CASE REPORT

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Tissue Distribution of Ethosuximide and Clobazam in a Seizure Related Fatality

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ABSTRACT: The case of a six-year-old male who died in a hospital while receiving several anticonvulsant drugs is described. Phenytoin, desmethyldiazepam, clobazam (an experimental 1,5 benzodiazepine), and desmethylclobazam were quantitated in serum, liver, and brain tissue by high performance liquid chromatography. Ethosuximide was quantitated by gas chromatography. To our knowledge, this is one of few reports describing tissue concentrations of ethosuximide collected at autopsy and the first report of clobazam/desmethylclobazam tissue distribution in man.

KEYWORDS: pathology and biology, epilepsy, ethosuximide, clobazam, chromatographic analysis

The 1,4 benzodiazepines have an established role in the treatment of epilepsy. Diazepam administered intravenously or rectally is used in the management of status epilepticus, and several of the benzodiazepines, including clonazepam and nitrazepam, have been used orally for the long-term management of intractable seizures [*1-4*].

Anticonvulsant properties of clobazam, a 1,5 benzodiazepine, were recognized in animals in 1973 by Barzaghi [*5*]. Clobazam (Fig. 1) was recently introduced in Europe and claimed to have fewer side effects than other benzodiazepines. In several double blind clinical trials [*3,6,7*] in poorly controlled epileptic patients, there was a 50% decrease in number, duration, and severity of seizures compared to the placebo group.

Ethosuximide was first described for treatment of absence (petit mal) seizures in 1958. The incidence of serious toxic effects has been relatively low, although one case of a suicidal overdose of ethosuximide has been reported [*8*].

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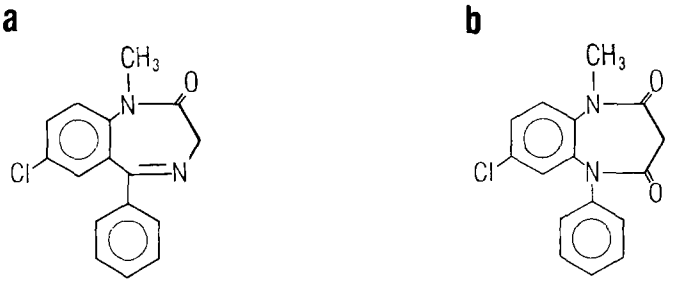

FIG. 1—*Structural differences between diazepam* (a), *a 1,4-benzodiazepine and clobazam* (b), *a 1,5-benzodiazepine.*

This laboratory was recently asked to analyze serum, liver, and brain for several anticonvulsant drugs and metabolites from a six-year-old boy who died of intractable seizures ten days after initial treatment with clobazam. Because of the clinical history and development of seizures after introduction of this experimental benzodiazepine, it was considered of interest to report this case, relevant pathology, and analytical methodology.

Case History

A six-year-old male was admitted to a children's hospital for introduction of the 1,5 benzodiazepine, clobazam, to aid in the control of his long-standing seizure disorder. On admission, medications included nitrazepam, carbamazepine, ethosuximide, acetazolamide, and lorazepam. His pattern of seizure activity worsened two months before admission, but an increase in ethosuximide dose was beneficial. Nitrazepam was gradually discontinued, over eight days, with clobazam being introduced at 10 mg three times a day (t.i.d.). Except for a generalized seizure, within 36 h of the first clobazam dose, he remained seizure free for nine days. Within a 4-h period, two to four days later, he had several generalized seizures which were treated with diazepam and phenytoin intravenously and clobazam, ethosuximide orally. Respirations ceased at 0230 h and cardiac arrest occurred. On transfer to the Intensive Care Unit (ICU), no brain stem function was found and life support was discontinued.

Pathological Findings

Pathological examination of the brain including transmission electron microscopy failed to reveal the cause of the seizure disorder. An important finding at autopsy was an acute suppurative appendicitis. A patchy early acute bronchopneumonia was also present but felt to be related to aspiration rather than secondary to sepsis from the appendicitis.

Toxicological Analysis

High Performance Liquid Chromatography (HPLC)

Liquid chromatography was performed on a Spectra Physics liquid chromatograph (SP 8770 pump, SP 8780 XR autosampler, and SP 4270 integrator) attached to an LKB 2140 rapid scanning spectral detector. The optical and data units of the LKB 2140 system were interfaced with an IBM-XT personal computer (all components obtained from Technical Marketing Associates, Halifax, Nova Scotia).

Clobazam, desmethylclobazam, and desmethyldiazepam were analyzed simultaneously by modification of the methods of Zilli [*9*] and Ratnaraj [*10*]. To 1.0 mL of serum was added

0.4 mL of aqueous prazepam internal standard (4 mg/dL), 0.1 mL of saturated sodium carbonate, and 5 mL of methylene chloride. After mixing and centrifugation, the extract was evaporated before reconstitution in the mobile phase (0.1*M* sodium phosphate, monobasic [NaH_2PO_4]: acetonitrile (1 : 1) at pH 3.0) and chromatographed at a flow rate of 1.6 mL/min on a Brownlee 250- by 4.6-mm RP-8 column (5 μm). Detector wavelength was set at 228 to 232 nm. Calibration curves were prepared by obtaining peak height ratios for each analyte against prazepam internal standard. For clobazam and desmethyldiazepam, a single series of standards were used from 0.1 to 0.43 mg/L. Two standard curves were prepared for desmethylclobazam (0.08 to 0.30 and 0.50 to 2.0 mg/L).

Phenytoin was quantitated in serum using a modification of the Soldin method [*11*] and tissue analysis after extraction as described previously [*12*].

Gas Liquid Chromatography

Ethosuximide was analyzed by gas chromatography with a nitrogen/phosphorus detector. Drug free serum standards were prepared from 38 to 190 mg/L. To a 0.5-mL sample or standard were added 0.5 mL of aqueous α-methyl, α-propyl succinamide internal standard (160 mg/L), 1.0 mL of 0.05*M* phosphate buffer (pH 3.0), and 0.5 mL of ethyl acetate. The tubes were mixed for 5 min, centrifuged, and 1 to 2 μL of organic layer injected onto the gas chromatograph.

An HP 5730A gas chromatograph fitted with a nitrogen/phosphorus detector (Hewlett Packard Canada Ltd., Montreal, Quebec) was used for ethosuximide quantitation. A 30-m by 0.75-mm inner diameter wide-bore capillary column coated with SPB-1 (Supelco Inc., Bellefonte, PA) was operated at 120°C. Peak height ratios relative to the internal standard was used for quantitation.

Standards and Reagents

Acetonitrile and methanol were HPLC grade and glass distilled (Caledon Laboratories Ltd., Georgetown, Ontario and Fisher Scientific, Dartmouth, Nova Scotia). Clobazam and desmethylclobazam were obtained from Hoechst Canada, Inc., Montreal, Quebec. Desmethyldiazepam and nitrazepam were supplied by Hoffman La Roche Ltd., Etobicoke, Ontario. Park Davis Canada Inc. provided ethosuximide, sodium phenytoin, and prazepam. Alphamethyl alphapropyl succinimide was purchased from Aldrich Chemical Co., Milwaukee, WI.

Results

A summary of the quantitative serum and tissue analysis appears in Table 1.

The chromatogram of the serum extract for clobazam, desmethylclobazam, and desmethyldiazepam is found in Fig. 2. In Fig. 3, the chromatogram of the serum extract for ethosuximide quantitation is presented.

Discussion

Dulac [*13*] and Shimazu [*14*] reported mean concentrations of clobazam of 0.32 and 0.73 mg/L in successfully controlled children with epilepsy. At steadystate, the concentration of the major metabolite desmethylclobazam is approximately eightfold greater than the parent drug, but has only 25% of its potency as a seizure controlling drug. Although there have been very few studies [*2,14,15*] comparing concentrations of clobazam/desmethylclobazam with therapeutic or toxic effects, it is our opinion that the concentrations measured in this case were within the concentrations normally seen at steadystate after therapeutic doses for

TABLE 1—*Summary of toxicological analysis.*

	Concentration		
	Serum, mg/L	Liver, mg/kg	Brain, mg/kg
Clobazam	0.1	0.37	
Desmethylclobazam	1.5	8.6	3.6
Desmethyldiazepam	0.05	0.29	0.05
Phenytoin	9.3	35.0	21.0
Ethosuximide	130	200	160
Nitrazepam	0.05	0.1	0.1

seven to ten days. No one has previously reported human tissue concentrations of clobazam and desmethylclobazam.

The subtherapeutic concentration of desmethyldiazepam was considered appropriate since diazepam was only administered intravenously (2 mg on two separate occasions) approximately 10 to 12 h before death.

For seizure control, the most often quoted therapeutic range for phenytoin is 10 to 20 mg/L [*15*]. The serum concentration of 9.3 mg/L at autopsy and tissue concentrations of

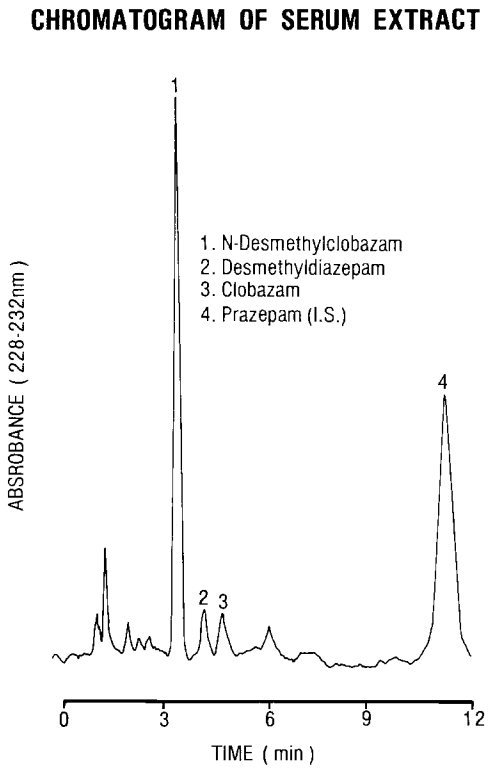

FIG. 2—*Chromatogram of serum extract used for benzodiazepine analysis:* (1) *desmethylclobazam (1.5 mg/L),* (2) *desmethyldiazepam (0.05 mg/L),* (3) *clobazam (0.1 mg/L), and* (4) *prazepam internal standard. For chromatographic conditions, see text.*

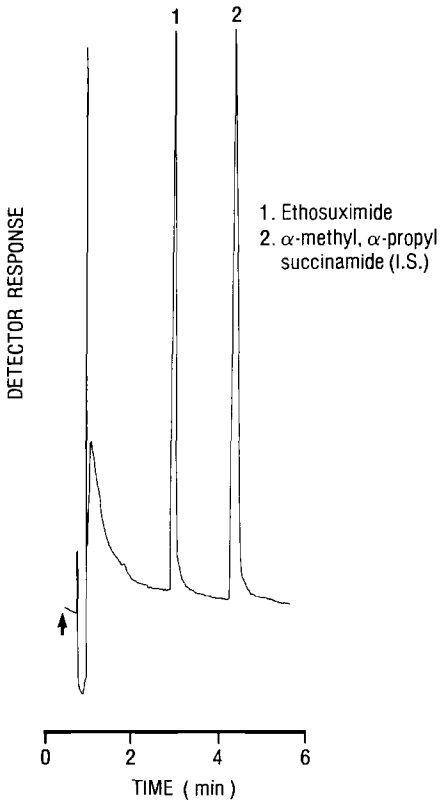

FIG. 3—*Chromatogram of serum extract used for ethosuximide quantitation:* (1) *ethosuximide (130 mg/L) and* (2) *α methyl α propyl succinamide internal standard. For chromatographic conditions, see text.*

35 mg/kg (liver) and 21 mg/kg (brain) were considered within the concentrations seen after chronic drug administration. By comparison, a 4.5-year-old who ingested 2.0 g of phenytoin and died after 48 h had postmortem concentrations of: blood 45 mg/L, liver 272 mg/kg, and brain 78 mg/kg [*16*].

In a group of pediatric patients, chronic daily doses of 11 to 40 mg/kg of ethosuximide resulted in an average steadystate concentration of 50 mg/L (range 28 to 83 mg/L). Most laboratories quote an ethosuximide therapeutic range of 40 to 100 mg/L. Concentrations greater than 100 mg/L are considered in the toxic range.

In a fatality involving an ethosuximide overdose, the following postmortem concentrations were reported: blood 250 mg/L, liver 280 mg/kg, and urine 120 mg/L [*17*]. The results in this case were considered to be in the toxic range (Table 1).

In a large study in the State of New Mexico, where death was suspected to be due to a seizure disorder or complication thereof, no focus for the origination of seizures was found on neuropathologic examination in 50% of the cases [*18*].

Conclusion

In conclusion, the major factor(s) responsible for the terminal seizure was not determined by toxicological or pathological investigation. It was considered of interest to report this case partly for the analytical methodology used for clobazam/desmethylclobazam, and to em-

phasize the importance of further investigations in young epileptic patients who die with intractable seizures.

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