Short communication

Aluminium overload after 5 years in skin biopsy following post-vaccination with subcutaneous pseudolymphoma

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A B S T R A C T

Aluminium hydroxide is used as an effective adjuvant in a wide range of vaccines for enhancing immune response to the antigen. The pathogenic role of aluminium hydroxide is now recognized by the presence of chronic fatigue syndrome, macrophagic myofasciitis and subcutaneous pseudolymphoma, linked to intramuscular injection of aluminium hydroxide-containing vaccines. The aim of this study is to verify if the subcutaneous pseudolymphoma observed in this patient in the site of vaccine injection is linked to an aluminium overload. Many years after vaccination, a subcutaneous nodule was discovered in a 45-year-old woman with subcutaneous pseudolymphoma. In skin biopsy at the injection site for vaccines, aluminium (Al) deposits are assessed by Morin stain and quantification of Al is performed by Zeeman Electrothermal Atomic Absorption Spectrophotometry. Morin stain shows Al deposits in the macrophages, and Al assays (in μg/g, dry weight) were 768.10 ± 18 for the patient compared with the two control patients, 5.61 ± 0.59 and 9.13 ± 0.057. Given the pathology of this patient and the high Al concentration in skin biopsy, the authors wish to draw attention when using the Al salts known to be particularly effective as adjuvants in single or repeated vaccinations. The possible release of Al may induce other pathologies ascribed to the well-known toxicity of this metal.

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Introduction

Aluminium hydroxide is currently used as an effective adjuvant in a wide range of vaccines, including those against hepatitis A, hepatitis B, diphtheria, tetanus and whooping cough [1]. Aluminium (Al) compounds are well-known for enhancing immune response to the antigen and allergen and the role of Al as an adjuvant and for antigens has already been studied by Exley et al. [2,3]. The pathogenic role of aluminium hydroxide is now recognized by the presence of chronic fatigue syndrome (CFS) with myalgia, macrophagic myofasciitis (MMF), and chronic cognitive dysfunction linked to intramuscular injection of aluminium hydroxide-containing vaccines [4–6]. In light of these observations, inflammatory nodular reactions [7] and cases of late onset cutaneous lymphoid nodules after aluminium-adsorbed vaccination have likewise been described [8]. Maubec et al. [9] reported on a first demonstration of antihepatitis B and hepatitis A vaccination-derived aluminium deposits in lesions of cutaneous lymphoid hyperplasia. In our study, after having developed a post-vaccination subcutaneous pseudolymphoma, we describe aluminium overload in skin biopsy in a woman after 5 years injection of aluminium-adsorbed vaccines including diphtheria, tetanus and poliomyelitis (Revaxis, Pasteur, France). Twelve years earlier and in the same lesion area, this woman had already received an antihepatitis B vaccine injection (Genhevac, Pasteur, France). As a supplement to a usual histochemical technique such as Morin colouration, we decided several years after the vaccines to quantify the by ZEAAS (Zeeman Electrothermal Atomic Absorption Spectrophotometry) the Al in skin lesions and to assess the possibly pathological consequences of a clearly toxic form of metal overload.

Materials and methods

Case

The patient was a 45-year-old woman presenting on the outer side of the left arm with a subcutaneous nodule accompanied by pruritis and red blotsches. A dermatofibrosarcoma was suspected and occasioned exeresis. The clinical site and the record of a recent vaccination may help a clinician to make a correct diagnosis of an
injection site reaction to aluminium, but in this case, there was no evidence of recent vaccination. Subsequently, during clinical questioning, it was found that the patient had received an antihepatitis B vaccine injection in the area of the lesion (Genhevac, Pasteur, France) as well as vaccinations against diphtheria, tetanus and poliomyelitis (Revaxis, Pasteur, France) 12 years and 5 years before surgery, respectively. From a histological standpoint, the lesion consisted in lymphocytic follicular infiltrates with germinal centres composed of clear B cells surrounded by inflammatory elements including plasma cells, eosinophils and PAS-positive macrophages, suggesting post-vaccination subcutaneous pseudolymphoma (data not shown).

Detection of aluminium in skin biopsy

For aluminium deposits, Morin colouration is used (pentahydroxylavone) with elimination of calcium salts and lecture in fluorescent microscopy [9].

Quantification of aluminium in skin biopsy by ZEAS

As Al is a ubiquitous element, great care was taken at all stages of preparation to avoid any risk of sample contamination. Details of the procedure have been described by Pineau et al. [10]. Sample preparations were carried out and analysed in a clean laboratory environment equipped with a laminar flow bench and fume cupboards. Aluminium levels in skin biopsy (in triplicate analysis, mean ± SD) were determined by ZEAS (ZL, Perkin-Elmer, USA). The graphite furnace program was adapted to the stabilized temperature platform furnace concept according to Guillard et al. [11]. Skin biopsies were dried and digested according to the method of Van Gikel et al. [12] which we have validated for dry-weight samples [13]. Detection limits (LOD: μg/L) and quantification limits (LOQ: μg/L) were for tissues 0.87 and 2.9 respectively. Analytical performance was monitored by participation in two interlaboratory surveys, the Quebec Toxicology Center Interlaboratory Comparison Program [14] and the Worldwide Interlaboratory Aluminium Quality Control [15].

Results and discussion

Previous reports have shown that vaccines containing aluminium hydroxide are a potential causal factor of cutaneous pseudolymphoma [8,9]. In our case, Al could be identified in skin biopsy of the patient through usual histochemical techniques such as Morin colouration. Fig. 1 underscores the presence in fluorescent particles of intra-cytoplasmic Al deposits in interfollicular macrophages. In a second phase, we wished to precisely quantify the Al concentration at the vaccine injection sites several years after the actual injection. Most studies that have confirmed the presence of Al in skin lesions from vaccines have used energy-dispersive X-ray (EDX) microanalysis of the lysed tissue sample which revealed an absorption peak characteristic of aluminium salts [9]. Our measurement of Al in skin biopsy was performed by ZEAS. We found a pronouncedly high Al concentration in the skin biopsy of the patient (803.0 ± 20.4 μg/g dry weight) as compared to two controls (40.61 ± 2.60 and 44.13 ± 3.10 μg/g dry weight). Given these observations, it was far from normal that the two controls showed concentrations in skin biopsy of about 40 μg/g (in dry weight) without ever having received an injection of vaccines based on aluminium in the form of hydroxide. It is worth noting that during initial tissue preparation (patient and two controls) were painted over with a veterinary tattoo paste used to identify the resection margins. As Al is a ubiquitous element present in numerous colourants, we wished to analyse this colourant in the same pre-analytical and analytical conditions as skin biopsies.

Actually, we found a surprising concentration of 35 ± 2.7 μg/g, dry weight Al in the tattoo paste (n = 3). Consequently, the results of Al (μg/g, dry weight) in skin biopsies removing the Al concentration in colourant are presented in Table 1.

Multiple aluminium hydroxide injections used in vaccines may help explain the MMF with histopathological lesions detected in human arthromyalgia and CFS that has recently been described [4–6]. Electron microscopy shows the presence in the cytoplasm of macrophages of a crystalline material that is a form of aluminium hydroxide known for the way it stimulates the immune system and shifts immune responses [3]. Even though the patient in this study did not present symptoms such as CFS the results (Table 1) suggest that the patient had a particularly high skin burden of Al. Exley et al. [2] have described the first report of vaccine-associated MMF and CFS, which was coincident with an aluminium overload diagnosed 5 years post-vaccination (hepatitis A, hepatitis B, poliomyelitis and tetanus/diphtheria) through analysis of Al in urine over five consecutive days. Moreover, Shaw and Petrik [16] revealed in mice having received subcutaneous injection of multiple doses of aluminium hydroxide a significant impairment of motor function as well as diminished spatial memory capacity. While demonstrating the neurotoxicity of aluminium hydroxide and its relative ubiquity as an adjuvant, these authors have suggested that further scrutiny by the scientific community is surely warranted.

One possible complication resides in the mechanisms by which Al is released and, once it is released, the uncertain consequences of its redistribution in the body through accumulation in different kinds of cells. It bears remembering that strong suspicious exist on the role of Al in neurodegenerative disorders [17] as well as the potentially toxic role of a metal such as aluminium chlorohydrate when used as an active antiperspirant; it has been shown that the skin constitutes the main entranceway into the organism of Al [18–20].

Conclusion

In light of these different findings and suggestions, it is important to be extremely prudent when using the aluminium salts.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Aluminium concentration (μg/g dry, weight) in skin biopsy.</th>
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<tr>
<td>Control (1)</td>
<td>5.61 ± 0.59 (CV%= 10.5)</td>
</tr>
<tr>
<td>Control (2)</td>
<td>9.13 ± 0.057 (CV%= 0.62)</td>
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* Mean ± SD of triplicate analysis.

Fig. 1. Results from Morin staining showing granular cytoplasmic fluorescence of aluminium deposits in interfollicular macrophages (X400).
known to be particularly effective as adjuvants in single or repeated vaccinations. Though insightfully research into the possible mechanisms of action of Al has progressed significantly, all individuals will not respond in an identical manner to injection of Al in tissues [3]. However, precautions must likewise be taken as concerns its introduction in vaccines and other products such as antacids or antiperspirants [19,21]. The potential adverse effects are underestimated and have not been rigorously evaluated in the medical and scientific community [22]. Since we now know that Al is highly toxic, we should be alerting manufacturer laboratories and encouraging them to discontinue their use of a metal that undoubtedly endangers human health.

Conflict of interest statement

We declare that we have no conflicts of interest.

References