CASE REPORT

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Tungsten Determination in Biological Fluids, Hair and Nails by Plasma Emission Spectrometry in a Case of Severe Acute Intoxication in Man

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ABSTRACT: A healthy 19-year-old recruit in a French artillery regiment drank 250 mL of a mixture of beer and wine that had rinsed in a hot 155-mm gun-barrel. Fifteen minutes later, he complained of nausea followed by seizures. He was comatous for 24 h, presenting signs of encephalopathy. A moderate renal failure was noted initially and worsened to an extensive tubular necrosis with anuria on the day after the incident. The first toxicological investigations only showed a 0.31 g/L blood ethanol. Then inductively-coupled plasma (ICP) emission-spectrometry revealed very high concentrations of tungsten in the "beverage" as well as in gastric content, blood and urine (1540 mg/L, 8 mg/L, 5 mg/L, and 101 mg/L, respectively). The nature of the metal was confirmed by ICP coupled to mass spectrometry. A simple and reliable ICP quantitative assay of tungsten in biological fluids, hair and nails was then developed. It showed high blood levels (>0.005 mg/L) until day 13 in spite of six hemodialyses, and in urine until D_{33}. Tungsten was also incorporated in hair and nails.

To the best of our knowledge, such an intoxication has never been reported before though this drinking seems to be traditional in the French Artillery. It has probably been favored by the unusually high volume of beverage absorbed and by the new alloy of the gun, containing tungsten. The clinical evolution was satisfactory over weeks and the patient was declared totally cured after five months.

KEYWORDS: forensic science, forensic toxicology, tungsten, inductively-coupled-plasma-emission spectrometry

Tungsten (W) is a heavy metal (molecular weight = 184), naturally occurring as a mineral, named Wolfram (FeWO4, MnWO4). In its metallic form, tungsten is used to harden steels, whereas tungsten oxides are used in chemical industry, and tungsten carbide in metallurgy to manufacture cutting and perforating tools.

Only chronic intoxications with these different forms of tungsten have been described in man. They usually concerned metallurgy workers after long-term respiratory or cutaneous contact with tungsten carbide particles (1,2). Clinically, they induce pulmonary fibrosis (3,4) or allergic dermatitis (5) favored by cobalt, often associated with tungsten. The only treatment so far seems to be corticoids, as chelating agents did not prove efficient (6). Acute intoxication, per os or parenterally, is only known from already ancient experiments in rats (7–9) or in rabbits (10). We probably report here the first case of a severe acute tungsten intoxication in human, following an accidental ingestion of an important quantity of this metal.

Case History

At the end of their training, the young conscripts in several French artillery-companies receive the "artilleryman's baptism." After guns have been fired several times, a beverage is prepared by rinsing the still hot gun-barrels with wine or beer and is given to drink to the new artillerymen, who usually spit or vomit it. Recently, a 19 year-old conscript drank—and kept—two glasses (approximately 250 mL) of such a drink, prepared from a mixture of wine and beer. Fifteen minutes later, he complained of nausea, followed by seizures. Clonic convulsions persisted until the physician administered him 10-mg diazepam intravenously 20 min later. The patient presented then with a first stage coma, a tongue bite but no loss of urine. The rest of the examination was normal. He was mechanically ventilated through a laryngeal canula and transferred to the intensive care unit of the closest tertiary teaching hospital. There was noted a stage II coma, dark vomiting and a polyuria (4 L in the first hours). The first biological findings were a deep metabolic acidosis (pH = 7.17, bicarbonates = 10 mmol/L) a moderate renal failure (blood creatinine = 160 μmol/L, blood urea = 6.4 mmol/L), a hyperkaliemia (6 mmol/L), a hepatic cytolysis (GOT = 153 IU/L, GPT = 67 IU/L) and a moderate rhabdomyolysis (CPK = 1287 IU/L). Gastric content, urine, and blood were immediately sampled for a toxicological screening.

Brain computed tomography and cerebrospinal fluid examination were unremarkable, whereas electroencephalogram revealed

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diffuse slow waves. Pulmonary and abdominal radiographs were normal.

A progressive wake-up of the patient allowed his extubation on day 2 (D_2) of his hospitalization. Renal failure worsened, inducing an anuria, which required a first hemodialysis on D_3. Abdominal ultrasound demonstrated normal sized hyperechogenic kidneys and was otherwise unremarkable. Optic microscopy of a kidney biopsy revealed extensive tubular necrosis, with an interstitial inflammatory infiltrate. Electronic microscopy confirmed a major tubular necrosis, without associated glomerular injuries. Rhabdomyolysis was probably not responsible for these lesions because it always remained moderate and total CPK was normalized on D_{12} (42 IU/L).

Nine dialyses were then performed between D_3 and D_{19}, whereas the administration of a diuretic (bumetanide: 30 mg/day) induced a progressive recovery of diuresis on D_{12}.

Experimental

Screening Procedures

Gastric content, plasma, and urine were screened for drugs and toxicants by a number of published or laboratory-developed techniques: a) High performance liquid chromatography with diode array spectrophotometric detection (HPLC/DAD) and gas chromatography-mass spectrometry (GC/MS) after non-selective acidic or basic extraction of the samples, or after more selective extractions for neuroleptics, antidepressants, benzodiazepines or others; b) headspace GC/MS for volatile compounds; c) GC with flame ionization detection for alcohols and glycols; d) optical microscopy to search for oxalate crystals in urine; e) colorimetry for cyanide detection; f) inductively coupled plasma (ICP) emission spectrometry and atomic absorption spectrometry for metals or elements (particularly mercury and lead, which are commonly used in shell initiators).

Tungsten Determination

A simple, quantitative assay of tungsten in biological samples was rapidly developed and validated. Analyses were performed on an ICP atomic emission spectrometer JY24 (Jobin Yvon, France), consisting of a Plasmatherm source inductively coupled to a high frequency (40.68 MHz) oscillator operating at 1 kW, a monochromator including a 3600 grooves/mm holographic grating. Focal length is 0.64 m and dispersion 0.4 nm/mm. The electronic measuring instrument is connected to a PC-type microcomputer. Samples on a 221–222 automatic sampler (Gilson, Villiers, France) are pumped with a Perimex pump (Petec, Erding, Germany) and nebulized by means of a concentric pneumatic nebulizer. Argon was used as coolant, nebulizer and sheath gas at respective flow rates of 15 L/min, 0.4 L/min and 0.35 L/min. Tungsten was quantified at a wavelength of 207.91 nm, with the 193.03 nm wavelength of carbon as reference.

Tungsten standard solution (1.000 mg/L) was purchased from Spex Industries (France), nitric acid Suprapur from Merck (France), and ultrapure deionized water obtained by filtration on Milli-Q Standard System, Millipore (France).

Liquid samples were diluted to 1/2 in deionized water before analysis by ICP. For hair and nails, this procedure was preceded by a 12 h hydrolysis at 60°C in 500 μL of pure nitric acid. Each analysis was realized in triplicate and the average result computed against a six point standard curve (blank; 100; 200; 1000; 5000 and 10,000 μg/L), prepared by spiking tungsten-free serum and analyzed on the same run.

Linearity and reproducibility were evaluated by means of five such standard curves prepared and quantified each on a different day. Repeatability was assessed for the 100 μg/L and 10,000 μg/L levels, by analyzing six spiked standards on a same day, for each concentration.

Results

The only findings of the first toxicological screening were ethanol in blood (0.31 g/L) and glycerol in gastric content. Analyses were then orientated towards some gun-powder constituents or combustion residues: Head-space GC/MS showed no metaldehyde nor mercaptans; the determination of sulfur, by a semi-quantitative ICP screening of 32 metals and elements, showed its presence at a rather high concentration in gastric content, but at normal concentrations in blood and urine; but the major finding of this last method was the presence of tungsten at very high concentrations in all the samples.

As this last result was not expected, a possible analytical interference was investigated: First, the atomic emission spectrum of tungsten in the patient's samples was pure, centered at 207.911 nm and showed no interference from other hard metals; second, the same ICP screening procedure found no tungsten in blood and urine samples of normal subjects; finally, the nature and the concentration of the toxicant in urine and beverage were confirmed by ICP coupled to mass spectrometry (Elan 5000, Perkin-Elmer Sciex, France) which showed typical tungsten isotopic contributions between m/z 182 and 186 (Fig. 1). This technique also revealed the presence of tungsten oxide, WO (m/z 198 to 202 peaks) which is probably an artifact produced in the plasma source but might be confused with Hg, which has almost the same isotopic distribution.

The ICP quantitative assay developed to confirm the initial concentrations and to monitor the excretion of tungsten, had a 50 μg/L limit of detection in biological fluids and an excellent linearity between 100 and 10,000 μg/L ($r^2 > 0.998$) (Table 1). Repeatability and reproducibility were satisfactory over this range (CV < 10% for both).

Concentrations in the first day samples were as follow: Gastric content: 8 mg/L, serum: 5 mg/L (N = 0.0058 ± 0.0035 mg/L in normotensive and 0.011 ± 0.013 mg/L in hypertensive non-exposed volunteers (12)), urine: 101 mg/L and 344 mg/24h (N < 0.5 mg/24 h for exposed workers (6)), as no beverage ingested by the patient was kept for analysis, an almost similar drink was prepared several days after the intoxication by rinsing the same gun-barrel with 1 L of water (instead of wine and beer) after the gun had been fired. The concentration of tungsten in this beverage was 1540 mg/L, whereas the normal daily intake in food is approximately 8 to 13 μg (13).

Tungsten levels in biological fluids from D_1 to D_{53} are reported in Table 2. Unhappily, blood samples collected between D_1 and D_{11} were used up for the toxicological screening, before tungsten was at last incriminated. However, tungsten could still be determined in serum until D_{12} and in plasma until D_{13}, in spite of 6 hemodialyses. After diuresis had recovered, urinary excretion of tungsten increased from D_{14} to D_{19} and this metal was detectable in urine until D_{33}. No tungsten was found in urine, collected on D_{12}, of two other freshmen who had tasted (but spit) the same beverage as our patient, on the same day.

In the patient's nails, tungsten was undetectable on D_{41} but

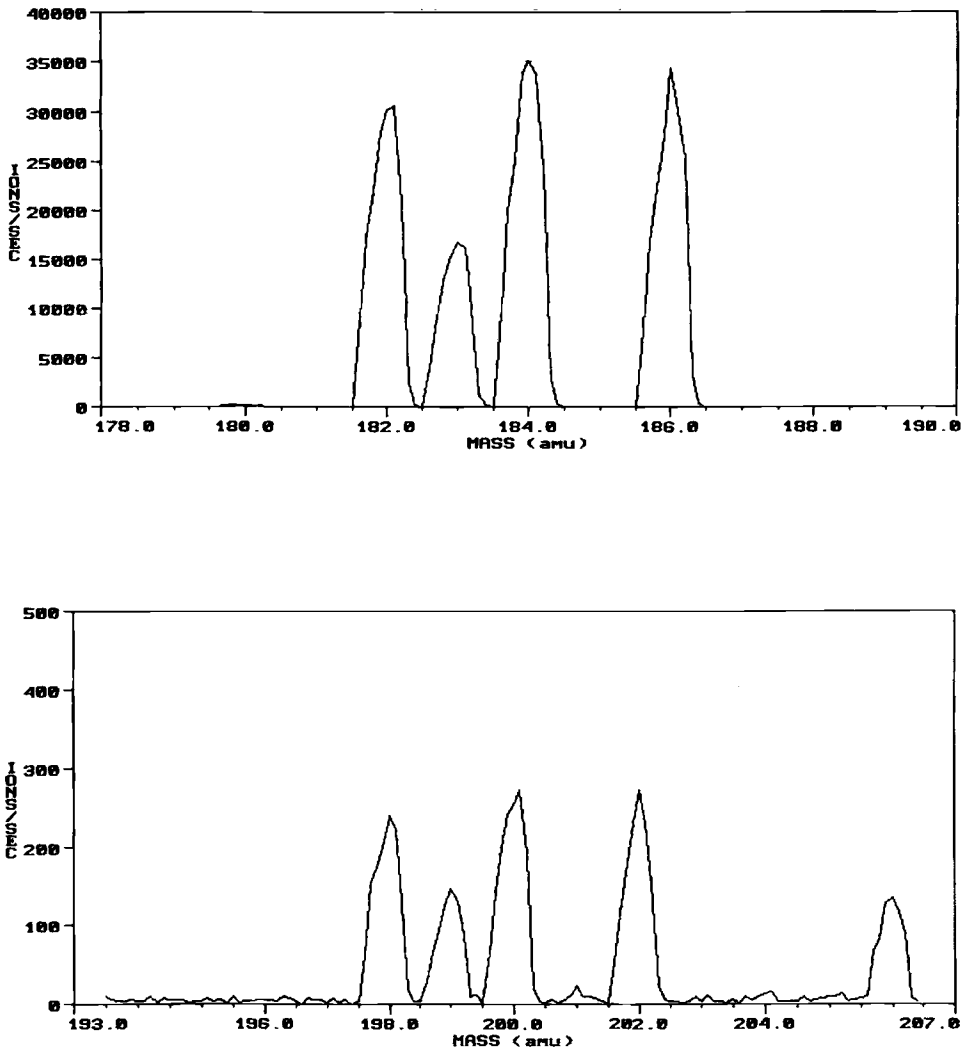

FIG. 1—*Mass spectra of tungsten (in the 181 to 187 mass range) and of tungsten-oxide (in the 197 to 203 mass range), obtained by ICP-MS analysis of 1/3000 diluted urine.*

TABLE 1—*Repeatability and reproducibility of tungsten determination in serum.*

	Repeatability (n = 6)		Reproducibility (n = 5)		
Added Concentration (μg/L)	Mean Concentration Found (μg/L)	CV (%)	Mean Concentration Found (μg/L)	CV (%)	Inaccuracy (%)
0	—	—	−9	163.7	—
100	97	9.8	106	16.5	6.5
200	—	—	205	8.4	2.6
400	—	—	393	4.0	1.8
2,000	—	—	2,024	3.8	1.2
10,000	9843	2.2	10,006	1.3	0.6

present at very high concentration on D_{51} (3.81 μ/g, whereas undetectable in two non-exposed controls). In D_{51}, the level in hair was of the same order (4.26 μg/g; N < 0.1 μg/g) and 3 months later, it was undetectable in the short hair (1 to 1.5 cm long, representing 1 to 2 months of growth) of this military man.

Tungsten was absent or below detection limit in dialysates sampled on D_{16} and D_{19}, but at that time it was already undetectable in plasma.

Discussion

Though no previous observation of acute tungsten intoxication in humans could be found in the literature, there are strong arguments in favor of the imputability of tungsten in the observed clinical signs: a) extremely high concentrations of tungsten were determined in the control beverage, as well as in gastric content, blood and urine sampled on the first day, whereas no other toxicant could be found in spite of numerous screening procedures and specific assays; b) clinically, the patient presented with an encephalopathy resembling that of mercury for example, and very similar to the generalized convulsions with high amplitude slow waves described on the EEG of rats administered tungsten subdurally and intracortically on the left central area of the brain (9); c) experimental oral administration of tungsten to rats (7,8,13) or to pregnant mice (14) showed a rapid and important intestinal absorption (17% of the ingested dose in the first hour); a preferential distribution to spleen, bones, kidneys and, to a lesser extent to muscles; a long terminal half-life (mostly attributed to bone-fixation) in spite of a high urinary excretion in the first 24 h (40% of the ingested dose), d) LD_{50} in rabbits (105 mg/kg) (10) is consistent with the severe clinical signs observed in our patient. As a matter of fact, the dose he absorbed can be roughly evaluated

TABLE 2—*Concentrations in biological fluids and daily urinary excretion of tungsten.*

Date	Tungsten Concentrations (μg/L) Serum	Plasma	Urine	Diuresis (L/24 h)	Urinary Excretion (μg/24 h)
D1	4960		101,000	3.4	344,000
D11	89	133			
D12	140	119			
D13*					
b.h.†		111			
D13 a.h.‡		<50			
D14	<50	<50	756	0.3	227
D15		<50	478	0.4	191
D16		<50	506	1.3	658
D17		<50	262	N.D.§	N.D.
D18		<50	264	4.1	1082
D19		<50	435	2.9	1261
D22		<50	403	2.2	887
D23		<50	323	2	646
D24		<50	338	2.3	777
D25	<50	<50	281	2.4	674
D26		<50	278	1.9	542
D31			92	3	276
D33			131	1.5	196
D53			<50	N.D.	N.D.

*days in bold characters are those on which a hemodialysis was performed.

†b.h.: before hemodialysis. ‡a.h.: After hemodialysis.

§N.D.: not determined.

as 5 to 12 mg/kg, from the concentration found in the control beverage and from the first day urinary excretion, respectively; 250 mL of beverage containing at least 1500 mg/L tungsten (concentration probably underestimated by the control beverage) would give 375 mg ingested, i.e., 5.4 mg/kg for a 70 kg body-weight; 344 mg of tungsten excreted in urine during the first 24 h, supposed to be 40% of the oral dose (as determined in rats), would give 860 mg ingested, i.e., 12.3 mg/kg.

The limit of detection of the present method was equal to the physiological tungsten concentration in the plasma of non-exposed man and thus elicited a long-term monitoring of the intoxication, even after clinical signs had disappeared.

High concentrations of tungsten in hair and nails have already been described in workers chronically exposed to hard metal dusts, confirming the incorporation of the metal in these matrices (15). As shown in the present case, determination of tungsten in hair may thus allow a retrospective diagnosis of either a chronic or an acute exposure.

The reason why such an intoxication never happened before despite a long tradition, nor happened to the other persons having drunk the same mixture on the same day, is not fully understood. Some clues were given by the regiment authorities and by the general staff: The young artillerymen do not usually swallow or keep such beverages, so drinking 250 mL of it was very unusual, if not the first time; in the patient's regiment, the 155-mm guns had been changed recently for new models, the steel of which contained tungsten.

One can also suspect one or several co-factors, either external, physiological, or pathological, to have enhanced considerably tungsten biodisponibility or molecular toxicity, but none of these could be identified. Nevertheless, the general staff has forbidden such a practice in the French Army.

The patient's clinical evolution was satisfactory over weeks, so he was discharged from the hospital on day 35; he had then fully recovered from renal failure and the only remaining signs were slow waves on EEG. He joined his regiment on 8th week, then he definitively enlisted in the Army. He was declared totally cured at 5th month, when EEG had normalized.

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References

1. Bech AO, Kipling MD, Heather JC. Hard metal disease. Br J Industr Med 1962;19(4):239–51.
2. Einbrodt HJ, Fitzek J. Untersuchungen des Lungenstaubes bei Hartmetallfibrose. Int Arch Gewerbepathol Gewerbehyg 1962; 19(6):651–9.
3. Coates EO Jr, Watson JHL. Diffuse interstitial lung disease in tungsten carbide workers. Ann Int Med 1971;75(5):709–16.
4. Coates EO Jr, Watson JHL. Pathology of the lung in tungsten carbide workers, using light and electron microscopy. J Occup Med 1973;15(3):280–6.
5. Skog E. Skin affections caused by hard metal dust. Industr Med Surg 1963;32(7):266–8.
6. Barborik M. Excretion of cobalt and tungsten in workers in the production of hard metals. II- The daily excretion of tungsten in urine. The influence of $CaNa_2$ EDTA and penicillamine on its excretion. Pracov Lek 1972;24(8):295–7.
7. Kinard FW, Van de Erve J. Toxicity of orally ingested tungsten compounds in the rat. J Pharm Exp Ther 1941;72:196–201.
8. Kinard FW, Aull JC. Distribution of tungsten in the rat. J Pharm Exp Ther 1945;83:53–5.
9. Takahashi S, Yamauchi T, Hirabayashi Y. Experimental seizures produced by tungstic acid gel in rats. Life Sci 1972;11(1):1011–20.
10. Lusky LB, Braun HA, Laug EP. Effects of BAL on experimental lead, tungsten, vanadium poisoning. J Industr Hyg 1949;31:301–8.
11. Wester PO. Trace elements in serum and urine from hypertensive patients before and during treatment with chlorthalidone. Acta Med Scand 1973;194:505–12.
12. Wester PO. Trace element balances in relation to variations in calcium intake. Atherosclerosis 1974;20:207–15.
13. Kaye SV. Distribution and retention of orally administered radiotungsten in the rat. Health Phys 1968;15:398–417.
14. Wide M, Danielsson BRG, Dencker L. Distribution of tungstate in pregnant mice and effects on embryonic cells in vitro. Envir Res 1986;40:487–98.
15. Nicolaou G, Pietra R, Sabbioni E, Mosconi G, Cassina G, Seghizzi P. Multielement determination of metals in biological specimens of hard metal workers: A study carried out by neutron activation analysis. J Trace Elem Electrolytes Health Dis 1987;1(2):73–7.

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