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Clinical parameters, postmortem analysis and estimation of lethal dose in victims of a massive intoxication with diethylene glycol

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Abstract

This work analyzes a massive intoxication that occurred in 1992 in Argentina as a result of the use of propolis syrup as a popular upper respiratory infection medicinal agent. The intoxicating agent was diethylene glycol (DEG), which caused metabolic acidosis, anuria, renal failure and death in 15 out of the 29 studied victims. DEG poisoning cases were classified in three groups according to survival time: Group 1—patients that survived up to 3 days; Group 2—patients that survived between 4 and 5 days; Group 3—patients that survived between 6 and 21 days. Patients from Group 1 showed the highest values of anion gap, the lowest measures of base excess (BE) and more severe clinical manifestations. Correlation between pH and BE was $r^2 = 0.68$, 0.99 and 0.55 for Groups 1, 2 and 3, respectively. A methanolic extraction was performed on the fatal victims' viscera and blood, with subsequent concentration and purification. The semi-crystalline fraction obtained retained DEG by means of co-dissolution and adsorption as demonstrated by thin lay chromatography/flame ionisation detection (TLC/FID). In 3 out of the 15 fatal cases (from Group 1), DEG was isolated from viscera and blood (femoral venous), between 48 and 72 h post ingestion. The concentration relation $(DEG)_{viscera}/(DEG)_{blood}$ ranged from 1.45 to 1.55 with a coefficient correlation $r^2 = 0.96$ ($n = 3$). In the other victims, DEG could not be detected. The reason for this could be the long survival period of the victims after their ingestion of the syrup. Additionally, putrefying mechanisms could have been operating. Samples of the propolis syrup of each victim were studied by means of nuclear magnetic resonance (NMR) and quantified by gas chromatography/flame ionisation detection (GC/FID). Results showed that syrup samples contained 65.0% (w/v) of diethylene glycol (DEG) and 32.0% (w/v) of propylene glycol (PG). A good correlation between the amount of DEG ingested and the anion gap ($r^2 = 0.63$) for the 15 victims studied could be observed. The lethal dose for human beings estimated in this work ranged from 0.014 to 0.170 mg DEG/kg body weight. This is a lower lethal dose than reported in a separate incident in Haiti. These results may contribute to the understanding of DEG's metabolic pathway and provides data from lethal doses in humans.

Keywords: Diethylene glycol; Clinical parameters; Postmortem analysis; Lethal dose

1. Introduction

Diethylene glycol (2-2 oxybisethanol, DEG) is produced in large amounts for domestic use in antifreeze blends, as a solvent, and as an intermediary in the production of poly-

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mers, higher glycols, morpholine and dioxane. There is high potential for consumer and worker exposure, and ingestion of sweet tasting glycol by children and pets is common [1]. The main effects produced by DEG after acute overexposure is kidney injury and metabolic acidosis. DEG may cause pain or discomfort in the abdomen, nausea, vomiting, diarrhea, dizziness, drowsiness, decreased urine production, malaise and coma. Severe kidney damages may occur that can be fatal if not promptly and adequately treated. Liver injury may also occur. Appropriate biochemical monitoring may be required. [2]

There have been few isolated cases of DEG intoxication for suicidal purposes or by domestic accidental ingestion have been reported. Other cases of massive intoxication episodes have also been described. Reports of 105 deaths following acute or chronic ingestion of DEG in a sulphanilamide elixir have demonstrated little dose–toxicity relationship. Oxaluria has not been observed in overdoses [3–4]. Another massive intoxication occurred in South Africa in 1967, with seven children as fatal victims [5]. Pandya [6] described 14 patients (ages 10–76 years) who had received glycerin contaminated with 18.5% diethylene glycol and 51% polyethylene glycol. Vomiting, diarrhea, gastrointestinal bleeding, abdominal pain, guarding, rigidity and abdominal distension developed within 4–5 days of administration. Over the next 2–3 days, the patients experienced oliguria, anuria, acidosis and hypotension. The doses of glycerin administered were not recorded. Later, two outbreaks were reported in which DEG replaced the regular vehicle in antipyretic preparations of acetaminophen. Both outbreaks occurred at the beginning of the 1990s in Nigeria and Bangladesh, causing 47 and 51 deaths, respectively [7–8]. Finally, between 1995 and 1996, more than 80 children in Haiti died because of the ingestion of locally manufactured acetaminophen syrup. The median concentration of DEG was 14.4% [9]. In this outbreak, 109 cases of acute renal failure among children were identified by the reports of hospital pediatricians and by the disease surveillance list collected by the Ministry of Health of Haiti.

In 1992, 29 people died due to acute renal failure with oliguria followed by anuria, between 24 and 48 h after consuming a propolis syrup that was commercialized in Argentina as a medicinal product for mild upper respiratory tract infections [10]. In the 20 cases studied, fatal victims ingested propolis syrup with high DEG levels. Propolis syrup has been used by many people to prevent or treat common upper respiratory infections. Propolis has been shown to exert antimicrobial activities, act as a healing product, and may have immunomodulatory properties [11,12]

The aim of this work is to study the interrelationships between the internal medium biochemical parameters modified by the ingestion of DEG, the relevant clinical manifestations and symptoms, the estimated lethal dose, and the correlation between the dose ingested and the anion gap. The results of the analysis of the victims' postmortem blood and organs were considered, as well.

2. Material and methods

2.1. Samples

Fifteen adult subjects were admitted to the hospital 1–3 days after the ingestion of contaminated propolis syrup (containing 65.0% (w/v) DEG and 32.0% (w/v) PG). Their sex and age are shown in Table 1.

The clinical history of each patient was studied. Blood chemistries were performed on samples collected when the patients were admitted to the hospital.

Autopsy blood and viscera (kidney and liver) of each victim were also analyzed to determine DEG. Postmortem blood specimens were taken from the femoral region and were placed in tubes without preservatives at 4 °C during 24 h before analysis. The viscera were stored frozen until analyzed. Syrup samples provided by relatives of the fatal victims were also analyzed.

2.2. Methods

2.2.1. Assay of biochemical parameters in patients

Na^+, K^+ and Cl^- ions were analyzed by flame photometry with a Radiometer FLM 3, Chirom Rapid Lab 348, ILAIT. The pH, pCO_2, bicarbonate (HCO_3^-) and hemoglobin measures were obtained by an AADEE Analyzer (CCI). The BE derived parameter (mEq/L) was estimated with the pH, bicarbonate and hemoglobin data.

The following formula was used in order to estimate the anion gap:

$$\text{Anion gap (mEq/L)} = (Na^+) + (K^+) - (HCO_3^-)$$

2.2.2. Isolation and assay conditions for DEG in blood and viscera

Homogenized tissue (kidney and liver, 10 g each) was dehydrated with anhydrous sodium sulfate (analytical grade) and left for 24 h at 40 °C. Visceral powder was placed in a Whatman paper No. 3 and extracted in a Soxhlet for 12 h in methanol. An aliquot was concentrated at low temperature in a vacuum evaporator. Another aliquot was placed at 4 °C. Blood was treated with methanol (1:10), vortexed and centrifuged. Part of the clear supernatant was conditioned for the qualitative assay. Ethylene glycol (EG) was used as an internal standard (IS) [13–14].

Gas chromatography was performed in a Shimadzu GC-14 equipped with a Shimadzu CR 4A integrator. A J&W DB-Wax column (30 m × 53 mm i.d. × 1.5 μm film thickness) was used. The injector temperature was set at 250 °C and the flame-ionization detector (FID) was set at 250 °C. An initial oven temperature of 110 °C was held for 2 min before a temperature ramp of 8 °C/min was used to reach a final

temperature of 210 °C. The carrier gas was nitrogen (12 cm^3/min).

2.2.3. Assay conditions for propolis syrup analysis

Syrup samples from fatal victims were diluted 1:10 in methanol (chromatographic grade (Merck) and qualitatively and quantitatively analyzed in a Shimadzu GC 14 under the same conditions as described above.

Compound identifications were performed by comparing the retention times of the sample constituents with respect to reference standards of propylene glycol (PG), diethylene glycol (DEG) and ethylene glycol (EG). These reference standards were in methanol and kindly provided by the Organic Chemistry Department, National University of La Plata, Argentina. In all cases, EG was used as the internal standard. The calibration curve for DEG was linear from 50 to 500 μg/mL and it was constructed of standards with a concentration of 0.05, 0.10, 0.25, 0.50 and 1 mg/mL. The limit of detection (LOD) was determined as 5 μg/mL (the concentration at which the signal to noise ratio >3). The limit of quantitation (LOQ) was 50 μg/mL (the concentration at which the signal to noise ratio >6). Three blank plasma samples were analyzed for peaks interfering with DEG and EG.

As a complementary study, the samples were also analyzed in a Brucker ACE 200 NMR spectrometer. Aliquots (0.3 mL) of each sample were diluted in an equal volume of deuterated water, measuring the C13 NMR spectrum at 50.3 MHz, completely uncoupled of H^+, by means of Distorsionless Enhancement by Polarization Transfer technique (DEPT)

2.3. Classification of victims in groups according to survival time

The 15 victims were classified into three groups according to survival time from hospital admission up to the moment of death. Three groups were defined as follows: Group 1—patients that survived up to 3 days (three cases); Group 2—patients that survived between 4 and 5 days (three cases); Group 3—patients that survived between 6 and 21 days (nine cases). In all patients, hemodialysis (HD) was performed with delays ranging from 1 to 6 days between ingestion of DEG and initiation of HD with development of anion gap metabolic acidosis.

3. Results and discussion

3.1. Analysis of internal medium biochemical parameters

The results of toxicological and clinical chemistry results from samples collected on admission into the hospital are presented in Table 1. In all cases, they are characteristic of DEG poisoning with metabolic acidosis. Other indexes related to metabolic acidosis, such as BE were calculated.

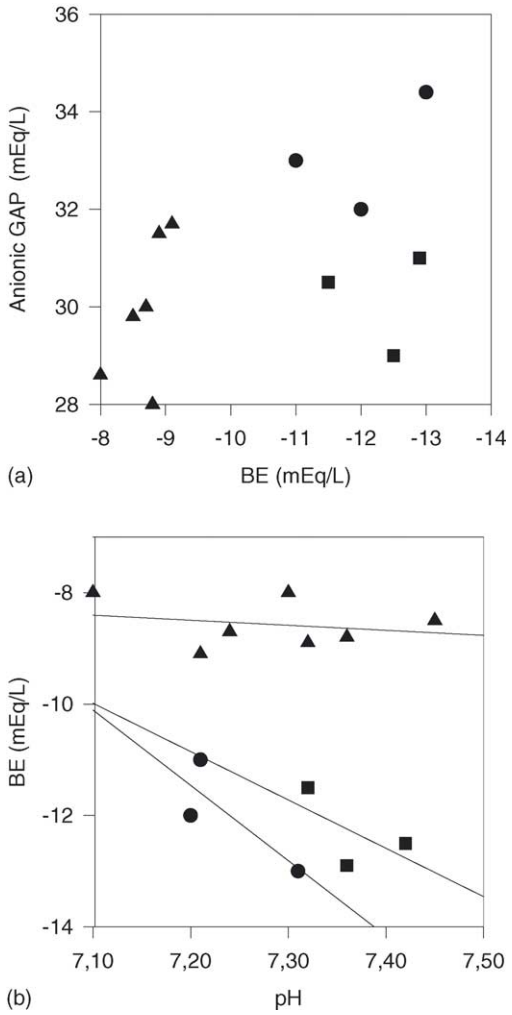

Fig. 1. Correlation between acid–base parameters for 15 victims with DEG poisoning (a) BE vs. anionic Gap, (b) pH vs. BE; (●) group 1, (■) group 2, (▲) group 3.

Fig. 1 shows the correlation between acid–base parameters. It can be observed that Group 1 patients showed the highest anion gap values and the lowest BE values (Fig. 1a; Table 1) and the more severe clinical manifestations caused by anuria, respiratory failure and rapid death (up to 3 days). Acidosis generates organic acid or acids yields markedly lower serum bicarbonate concentrations and an increased anion gap. Normal estimated anion gap ranges between 10 and 16 mmol/L or 12 and 16 mEq/L and BE = −2.3 and 2.3 mEq/L. This measure is obtained from the estimation in which negative charges from proteins (i.e., albumin, fatty acids and inorganic acids) have an effect.

Group 2 patients also showed low BE values but with lower anion gap values. They presented symptoms of renal failure and cardiac alterations (arrhythmia). Group 3 patients showed BE values not as negative as the previous cases.

Table 1
Acid–base status and DEG in blood and viscera in 15 victims with DEG poisoning

Group	Victim	Sex	Weight (kg)	Age (years)	DEG_{blood} (mg/L)	$DEG_{viscera}$ (mg/kg)	pH	Base excess (mEq/L)	Anion gap (mEq/L)	Survival time (days)
1[a]	1	M	77	70	0.22	0.32	7.31	−13.1	34.4	1
	2	M	80	59	0.20	0.31	7.21	−12.0	32.0	2
	3	F	76	57	0.27	0.42	7.21	−11.1	33.1	3
2[b]	4	M	91	60	–	–	7.42	−12.5	29.0	5
	5	F	75	93	–	NR	7.32	−11.5	30.5	4
	6	M	76	62	–	–	7.36	−12.9	31.0	5
3[c]	7	F	94	54	–	NR	7.21	−9.1	31.7	6
	8	F	77	60	–	–	7.24	−8.7	30.0	7
	9	M	74	66	–	NR	7.32	−8.9	31.5	8
	10	M	86	50	–	–	7.45	−8.5	29.8	10
	11	F	93	56	–	–	7.10	−8.0	28.6	11
	12	M	89	83	–	–	7.30	−8.0	27.8	9
	13	F	92	59	–	–	7.36	−8.8	28.0	1
	14	F	71	65	–	NR	NR	NR	NR	–
	15	F	68	66	–	NR	NR	NR	NR	–

NR: not registered.
[a] Patient survived up to 3 days.
[b] Patient survived 4–5 days.
[c] Patient survived 6–21 days.

They showed symptoms of increased serum hepatic levels of SGOT, SGPT, Gamma-GT and LDH from medium to high. On the other hand, they presented significant total CK (creatinine kinase) levels and a progressive increase in blood leukocyte counts (up to 17,000 mm^{-3}). Hematocrit values decreased to 24%. Urea and creatinine levels increased, with a tendency to decrease after hemodialysis.

Fig. 1b shows the correlation between pH and BE for each patients group. r^2-Values were 0.68, 0.99 and 0.55 for Groups 1, 2 and 3, respectively, showing that there are differences for each group according to survival time and in the studied acid–base parameters.

Despite the severity of these anomalies and the mortality and morbidity that accompany DEG intoxication, few reports address the pathogenesis of the acidosis. Experimental models in rats show that high amounts of hydroxyethoxyacetic acid (HEAA) can be detected in urine [15–16], but other authors [17–18] suggest that DEG has the same metabolic pathway as EG, leading to the assumption that renal tubular damage is due to oxalate crystal depositions in the tubular walls. In the present work, histopathological examination of kidneys showed an intense tubular damage but no crystal deposits in the tubular wall. This would indicate that in human beings the metabolic pathway of DEG could be different form that of EG. In this sense, it may be that there is not any other linkage rupture of DEG, leading to HEAA formation instead of oxalate formation [10,15]. Therefore, HEAA may be the final compound that promotes hepatic and renal necrosis, with the consequent organ dysfunction causing the symptoms and eventual death.

3.2. Estimation of lethal doses of DEG

In this paper, the presence of DEG and PG (normal component in the propolis syrup) delivered in bottles by the victims' relatives was investigated. Table 2 shows the amounts (expressed in percentage) of DEG and PG in the propolis syrup samples. It can be observed that the syrup consumed by all victims contained high DEG concentrations.

Other syrup analyses performed on the vials supplied by survivors or collected by the investigators with the same serial numbers showed a DEG average concentration of 55.0% (w/v) and a PG average concentration of 32.0% (w/v). The rest of the contents was composed of solid residues (2.5%, w/v) and water.

Although in most cases the exact amount of syrup ingested is unknown, according to the interrogation of patients or relatives, the total ingestion was established between 5 and 20 mL. Based on that information, Table 2 also shows the estimate of DEG (mg) consumed by each victim. When there was evidence of larger syrup ingestion (20 mL), renal failure manifested earlier. In these cases, it is estimated that 4.8–13.3 mg of DEG have been consumed (Table 2). The same reasoning applies if there was evidence of low ingestion (assuming 5 mL of syrup). For this case, the estimated amount of DEG consumed would be 1.2–3.3 mg DEG. Therefore, the lethal dose range would be within 0.014–0.170 mg DEG/kg body weight. This dose range in the lowest range reported so far.

Fig. 2 shows the dose–effect relationship between DEG (mg/kg of body weight) and anion gap specific for each

Table 2
DEG (%, w/v) and PG (%, w/v) identified in samples of propolis syrup

Group	Victim	DEG (%, w/v)	PG (%, w/v)	DEG (mg), if 20 mL of syrup is consumed	DEG (mg), if it is consumed 20 mL of syrup	DEG (mg/kg body weight), if it is consumed 5 mL syrup	DEG (mg/kg body weight), if it is consumed 20 mL syrup
1	1	55.0	32	2.7	11.0	0.0355	0.1421
	2	50.5	42	2.5	10.1	0.0316	0.1266
	3	66.5	30	3.3	13.3	0.0371	0.1746
2	4	46.0	31	2.3	9.2	0.0253	0.1011
	5	58.0	51	2.9	11.6	0.0387	0.1547
	6	56.5	33	2.8	11.3	0.0333	0.1333
3	7	59.9	34	2.9	11.8	0.0312	0.1247
	8	52.0	29	2.6	10.4	0.0338	0.1351
	9	53.5	25	2.6	10.7	0.0361	0.1444
	10	24.0	53	1.2	4.8	0.0140	0.0558
	11	54.0	42	2.7	10.8	0.0290	0.1161
	12	38.0	43	1.7	7.6	0.0191	0.0764
	13	39.0	32	1.9	7.8	0.0211	0.0844
	14	28.5	42	1.4	5.7	0.0197	0.0817
	15	29.0	32	1.4	5.8	0.0206	0.0838

victim. Lethal dose is estimated assuming a consumption of 5–20 mL of syrup, which, divided by each victim's body weight, yields the DEG mg/kg body weight dose. A good correlation was observed between the amount of DEG consumed and the anion gap for the 13 victims studied ($r^2 = 0.63$ if 20 mL of syrup was consumed and $r^2 = 0.78$ if 5 mL of syrup was consumed).

These results are lower than those recorded for oral doses in human beings based on the death cases reported in other outbreaks. For example, in a case of sulphanilamide–Massengill poisoning [4,20] the lethal dose was in the range of 1–2 g/kg [21]. Also, these results are even lower than those reported in the Haiti outbreak [9], in which the estimated lethal dose ingested was 1.63 mg/kg body weight, with a range of 0.35–5.40 mg/kg. In the case of 12 children, the maximum doses were lower than 1.12 mg/kg. However, O'Brien et al. [9] observed that the toxicity mechanism of DEG in human beings is not well characterized and minimum toxic dose ranges have not been well established. Furthermore, the 1 mL/kg minimum lethal dose suggested by the literature is not very conclusive.

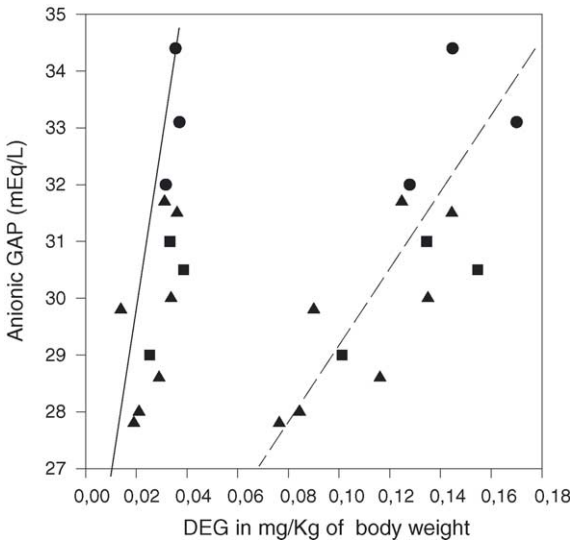

Fig. 2. Dose-reply relationship between DEG consumption and anionic Gap (full line if 5 mL of syrup were consumed, $n = 13$, $r^2 = 0.78$, cross line if 20 mL of syrup were consumed, $n = 13$, $r^2 = 0.63$; (●) group 1, (■) group 2, (▲) group 3.

The variations among the cases may be due to higher accuracy of the DEG quantification technique performed in this study compared to the older reports. On the other hand, it is also important to consider the age of the victims that may have been more affected by the hepatic and renal failure of the poisoning, as well as the hemodialysis treatment used.

3.3. Postmortem analysis of DEG disposition in acute intoxication

There have been no previous reports concerning DEG measurement in different postmortem human tissues (including blood). Tissue distribution after administration of DEG (5000 mg/kg oral dose) to rats showed the highest tissue to blood ratios (4–5) appearing in liver [1].

In the present work, GC/FID analysis showed that in 3 out of the 15 sets of victim samples, DEG was present in both the blood and viscera (kidney and liver). These three positive cases correspond to subjects who did not survive more than 3 days after the appearance of the symptoms (Group 1). Table 1 shows the values of DEG in blood and viscera in the victims of this massive intoxication. In these three cases,

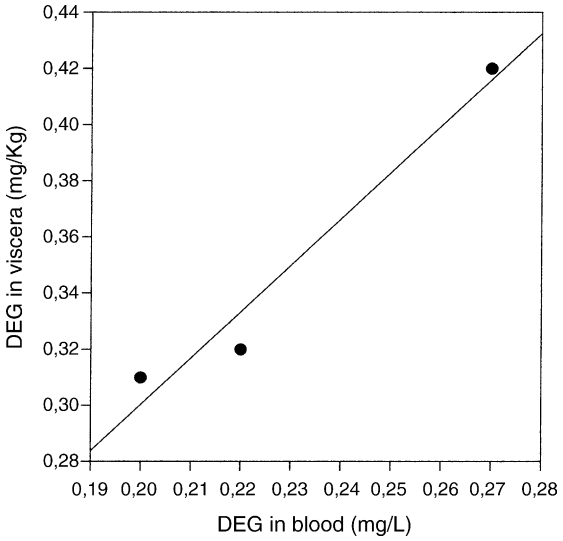

Fig. 3. Correlation between DEG in viscera and the corresponding blood concentration ($r^2 = 0.96$) for Group 1 victims.

the levels were higher in viscera than in blood. The concentration relation $(DEG_{viscera}/(DEG)_{blood}$ ranged from 1.45 to 1.55.

Fig. 3 shows the DEG concentration in blood vs. viscera concentration. A good correlation ($r^2 = 0.96$ ($n = 3$)) between blood and viscera can be observed in the subjects from Group 1. The fact that the DEG could not be isolated in the blood and viscera of the remaining 12 fatal victims may be attributed to putrefying processes operating in the corpses (the time elapsed between the deaths and the collection of samples was variable) or to the length of HD treatment applied. In these cases, the etiological diagnosis of DEG intoxication was based on the symptoms presented by the victims, the results of their clinical chemistries at the patients' admission to the hospital, and the time since propolis syrup ingestion.

On the other hand, we believe the possibility should be considered that DEG is retained by co-dissolution and co-absorption in putrefying tissue remains and tissues with a high level of complex lipids (mainly phospholipids) [19]. This especially applies to the cases with a high degree of putrefaction, even in exhumations, like many of the cases we analyzed.

4. Conclusion

It must be stressed that this massive intoxication had no precedents in Argentina, and few cases were reported worldwide. However, it must also be emphasized that more adequate controls should be performed to avoid new episodes. The propolis syrup that was later determined as the cause of the massive DEG poisonings was routinely used for prevention or treatment of common upper respiratory infections. Because the exipient was contaminated, it clearly demonstrates the need for adequate controls when manufacturing the product. From the forensic point of view, it is important to bear in mind that intoxication should not be attributed to the propolis.

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