 weeks at total daily doses of 80, 240, or 800 mg of cephradine/kg showed no significant signs of toxicity and no gross or microscopic changes that could be attributed to the compound. In a similar study, rhesus monkeys were given intravenous doses of cephradine or cephaloridine for 2 weeks at total daily doses of 60, 180, or 600 mg/kg. There were no drug-related toxic signs or gross micropathological changes in the monkeys given cephradine; the only change noted in these animals was loose feces during the first 9 days of treatment. In the animals given cephaloridine, one high-dose monkey died after 3 days of treatment, and the other high-dose animal and one intermediate-dose animal were sacrificed in poor condition during the first week of dosing. The latter two monkeys showed clinical and biochemical evidence of renal damage, and in all three animals there was histological evidence that indicated extensive necrosis of the tubules of the kidneys. As noted above, under similar experimental conditions, neither biochemical nor histological evidence of nephrotoxicity was found in the monkeys given cephradine.

Chronic toxicity studies in rats, dogs, and monkeys. The daily oral administration of cephradine to rats (100, 300, or 1,000 mg/kg), and to dogs and monkeys (50, 150, or 500 mg/kg) for 26, 26, and 13 weeks, respectively, caused no significant toxic effects. Grossly enlarged, but histologically normal, ceca were noted in rats sacrificed during treatment; however, this response, which is usually seen in rodents dosed with an antibiotic, was not observed in animals necropsied 3 weeks after the cessation of dosage. In the high-dose rats, sacrificed after 13 to 26 weeks of treatment, there was an increase in the absolute and relative weights of the adrenal glands; however, this response was not observed in animals maintained for 3 weeks after the end of the dosing period. In dogs, the only side effects noted (high- and intermediate-dose animals) were moderate abdominal tenderness, emesis, and loose stools; these effects were only observed during the first 3 weeks of treatment. In the monkeys, high- and intermediate-dose animals had loose stools throughout the study. In rats, dogs, and monkeys, hematological and blood chemical values were within the normal ranges.

Reproduction studies in mice and rats. The daily oral administration of cephradine to rats at doses of 100 or 300 mg/kg did not induce any adverse signs in (i) a fertility and general reproductive performance study, (ii) a teratology study, and (iii) a peri- and postnatal study. In another teratology study, in which Cephradine for Injection was administered intraperitoneally to rats at daily doses of 80 or 320 mg of cephradine/kg, the drug had no effect on

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* Bulk cephradine.
* Cephradine for Injection.
fetal development and did not induce teratogenic changes in the offspring. In a teratology study in which mice were given daily oral doses of 100 or 300 mg of cephradine/kg, anomalies were observed in some of the fetuses in both the treated and the control groups. Since the incidence of these anomalies was within the expected limits of the spontaneous changes usually found in the strain of mice used, it was concluded that these effects were due to chance, and were not caused by treatment with cephradine.

DISCUSSION

It has been reported (Gadebusch et al., in press) that, after oral administration in laboratory animals, cephradine is well tolerated, is absorbed efficiently, and is an effective therapeutic agent against experimental infections. On the basis of the studies reported here, it is also concluded that cephradine has a low order of acute, subacute, and chronic oral toxicity, and acute and subacute parenteral toxicity. After daily intravenous doses of cephradine had been given to monkeys for 2 weeks, these animals showed no signs of nephrotoxicity. Cephradine did not induce any teratogenic changes in the offspring of either mice or rats.

Heretofore, the route of administration has been a determinant in the choice of a cephalosporin antibiotic. Therefore, it is a distinct therapeutic advantage that a single cephalosporin antibiotic, cephradine, may be used in both oral and parenteral preparations.

Absorption, excretion, and distribution studies with cephradine in laboratory animals and in humans will be reported elsewhere.

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LITERATURE CITED