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Review article

Critical analysis of reference studies on the toxicokinetics of aluminum-based adjuvants☆

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ABSTRACT

We reviewed the three toxicokinetic reference studies commonly used to suggest that aluminum (Al)-based adjuvants are innocuous. A single experimental study was carried out using isotopic 26Al (Flarend et al., Vaccine, 1997). This study used aluminum salts resembling those used in vaccines but ignored adjuvant uptake by cells that was not fully documented at the time. It was conducted over a short period of time (28 days) and used only two rabbits per adjuvant. At the endpoint, Al elimination in the urine accounted for 6% for Al hydroxide and 22% for Al phosphate, both results being incompatible with rapid elimination of vaccine-derived Al in urine. Two theoretical studies have evaluated the potential risk of vaccine Al in infants, by reference to an oral "minimal risk level" (MRL) extrapolated from animal studies. Keith et al. (Vaccine, 2002) used a high MRL (2 mg/kg/d), an erroneous model of 100% immediate absorption of vaccine Al, and did not consider renal and blood-brain barrier immaturity. Mitkus et al. (Vaccine, 2011) only considered solubilized Al, with erroneous calculations of absorption duration. Systemic Al particle diffusion and neuro-inflammatory potential were omitted. The MRL they used was both inappropriate (oral Al vs. injected adjuvant) and still too high (1 mg/kg/d) regarding recent animal studies. Both paucity and serious weaknesses of reference studies strongly suggest that novel experimental studies of Al adjuvants toxicokinetics should be performed on the long-term, including both neonatal and adult exposures, to ensure their safety and restore population confidence in Al-containing vaccines.

1. Introduction

Vaccination helped with the eradication of smallpox, a 99% decline in poliomyelitis between 1988 and 2003, and a 40% decrease in measles cases between 1999 and 2003 worldwide, as well as a decrease in cases of mumps of 859 to 9 per 100,000 inhabitants between 1986 and 2013 in France [1]. The maintenance of good vaccination coverage, i.e. a high rate of vaccinated persons in the population, is necessary to avoid the resurgence of other infectious diseases, as was observed for pertussis or rubella, with a double benefit, both individually and collectively, by reducing the number of people who can transmit infectious diseases [1].

Although the success of many vaccines has been amply demonstrated, a growing public distrust of vaccination has emerged in recent years. This reluctance, of varying degrees, appears concomitantly with an expanding global World Health Organization (WHO) policy for burgeoning vaccination programs with > 120 new vaccines currently being developed and an annual growth of 20% of vaccine business is expected, realizing a turnover which has increased from 5 to 43 billion dollars between 2000 and 2016, and will be > 100 billion dollars in 2025 [2].

Unlike conventional medicines, vaccines are administered to healthy subjects that need to be convinced of their value and safety. In this context, the vaccine issue has become a major societal issue, leading to the establishment of a national citizen consultation on vaccination chaired by Alain Fischer in France [3]. According to the findings of its final report of 30th of November 2016, several factors contribute to mistrust of vaccination, *especially*:

- Suspicions of collusion between health authorities and the drug industry as a result of mediated scandals;

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- The disappearance of many infectious diseases that question the appropriateness of continuing vaccination;
- The issue of adjuvants in vaccines;
- The position of doctors who complain of a lack of training to convince reluctant patients;
- The complexity of the vaccination course (mandatory medical prescription, pharmacy purchase of the vaccine, medical vaccination, etc.);
- Lack of information from doctors on the immunization status of their patients (health book lost or not presented);
- Health crises (mediator, contaminated blood, etc.) and the insufficient responsiveness of the answer and the commitment of the public authorities which have left the field open to anti-vaccination propaganda [3].

A key question in the debate on vaccine safety concerns the adjuvants, compounds essential for strong and lasting immunization [4]. The controversy focuses on the aluminum salts which were empirically introduced by Alexander Glenny as adjuvants to vaccines in 1926 [5]. It has resulted in various actions brought by patient associations [6,7], publication of books for the general public, either critical [8] or reassuring [9], scientific blogs [10], drafting of institutional technical reports [4,11–13], and holding of parliamentary initiative discussion meetings [14,15]. Although the principle of vaccination has never been questioned during these exchanges, the exact degree of safety of aluminum-containing vaccines has remained the subject of persistent disagreement.

The occurrence of myalgia and arthralgia, chronic fatigue and neurological disorders following multiple injections of aluminum-containing vaccines against hepatitis B, tetanus and human papilloma virus (HPV) has been reported in many countries: Australia [16], Canada [17,18], Denmark [19,20], France [21–23], United Kingdom [24,25], Italy [26], Israel [27], Japan [28–29], Mexico [30], Portugal [31], and USA [32]. Nevertheless, beyond the temporal association, the existence of a causal link remains debated. For vaccination against HPV for example, the risk of occurrence of adverse events, which may form part of one or more of the clinical entities [19] - chronic fatigue syndrome (CFS), regional pain syndrome (RPS), orthostatic postural tachycardia syndrome (POTS) – emerges from an epidemiologic point of view [33]. A systematic cross-sectional study of 12 published studies showed a slight increase of adverse events in the HPV-vaccinated group, but this information must take account of the quasi-systematic use of control groups that received aluminum adjuvants in the form of a placebo containing the adjuvant or, more rarely, the hepatitis A vaccine (11 of the 12 publications analyzed, comprising 29,533 of the 29,600 patients studied) [34]. Despite this major bias [35], European Medicines Agency (EMA) issued a negative opinion on the existence of an association between HPV-vaccination and increasing of adverse events [36]. Some pharmaco-epidemiological studies were seemingly in support of this opinion [37,38], but having focused on most specific auto-immune diseases, they have excluded CFS, RPS, and POTS from their investigations. The EMA's decision caused strong dissatisfaction of Cochrane Nordic and a complaint was lodged against EMA [39]. The question of the existence of a causal link, and thus of an authentic adjuvant syndrome [40,41], may never be resolved by epidemiological approaches [42]. The performance of epidemiology to establish causality is notoriously limited, as it can be conceived for multi-systemic effects in the more or less long term of low cumulative doses administered in a context of multiple exposures. Failing this, the debate can be enlightened only by establishing the existence or not of an unequivocal biological plausibility of a causal link.

To date, aluminum adjuvants per se have, perhaps surprisingly, not been the subject of any official experimental investigation, and this being in spite of the well-established neurotoxicity of aluminum. The WHO also notes: “Adjuvant safety is an important and neglected field. Since adjuvants have their own pharmacological properties, which might affect both the immunogenicity and the safety of vaccines, safety assessment is essential” [43]. For its part, the National French Academy of Pharmacy asked that studies on the safety of the aluminum-based adjuvants be carried out taking into account a set of parameters so far little studied, which can contribute to the appearance of risk [13]. In the following review, we have examined in detail in the light of recent findings the few articles of classical toxicokinetics in the literature that serve as a reference for health regulators and industrialists to apparently confirm the safety of aluminum adjuvants.

2. Generality on Al adjuvants

The two main aluminum salts used as adjuvants are Al oxy-hydroxide (AlOOH, Alhydrogel®) and Al hydroxyphosphate ($AlOHPO_4$, AdjuPhos®). They are present in about 60% of human vaccines (Table 1) and veterinary vaccines [44]. The oxy-hydroxide form is the most widely used adjuvant in vaccines distributed in France (the most commonly used vaccines against hepatitis B, hepatitis A, or tetanus, many other vaccines, as well as products for immunotherapy subcutaneous desensitization). For HPV vaccines, the adjuvants are Al-oxy-hydroxide for the divalent 16/18 Cervarix® (combined with a second adjuvant, monophosphoryl lipid A, detoxified derivative of lipopolysaccharide [45]), and amorphous Al hydroxyphosphate sulfate for the quadrivalent 6/11/16/18/ Gardasil® (an adjuvant more immunostimulating than conventional aluminum-based adjuvants) [46].

The two major types of aluminum adjuvant strongly potentiate the production of antibodies (humoral response by activation of CD4 + Th2 lymphocytes and B-cell priming) and not, or very little, production of cytotoxic T lymphocytes. The mechanisms involved are still incompletely understood [47,48]. The Food and Drug Administration (FDA) empirically fixed the authorized level of adjuvant at 0.85 mg of aluminum per dose of vaccine, based on results showing a good adjuvant effect at this concentration (according to Joan May, FDA/CBER, quoted in [49]).

The two Al-adjuvants have different physicochemical properties in the native state. The oxyhydroxide (commonly called Al hydroxide) has a crystalline morphology, known as Boehmite, while hydroxyphosphate (commonly called Al phosphate) is amorphous. Al hydroxide is composed of nanoparticles of about $2.2\,nm \times 4.5\,nm \times 10\,nm$ which spontaneously form micron-sized aggregates having a nano-fibrous appearance under transmission electron microscopy [50,51]. This adjuvant is highly hydrated, forming a stable gel whose antigenic adsorption capacities are uniformly high. Hydrostatic interactions and exchange of hydroxyl groups with phosphate are the main forces explaining the adsorption at the surface of the adjuvant. Al phosphate has fewer hydroxyl groups and therefore its antigenic adsorption capacities are lower than those of Al hydroxide. Al hydroxide has a positive surface charge, Al phosphate a negative charge. The kinetics of biodisposition of the two adjuvants are also significantly different: Al hydroxide is much slower solubilized, more avidly internalized and less toxic to the phagocytic cells [51] than Al phosphate, suggesting notable differences in the reactions of the two adjuvants during the interactions with phosphate, organic acids, protein environments and immune cells encountered in vivo.

3. Critical analysis of reference articles on the toxicokinetics of Al adjuvants

3.1. Study of absorption and elimination of vaccine aluminum [52]

For a long time specialized international meetings have held that Al injected by the vaccine route was essentially rapidly eliminated from the body in the urine [53] and this message was relayed by general public official information sites, until recent withdrawal [54]. This claim has its roots in studies from the 1990s using a new technique to study Al toxicokinetics. Indeed, until 1990, it was difficult to know the