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Aluminium assay and evaluation of the local reaction at several time points after intramuscular administration of aluminium containing vaccines in the Cynomolgus monkey

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Abstract

Aluminium hydroxide and aluminium phosphate have been widely used as vaccine adjuvants with a good safety record for several decades. The recent observation in human deltoid muscle of macrophage aggregates containing aluminium hydroxide spicules and termed Macrophagic Myofasciitis (MMF) has encouraged research on aluminium salts. This study was conducted in order to further investigate the clearance of aluminium at the vaccine injection site and the features of induced histopathological lesions. Two groups of 12 monkeys were immunised in the quadriceps muscle with Diphtheria–Tetanus vaccines, which were adjuvanted with either aluminium hydroxide or aluminium phosphate. Three, six or twelve months after vaccination, four monkeys from each group were sacrificed and histopathological examination and aluminium assays were performed on quadriceps muscle sections.

Histopathological lesions, similar to the MMF described in humans, were observed and were still present 3 months after aluminium phosphate and 12 months after aluminium hydroxide adjuvanted vaccine administration. An increase in aluminium concentration, more marked in the area of the lesions, was also observed at the 3- and 6-month time points. These findings were localised at the injection site and no similar changes were observed in the distal or proximal muscle fragments.

We conclude from this study that aluminium adjuvanted vaccines administered by the intramuscular route trigger histopathological changes restricted to the area around the injection site which persist for several months but are not associated with abnormal clinical signs.

Keywords: Aluminium; Macrophagic myofasciitis; Animal study

1. Introduction

Aluminium salts have been used as vaccine adjuvants since the initial proof of concept in an animal model by Glenny et al. [1] in 1926. This type of metal salt remains the only class of adjuvant accepted in a wide range of vaccines such as Tetanus, Diphtheria, Pertussis, Hepatitis A and Hepatitis B [2]. The unique exception to this broad use of aluminium is a lipid-based adjuvant, MF 59, the adjuvant used in a European flu vaccine.

There are several potential mechanisms for the mode of action of aluminium adjuvant [3] which are still being investigated [4,5]. These mechanisms are as follows: (a) depot formation allowing a slow release of the antigen, (b) arrangement of the aluminium adjuvanted vaccine in a particulate form which is better processed by antigen presenting cells, and (c) stimulation of the immune system via an inflammatory reaction with the release of immune mediators.

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Due to the extensive use of this adjuvant, there is a large amount of data indicating its good safety profile. Some case studies reporting local reactions after administration of vaccines using aluminium as the adjuvant either by the subcutaneous or intramuscular routes have been published [6–10]. However, as aluminium has never been administered separately from the vaccine formulation, all data should always be considered to be related to the adjuvanted vaccine as a single entity, which is a mixture of one or several antigens plus the adjuvant [11]. Consequently definitive correlation of any of the reported findings to aluminium itself can be challenged. The metal aluminium, related assay methods, sources of human and environmental exposure, kinetics, metabolism and toxicity have also been studied in detail [12]. These findings state that aluminium is widely distributed in water, air, food, cosmetics and pharmaceuticals in relatively high concentrations. By comparison with natural or environmental exposure as stated in this review from the WHO [12], exposure to the very low quantity of aluminium administered as an adjuvant in a vaccine would not seem to raise major safety concerns.

Despite this reassuring comparison with natural exposure, French scientists (former GERMMAD) recently described a focal histological lesion observed in biopsy samples from the deltoid muscle of the non-dominant arm, which they termed Macrophagic Myofasciitis (MMF). These biopsies were conducted following patients' reports of clinical symptoms observed in muscular disorders, which generally combined persistent myalgias, arthralgias and marked fatigue [13]. Interestingly, there were no apparent links between the anatomical distribution of muscular weakness and the localised deltoid lesion. In these biopsies of the deltoid muscle aluminium hydroxide spicules [14] were identified in the macrophages of the lesions, potentially incriminating aluminium adjuvants in the aetiology of this local histopathological entity in the muscle [15]. However, due to the lack of appropriate controls and the very limited number of cases, the role of aluminium and the causal relationship between focal MMF in the deltoid muscle and a more widespread muscle weakness are still being disputed.

A WHO meeting [16] dedicated to this issue associated with aluminium, emphasised the need for more research on this topic. Direct investigation in humans is difficult; both the pain and the remaining scar associated with a muscular biopsy are barriers to studying potential local lesions in the injected muscles of vaccinated people. In addition, epidemiological studies are complicated by the fact that the occurrence of this set of reactions is very low (i.e., approximately 200 cases in several tens of millions of vaccinated people) and by the lack of a case definition. Therefore, non-clinical studies may usefully contribute to confirmation or invalidation of the potential association between aluminium salts and the local histological lesions, termed MMF, and also between local deposits of aluminium salts and generalised clinical symptoms. In addition preclinical studies may provide information on the distribution of aluminium following administration of adjuvanted vaccine. Distribution has been reported [17,18]; however, these studies lacked the sensitivity of detection possible with modern apparatus. Fortunately, Stanley Hem and his collaborators [19,20] recently addressed this issue and applied a radioactive method using ^{26}Al and accelerator mass spectrometry to compare the deposition following intramuscular administration of aluminium oxyhydroxide (AlOOH) and aluminium phosphate (AlPO4). However, this work did not address the question of clearance of aluminium from the site of injection in the muscle nor did they use a complete vaccine formulation including the adjuvant to test for aluminium deposition in conditions similar to those used in man.

Several critical questions remain:

- How long does the aluminium stay in the muscle after intramuscular administration of adjuvanted vaccines?
- Does aluminium adjuvanted vaccine "in essence" trigger a histological reaction, which can be termed MMF?
- If there is such a reaction what are its features (size, identification of the cells, persistence)
- Does such a local muscular lesion characterise a more widespread muscular disease?

This study was conducted to address these questions to a certain extent by the evaluation of the local reaction and aluminium concentration after intramuscular injection of aluminium adjuvanted vaccines in Cynomolgus monkeys.

2. Materials and methods

2.1. Vaccines

Combined Diphtheria–Tetanus vaccines were prepared by Aventis Pasteur with either AlOOH from Reheis (Ireland) or AlPO4 from Biosector (Denmark). The two Diphtheria–Tetanus vaccines, identical in all respects except for the aluminium salts, contained 30 Lf/ml of Diphtheria, 10 Lf/ml of Tetanus toxoid, and adjuvants (AlPO4 or AlOOH) corresponding to a final concentration of 0.6 mg/ml Al. These vaccines also both contained Merthiolate as a preservative.

2.2. Animal immunisation

Two groups of 12 male Cynomolgus monkeys (*Macaca fasciculata*) supplied by CRP le vallon (Mauritius), weighing 2.3–3.9 kg at the beginning of the study, were given a single intramuscular vaccine injection with a 10 mm needle carefully oriented perpendicular to the skin at the midshaft femoral area of the quadriceps muscle. Either AlPO4 adjuvanted DT or AlOOH adjuvanted DT vaccine (as detailed above) was administered at a dose volume of 0.5 ml per monkey. The injection site was identified by an ink tattoo on the skin to increase the precision of muscle sampling. Either the left or right quadriceps muscle was used in a random manner. The primates were maintained in a temperature and humidity regulated room and allowed free access to water and to expanded complete primate diet with additional daily fruit supplement and were examined daily to monitor for any