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Short Communication

Aluminium in antihaemophilia preparations as determined by electrothermal atomic absorption spectrophotometry

JAN RUD ANDERSEN*[1] and PER HELBOE[2]

[1] *The Royal Danish School of Pharmacy, Department of Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen, Denmark*

[2] *National Board of Health, Drug Standardization Laboratory, 378 Frederikssundsvej, DK-2700 Brønshøj, Denmark*

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Introduction

During the last decade increasing attention has been paid to the toxic effects of aluminium in patients suffering from chronic renal failure. In 1976 Alfrey *et al.* [1] suggested that aluminium is responsible for a fatal neurological syndrome seen in patients on regular hemodialysis, as elevated tissue aluminium levels were found in autopsies from victims of this syndrome. The aluminium accumulated in the organism is supposed to stem either from the water used in the hemodialysis treatment [2], or from the aluminium-containing phosphate binders used to control blood phosphate levels in uremic patients [3], or from a combination of the two. Serum or plasma aluminium concentrations are most often used to assess the body burden, and concentrations above 100–150 $\mu g\ l^{-1}$ may cause adverse effects [4].

Patients suffering from haemophilia are often treated for long periods with large doses of the antihaemophilic factor VIII and factor IX protein fractions obtained from human plasma. As these fractions containing factor VIII and IX are administered intravenously, and as precipitation with aluminium hydroxide may be utilized in their isolation, the patients risk the possibility of elevated blood aluminium levels and the consequent toxic effects.

The aim of the present investigation has been to examine the levels of aluminium in such preparations. At present monographs on factors VIII and IX such as the *British Pharmacopoeia* [5] do not contain any tests to limit the content of aluminium.

*To whom correspondence should be addressed.

Experimental

Apparatus

A Perkin–Elmer model 460 atomic absorption spectrophotometer equipped with the HGA 74 furnace, HGA 2100 furnace controller including a ramp module, and a home-built autosampler/sequencer was used. Atomization signals were recorded on a Radiometer REC 61 strip chart recorder. The light source was an aluminium hollow-cathode lamp. Pyrolytically coated graphite tubes equipped with the L'vov platform were used. The instrument settings are given in Table 1.

Table 1
Instrument settings employed for Perkin–Elmer Model 460 AAS equipment

Spectrophotometer:		
Hollow cathode lamp current		25 mA
Wavelength		309.3 nm
Slit width		0.7 nm
Mode of measurement		peak height
Integration time		4 s
Background correction		off
Graphite furnace:*		
Drying phase;	temperature	200°C
	ramp	40 s
	hold	20 s
Charring phase;	temperature	1500°C
	ramp	40 s
	hold	30 s
Atomization phase;	temperature	2800°C
	ramp	0 s
	hold	5 s

Argon flow: 300 ml min^{-1}; flow interrupted during atomization.

*Pyrolytically coated graphite tubes equipped with the L'vov platform were used.

Chemicals

Nitric acid Suprapur®; Triton® X-100 scintillation grade, and a certified 1 g l^{-1} (1000 ppm) aluminium working standard solution (Titrisol®) were obtained from E. Merck (Darmstadt, FRG). Milli-Q water is a trade mark of Millipore Corp. (Bedford, MA, USA) for class 1 purified water.

Contamination control

All containers, pipette tips, sample cups, etc. used were made of polyethylene, polypropylene or polystyrene. They were cleaned by soaking in 4 M nitric acid for a week, followed by a rinse with Milli-Q water and renewed soaking for a week in Milli-Q water. They were dried in a laminar air flow clean bench providing a class 100 environment (Holten Laminair, Model 2448) and kept in polyethylene bags until use. In fact the contribution of aluminium from containers and reagents as determined by atomic absorption spectrophotometry was found to be insignificant.

Samples

The factor VIII and IX preparations investigated were received as sterile, freeze-dried concentrates. Prior to analysis they were reconstituted with water — in most cases supplied with the preparation; when no water was supplied Milli-Q water was used. The manufacturer's instructions were followed closely and all manipulations at this stage were carried out in a laminar air flow clean bench. After a test-run directly on the reconstituted samples, they were diluted with a 'modifier' containing 0.1% Triton-X 100 and 0.01 M nitric acid in Milli-Q water [6] to adjust to the linear range of the instrumentation used, i.e. aluminium in the range of approximately 30–50 $\mu g\ l^{-1}$. In a few samples precipitation occurred upon dilution; by reducing the amount of nitric acid added by a factor of four, clear solutions were obtained. Sample volumes of 20 µl were injected and the concentrations evaluated by the standard addition technique. The aluminium reference solution (1000 ppm) was used, aliquots of this being diluted with 'modifier' to yield working standards of 100 $\mu g\ l^{-1}$. When stored in purified and conditioned polyethylene containers these working standards were stable for months.

Results and Discussion

In Table 2 are shown the results obtained for eight different protein fraction preparations. Two batches of each were analysed. For four of the brands of preparation it appears that there is no correlation between batches as far as aluminium content is concerned. This may indicate that the manufacturers are unaware of an aluminium contamination problem.

Table 2
Aluminium in antihaemophilia preparations

Preparation	Purity*	i.u./vial	Volume of reconstituted solution	µg Al l†	µg Al/100 i.u.
Factor VIII					
1	II	525	20	1330	5.0
		370	20	1225	6.6
2	I	500	25	50	0.3
		500	25	283	1.4
3	III	275	10	397	1.4
		1100	30	113	0.3
4	II	500	20	110	0.4
		500	20	256	1.0
5	III	255	10	64	0.3
		480	20	47	0.2
Factor IX					
6		500	10	58	0.1
		500	10	482	1.0
7		540	20	248	0.9
		850	20	725	1.0
8		500	20	115	0.5
		500	20	130	0.5

*The purity rating is according to the recommendations of the European Public Health Committee [8]. I = Cryoprecipitate; II = Intermediate purity factor VIII concentrate; III = High purity factor VIII concentrate.
†Relative standard deviations for all determinations did not exceed 10%.

Apparently, aluminium is accumulated only in patients with renal failure, otherwise it is rapidly excreted [7]. Any proposed limit should, therefore, be related to the serum or plasma level which is expected to cause harm. The dosage administered to haemophiliacs prior, for example, to a surgical operation, is about 50 i.u. of factor VIII or 100 i.u. of factor IX per kg body weight per day. A few patients, however, described as so-called factor VIII 'inhibitors', may receive up to two times 100 i.u. per kg body weight per day. When administering these high doses, only high purity preparations should be used (cf. Table 2). To avoid toxic reactions serum or plasma concentrations of aluminium below 100 $\mu g\ l^{-1}$ should be maintained. For a patient weighing 70 kg with a plasma volume of 3 l this corresponds to approximately 4 μg aluminium per 100 i.u. for factor VIII preparations used in 'inhibitor' patients, and approximately 8 μg aluminium per 100 i.u. for preparations to other patients. Similarly, the limit for aluminium in factor IX preparations may be calculated as about 4 μg per 100 i.u. As no relevant data concerning the excretion rate of aluminium in humans with normal renal function exists, so far as the authors are aware, it is conservatively presumed in calculating the limits that all aluminium is excreted before the next dose is administered.

From Table 2 it appears that some brands have aluminium contents close to the limits defined above. Moreover, if a safety factor of, for example, 10 is employed, this being a realistic factor in the light of the high excretion rate observed, only one of the tested brands appears to be acceptable in both batches.

In conclusion, it seems that the manufacturers of factor VIII and factor IX preparations are not generally aware of the aluminium contamination problem. It is recommended that manufacturers, as well as the pharmacopoeial and registration authorities, should address this issue in order to eliminate the possible risk of exposing haemophiliac patients to seriously elevated blood aluminium levels.

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