DIMINAZENE ACETURATE

IDENTITY

Chemical name: 4,4'-(diazoamino)dibenzamidine diaceturate

1,3-bis(p-amidinophenyl)triazine bis(N-acetyl-glycinate)

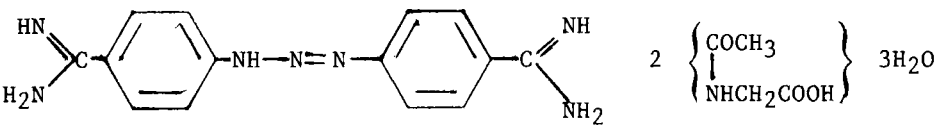
1,3-bis[4-guanylphenyl]triazine diaceturate

4,4'diamidinodiazminobenzene diaceturate

p,p'-diguanyldiazoaminobenzene diaceturate

Synonyms: Azidin; Ganasag; Berenil

Structural formula:



Molecular formula: $C_{22}H_{29}N_9O_6$

Molecular weight: 515.54

OTHER INFORMATION ON IDENTIY AND PROPERTIES

Pure active ingredient:

Appearance: Yellow solid

Melting Point: Decomposes at 217°C

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Diminazene is an antitrypansmal agent. Although it is principally used for the therapeutic treatment of trypanosomiasis in cattle, efficacy has been demonstrated in rabbits (Gilbert and Newton, 1982; Gilbert, 1983). In addition, pharmacokinetic evaluations have also conducted in sheep (Aliu and Odegaard, 1985), goats (Aliu, et al., 1984) and rats (Fussganger and Bauer, 1958; Raether et al., 1972). Activity has been demonstrated against Trypanosoma congolense, T. brucci, T. gambiense, T. rhodesiense, T. lewsisi, and Babesia canis (Fussganger and Bauer, 1958).

Dosage

The recommended therapeutic dose of diminazene aceturate is 3.5 mg/kg administered intramuscularly or 2.0 mg/kg administered intravenously as a sterile suspension.

Pharmacokinetics

The pharmacokinetics of diminazene have been investigated in the laboratory and target animal species. In general, the peak blood levels (Cmax) were reached within 1 hour after dosing (Aliu et al., 1985,; Gilbert and Newton, 1982; Gilbert, 1983; Kellner, et al., 1985; Raether, et al., 1972; Raether, et al., 1974; Klatt and Hajdu, 1971; and Aliu and Odegaard, 1985). The only exception was the dog, in which peak levels were not reached until 3 hours after dosing (Fussganger and Bauer, 1958). The peak blood levels were 1.1 ppm in the rabbit (Gilbert and Newton, 1982; Gilbert, 1983), 13 ppm in the rat (Raether et al., 1972), 4.6 to 6 ppm in cattle (Kellner et al., 1985; Klatt and Hajdu, 1971) and 3.9 to 6.7 in goats (Aliu et al., 1985) and sheep (Aliu and Odegaard, 1985).

The elimination from the blood was generally biphasic, with a half-life of approximately 1.3 hours for the initial phase and up to 188 hours for the terminal phase. However, the studies by Aliu and Odegaard (1985) indicated that the sheep displayed a triphasic elimination curve which is indicative of a three-compartment model. In cattle, the drug was primarily excreted in the urine (Kellner et al., 1985). More than seven times as much radioactivity was found in the urine than in the feces in this species. By day 20 after dosing, more than 80% of the dose had been eliminated.

METHODS OF RESIDUE ANALYSIS

Several techniques have been reported for the determination of diminazene in plasma, These methods include paper and thin-layer chromatography, (Clark, 1969), colorimetry (Raether, et al., 1972) and high performance liquid chromatography (Aliu and Odegaard, 1983). Except for the method of Aliu and Odegaard (1983), most assays were either non-specific, insensitive to submicrogram levels, or they involved tedious and protracted sample preparation steps.

Determinations of diminazene in milk have been conducted using a colorimetric method (Klatt and Hajdu, 1971) and a high performance liquid chromatographic (HPLC) method (Aliu and Odegaard, 1985). The colorimetric method reported a sensitivity of 0.07 ppm in milk using a wavelength of 540 nm. The HPLC method used paired-ion extraction and an internal standard which produced a sensitivity of 0.05 ppm in plasma.

The investigations of diminazene residues in tissues were generally conducted using either radio-tracer analysis (Gilbert, 1983) or colorimetry (Klatt and Hajdu, 1976).

METABOLISM AND RESIDUES STUDIES

Metabolism Studies

Investigations using radiolabeled diminazene administered intramuscularly to cattle at 3.5 mg/kg have shown that the compound is eliminated in the urine and feces according to a biphasic process (Kellner, et al., 1985). The terminal half-lives were 173 hours for the urine and 207 hours for the feces. Two metabolites, in addition to the parent compound, were found in the urine of one of the animals by thin layer chromatography. They were determined to be: 1) p-aminobenzamidine (22% of the radioactivity) and 2) p-amino-benzamide (4% of the radioactivity).

Residue Studies

Studies in rabbits given 3.5 mg/kg intramuscularly showed that liver contained the most residues after 7 days withdrawal. Those residues represented 35 to 50% of the dose (about 40 ppm). The residues in the kidneys after 7 days were approximately 3.3 ppm (Gilbert, 1985).

The residue pattern in the edible tissues of cattle 7 days after dosing with 3.5 mg/kg diminazene indicated that liver contained the highest concentrations of residues (75 ppm) followed by the kidneys at 55 ppm. The concentrations in the other tissues were much lower with the lowest values being found in the fatty tissue and skeletal muscle (Kellner et al., 1985). The pattern was similar 20 days after dosing but the concentrations were lower. The results of this study have been tabulated in Table I. A similar study was conducted in cattle using an 8 mg/kg dose (Klatt and Hajdu, 1976). The patterns found in that study were basically the same as those noted by Kellner et al.

TABLE I. Diminazene residues in calves (ppm Equivalents)

Tissue	7 days	20 days
Liver	75.49	24.42
Kidney	54.67	12.12
Spleen	2.51	1.00
Muscle	0.52	0.26
Injection Site	0.69	0.64
Fat	0.20	<0.18

The excretion of diminazene in cow's milk has been investigated after administration of a 3.5 mg/kg dose (Klatt and Hajdu, 1971). The highest milk levels were found 6 hours after dosing (0.2 - 0.5 ppm). The levels were below the limit of detection (0.07 ppm) 48 hours after dosing.

Peak levels (1.68 ppm) of diminazene were found in the milk of goats 4 hours after the intravenous administration of 2 mg/kg (Aliu et al., 1985). In the same study using a 3.5 mg/kg intramuscular dose, only trace amounts (0.05 ppm) of diminazene could be detected after 72 hours.

Recommended Withdrawal Times

Chronic (9-month) toxicity testing of the oral administration of diminazene in rats and dogs indicated a no-effect level of 20 mg/kg body weight (Baeder, et al., 1975; Scholz and Brunk, 1969). Allowing a safety factor of 100, the acceptable daily intake value (ADI) for a 60 kg human was calculated to be 12 mg. Based on this figure, the Federal Republic of Germany has set a withdrawal period of 20 days for cattle and sheep. By 20 days, the levels are lower than the ADI value.

Pharmacokinetic studies for diminazene in sheep were conducted by Aliu and Odegaard (1985) in Norway. Using a three-compartment open model the authors calculated the clearance of diminazene. Based on the data they obtained, a preslaughter withdrawal time of 14-26 days in sheep was recommended. However, the paper indicated that since liver contains the highest concentrations of the residues, the withdrawal period could be more accurately estimated from the time drug concentrations in the liver.

APPRAISAL

The intramuscular use of diminazene in food-producing animals produce significant residues in the liver and kidney tissues. However, the levels and persistence of these residues were significantly less than those found for a similar antitrypansmal agent, isometamidium.

With the chronic toxicity testing of the oral administration of diminazene, the acceptable daily intake (ADI) for man at 12 mg could be calculated. This provides for very high safe concentration levels and therefore minimal human food safety concerns. Therefore, the recommended withdrawal period prior to slaughter of 20 days for cattle and sheep as well as the 3 day milk discard period, should be adequate.

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