CASE REPORT

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A Case of Fatal Rotenone Poisoning in a Child

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ABSTRACT: A case of a fatal rotenone poisoning in a three-and-a-half-year-old girl is described. The case report and autopsy findings are mentioned. For the extraction of rotenone out of biological samples, a solvent partitioning and silica gel open column chromatographic cleanup procedure has been used. The determination of rotenone was performed by high pressure liquid chromatography.

KEYWORDS: pathology and biology, rotenone, poisons, chromatographic analysis

Rotenone [2-isopropenyl-8, 9-dimethoxy-6-oxo-1, 2, 6,6a, 12, 12a-hexahydrofuro (2,3-b) (1) benzopyrano (3,4-b)-(1) benzopyran], is the major insecticidal constituent of the rhizome and the roots of *Derris elliptica, D. malacensis* (Leguminosae), and other species of derris, containing not less than 3% of rotenone [*1*].

Derris is a widely used agricultural and horticultural insecticide and larvicide. According to literature, the fresh root is quite toxic to mammals, but, as employed in most insecticidal preparations, derris is relatively harmless. The lethal dose (orally) in mammals varies from 50 to 200 mg/kg in the Guinea pig (the most susceptible of the animals examined by Cutkomp [*2*], to 3.0 g/kg in the rabbit, the most resistant. A reasonable estimate for man is 0.3 to 0.5 g/kg [*3*]. Rotenone is generally accepted as being an inhibitor of mammalian cell respiration [*4,5*].

In man, cumulative toxic effects may appear such as skin sensitivity reactions, up to convulsions and coma [*6*].

Immediate cause of death is asphyxia from respiratory arrest [*3*].

Case Report

After she has been away for about half an hour, at about 10 a.m., a mother found her three-and-a-half-year-old little girl vomiting, not feeling well, and preferring to sleep.

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The mother put her to bed. Ten minutes later, she noticed that the child's respirations were becoming irregular and slow.

She tried to wake her daughter but was not able to do so. The child became comatose and her eyes were half open. At about noon, the girl was admitted into the hospital in a very deep coma; there was no more spontaneous breathing, no measurable heart rhythm, and no measurable blood pressure.

Other clinical findings were:

- bilateral mydriasis,
- anuria,
- total lack of reflexes, and
- blood: pH of 6.76.

Reanimation, including intubation, ventilation, and gastric lavage were to no avail. The girl died at 6 p.m. from respiratory arrest.

Meanwhile, the parents found out that she probably had swallowed a mouthful of a product called "Galicide." According to the label on the container, Galicide is an insecticide made of plant material only and appropriate for external use on animals.

The manufacturer Eurotonic, Mougins, France gave the product's exact composition as:

- ethereal oil of cinnamon, 18.5 g,
- ethereal oil of cloves, 27.5 g,
- ethereal oil of fir, 17.5 g,
- ethereal oil of rosemary, 1.0 g,
- ethereal oil of thyme, 1.0 g,
- rotenone (pure), 6.1 g, and
- emulsificator "arcaponic" sufficient quantity (q.s.) Ad 100 g.

The product however was labelled: "Natural Product-Non Toxic."

Autopsy Findings

The most significant postmortem findings were:

(1) anoxic hemorrhages in thymus, lungs, and heart;
(2) bilateral serohemorrhagic pleural effusion (± 60 cm^3), badly aerated lungs;
(3) bloody stomach content, several submucosal hemorrhages;
(4) bloody ascites liquor (± 80 cm^3);
(5) kidneys showing a pale cortex and a dark red medulla which could be a reflection of acute tubular necrosis; and
(6) histological alterations of the cerebrum suggesting a recent acute hypoxic episode.

Materials and Methods

Apparatus

The chromatographic cleanup columm with a length of 25 cm and an inner diameter of 0.7 cm was equipped with a 50-mL reservoir.

The high pressure liquid chromatograph (RPLC) was a Hewlett Packard (formerly Hupe and Busch) Model 1010B with variable wavelength with an ultraviolet (UV) detector 1030B Hewlett Packard.

The RPLC columns were RPLC-C_{18}: chemically bonded octadecylsilane (ODS), RSL, Ghent. They were 10 μm, with a length of 25 cm and an inner diameter of 0.41 cm. The material was stainless steel.

Reagents

The reagents were:

- Diethylether: peroxide free—Ether is mixed with hydroquinone and distilled over a glass column with KOH pellets. The condensed ether is stored over KOH pellets in brown bottles and distilled again immediately before use [*7*].
- *n*-hexane (analytical grade, UCB).
- Acetonitrile (analytical grade, Merck).
- Sea sand (acid washed, Merck).
- Anhydrous sodium sulfate (Na_2SO_4) (analytical grade, Merck).
- Benzene (analytical grade, Merck).
- Methanol (analytical grade, Merck).
- Double distilled water: water used for HPLC was double distilled in glass and filtered through a millipore (0.45-mm) membrane before use.
- Silica gel 40 (Merck) (0.063 to 0.200 mm).
- Rotenone (97% purity) was purchased from Aldrich Chemical Co., Inc.; Milwaukee, Wisconsin.

Isolation of Rotenone out of the Biological Material [7]

Liver (10 g), kidney (5 g), brain (5 g), thymus (5 g), and muscle (10 g) are mixed thoroughly with acid washed sea sand (10 g) and anhydrous sodium sulphate q.s. until a homogeneous dry powder was obtained. This mixture was extracted with 100 mL of diethylether. The fluid samples (blood 5 mL; stomach content 5 mL) were extracted with 50 mL of diethylether. The organic layers were decanted, filtered over anhydrous sodium sulphate, combined in a rotavapor flask, and evaporated to dryness in a rotavapor apparatus at 60°C.

The residue was subjected to a liquid liquid partitioning cleanup with *n*-hexane/acetonitrile: 1/1. The acetonitrile layer was transferred into a rotavapor flask with 10 mL of benzene, the mixture was again evaporated to dryness at 60°C in the rotavapor apparatus.

Using 5 mL of benzene, this residue was transferred to silica gel column prepared as described by Bowman et al. [*8*]. The flask and the column were washed with five additional portions of 5 mL of benzene. The eluate was discarded.

Rotenone was eluted with 70 mL of benzene-acetone (97/3), the eluate was collected in a rotavapor flask, and evaporated to approximately 5 mL at 60°C.

These 5 mL were evaporated to dryness under a N_2 stream and the residue was dissolved in 1 mL of methanol, for injection (20 μL) into the liquid chromatograph.

Recovery Experiments

To determine the recovery of the extraction procedure, we spiked homogenized postmortem samples (each tissue in fivefold) with 2.50 ppm of rotenone. After storing one night in an open flask in a light-free cabinet, we extracted the same way as described above (Figs. 1 and 2).

Detection

A Hewlett Packard 1010B (formerly Hupe and Busch) equipped with a Hewlett Packard 1030B UV/VIS variable wavelength detector was used.

The column is a 10-μm C_{18} reversed phase (RSL-Ghent) of 25-cm length and 4.1-mm

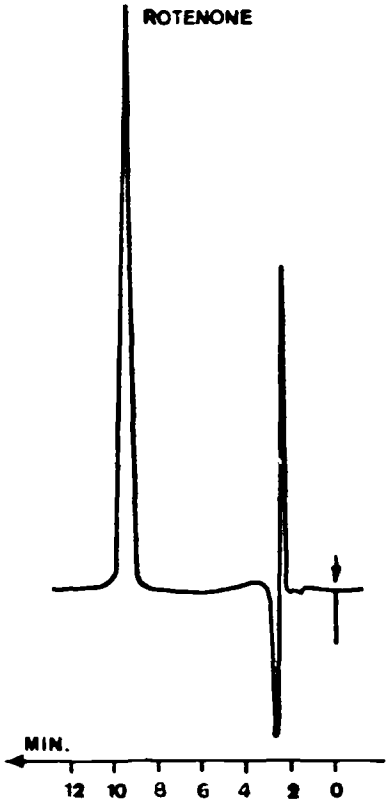

FIG. 1—*High pressure liquid chromatogram of a rotenone standard (25 μg/100 mL).*

internal diameter. As mobile phase we used a methanol-water mixture (75/25) at a temperature of 20°C (room temperature).

The solvent flow is 1 mL/min. Rotenone is detected at 295 nm. The injection system is a 20-μL loop connected on a Valco valve. The detection limit is 0.5 ppm.

Results

In Table 1, the results of the recovery experiments with control human biological material spiked with known amounts of rotenone are given.

In Table 2, the distribution and concentration of rotenone in different tissues of the victim are given.

We also determined the concentration of rotenone in the commercial product Galicide and found a value of 6 g/100 mL, which confirms the data given by the laboratories Eurotonic, Mougins, France.

Conclusion

Distribution of rotenone over the several tissues was not homogeneous. Values in the range of 2 to 4 ppm were present in blood, liver, and kidney, but we were not able to detect the presence of rotenone in brain, muscle, and thymus.

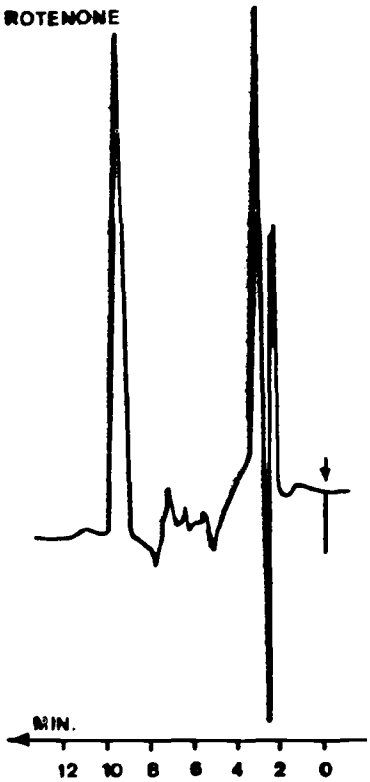

FIG. 2—*High pressure liquid chromatogram of a liver extract of the victim (att. 0.04).*

Although values of 2 to 4 ppm seem rather low, it is very likely that these amounts caused the death of the victim; she died from respiratory arrest, a probable cause of death in severe rotenone poisoning.

This hypothesis is strongly supported by autopsy findings such as histological alterations caused by asphyxia.

It must be stated that the possibility of an acute tubular necrosis associated with the ingestion of the ethereal oils may not be ruled out. The presence of ethereal oils in the Galicide solution might first have contributed to acute irreversible renal damage, dropping the clearance of rotenone nearly to zero and thus increasing serum levels, and secondly these oils promoted the absorption of the water insoluble rotenone out of the gastro-intestinal tract, again increasing serum levels and thus enhancing toxicity [*9*].

The estimated absorbed dose, orally, was about 10 mL, corresponding to 0.6 g of rotenone; the child's weight was about 15 kg so that the lethal dose can be estimated at 40 mg/kg.

In our knowledge, this is the first report of a fatal rotenone poisoning in man.

Medical history of the child revealed no particular preexisting pathology which could have contributed to the child's death.

TABLE 1—*Results of the recovery experiments with control human biological material spiked with known amounts of rotenone.*

Added ppm	Found ppm	% Recovery
	Blood	
2.50	2.48	
2.50	2.45	
2.50	2.42	
2.50	2.51	
2.50	2.49	
	2.47 ± 0.03	
	C.V.[a] = 1.4%	98.8% ± 1.2%
	Liver	
2.50	2.05	
2.50	2.15	
2.50	2.08	
2.50	2.02	
2.50	2.07	
	2.07 ± 0.05	
	C.V. = 2.3%	82.8% ± 2.0%
	Kidney	
2.50	2.00	
2.50	1.94	
2.50	2.05	
2.50	1.92	
2.50	1.93	
	1.97 ± 0.06	
	C.V. = 2.8%	78.7% ± 2.4%
	Brain	
2.50	1.03	
2.50	1.20	
2.50	1.15	
2.50	1.21	
2.50	1.12	
	1.14 ± 0.07	
	C.V. = 6.4%	45.6% ± 2.8%

[a]C.V. = coefficient of variation.

TABLE 2—*Distribution and concentration of rotenone in different tissues of the victim.*[a]

Biological Samples	Concentration of Rotenone, ppm
Stomach content	1260
Blood	2.40
Kidney	3.90
Liver	3.20
Thymus	neg or below detection limit
Brain	neg or below detection limit
Muscle	neg or below detection limit

[a]The concentration values are adapted from recovery experiments.

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