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In vivo absorption of aluminium-containing vaccine adjuvants using ^{26}Al

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Aluminium hydroxide (AH) and aluminium phosphate (AP) adjuvants, labelled with ^{26}Al, were injected intramuscularly (i.m.) in New Zealand White rabbits. Blood and urine samples were collected for 28 days and analysed for ^{26}Al using accelerator mass spectrometry to determine the absorption and elimination of AH and AP adjuvants. ^{26}Al was present in the first blood sample (1 h) for both adjuvants. The area under the blood level curve for 28 days indicates that three times more aluminium was absorbed from AP adjuvant than AH adjuvant. The distribution profile of aluminium to tissues was the same for both adjuvants (kidney > spleen > liver > heart > lymph node > brain). This study has demonstrated that in vivo *mechanisms are available to eliminate aluminium-containing adjuvants after i.m. administration. In addition, the pharmacokinetic profiles of AH and AP adjuvants are different. © 1997 Elsevier Science Ltd.*

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Vaccines usually contain an antigen and an adjuvant, which potentiates the immune response to the antigen. The adjuvant effect of aluminium-containing compounds was first observed in 1926[1]. Since that time aluminium hydroxide adjuvant and aluminium phosphate adjuvant have been widely used in both human and animal vaccines. These are the only adjuvants that are currently approved for use in human vaccines by the United States Food and Drug Administration (FDA).

A recent study[2] has shown that aluminium hydroxide (AH) adjuvant is crystalline aluminium oxyhydroxide, AlOOH. It has a fibrous morphology and dissolves very slowly in simulated interstitial fluid[3]. Aluminium phosphate (AP) adjuvant is amorphous aluminium hydroxyphosphate. It has a platy morphology and dissolves more rapidly in simulated interstitial fluid than AH adjuvant. Interstitial fluid contains three organic acids which have an α-hydroxy carboxylic acid group (citric, lactic and malic acids), and are therefore capable of chelating aluminium[4–6]. A recent *in vitro* study[3] showed that citrate anion was able to dissolve both AH and AP adjuvants, although AP adjuvant dissolved more rapidly.

Vaccines containing AH or AP adjuvants are usually administered intramuscularly. The FDA limits the quantity of the adjuvant to no > 0.85 mg aluminium per dose. The disposition of aluminium-containing adjuvants after intramuscular (i.m.) administration is not understood. This is largely because the low dose of aluminium does not cause detectable changes in the concentration of aluminium normally present in blood, urine or tissues. Measurement of ^{26}Al by accelerator mass spectrometry (AMS)[7,8] offers the first opportunity to directly determine if aluminium-containing adjuvants are removed from the site of injection by dissolution in interstitial fluid. In addition, AMS allows the absorption, distribution and elimination profiles of aluminium-containing adjuvants to be studied and optimized.

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MATERIALS AND METHODS

Adjuvants

^{26}Al-containing AH adjuvant was prepared by adding 0.596 g of an $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq $^{26}Al\ g^{-1}$ or 0.24 μg $^{26}Al\ g^{-1}$) to 45 ml of 0.2 M $AlCl_3$. Forty-five milliliters of a 0.6 N NaOH and 4 M NaCl solution was added dropwise over 30 min to the $AlCl_3$/$^{26}AlCl_3$ solution with vigorous agitation. The precipitate was repeatedly washed with 50 ml portions of double distilled water (ddH_2O) after centrifugation until the supernatant was free of chloride as determined by the absence of a precipitate when 0.1 M $AgNO_3$ was

added. The washed precipitate was resuspended in 50 ml of ddH_2O, filled into a sealed container and placed in an 80°C oven for 24 h. After heating, the volume was adjusted to 57.1 ml with ddH_2O. The adjuvant suspension was autoclaved at 121°C for 20 min. A dose of 0.20 ml contains 0.85 mg Al. The preceding procedure without the $^{26}AlCl_3$ was followed to produce an AH adjuvant for testing. The tests showed that the AH adjuvant prepared by this procedure exhibited the X-ray diffraction pattern and infrared spectrum which are typical of AH adjuvant[2].

^{26}Al-containing AP adjuvant was prepared by dissolving 3.7 g of alum [$KAl(SO_4)_2 \cdot 12\ H_2O$] in enough ddH_2O to make 68 ml and adding 0.519 g of the $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq ^{26}Al g^{-1} or 0.24 μg ^{26}Al g^{-1}). A phosphate solution was prepared (0.3403 g $NaH_2PO_4 \cdot H_2O$, 0.3501 g Na_2HPO_4 and 5.5796 g NaCl) in enough ddH_2O to make 800 ml. The alum solution was slowly added to the phosphate solution and agitated until the solution was clear. The solution was titrated with 1N NaOH with agitation until the pH was 7.1–7.2 to precipitate aluminium hydroxyphosphate. The suspension was agitated for 2 h and the pH readjusted to 7.1–7.2 with 1 N NaOH. The precipitate was washed three times with 0.9% NaCl by centrifugation. After the third wash, the sediment was dispersed in enough 0.9% NaCl to make 50 ml. The adjuvant suspension was autoclaved at 121°C for 20 min. A dose of 0.20 ml contains 0.85 mg Al. The preceding procedure without the $^{26}AlCl_3$ was followed to produce an AP adjuvant for testing. The tests showed that the AP adjuvant prepared by this procedure was amorphous by X-ray diffraction and the infrared spectrum was typical of AP adjuvant[2].

^{26}Al-containing aluminium citrate was prepared by dissolving 0.7606 g $AlCl_3 \cdot 6\ H_2O$ in enough ddH_2O to make 10 ml. Twenty-one microliters of the $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq ^{26}Al g^{-1} or 0.24 μg ^{26}Al g^{-1}) was added with mixing. A citric acid solution was prepared by dissolving 0.6620 g of citric acid in enough ddH_2O to make 10 ml. The citric acid solution was added to the $AlCl_3/^{26}AlCl_3$ solution and mixed. The pH was adjusted to 7.4 with 0.1 N NaOH.

The specific activity of the ^{26}Al-labelled adjuvants was 15.9 Bq ml^{-1} for the AH adjuvant and 15.5 Bq ml^{-1} for the AP adjuvant. The specific activity of the ^{26}Al-labelled aluminium citrate solution was 1.07 Bq ml^{-1}. Thus, the doses contained 3.2 Bq for the AH adjuvant (i.m.), 3.1 Bq for the AP adjuvant (i.m.) and 0.32 Bq for the aluminium citrate solution (intravenous; i.v.). Calibration errors were 3–5%.

Rabbits

Six female New Zealand White rabbits were used to determine the *in vivo* absorption of the ^{26}Al-labelled adjuvants. They were conditioned for 21 days before the study and their weights were 2.5–2.8 kg at the beginning of the study and 3.2–3.7 kg at the end of the study.

Two rabbits received an i.m. injection (0.2 ml of ^{26}Al-labelled adjuvant followed by 0.1 ml of sterile 0.9% NaCl to wash the syringe) of ^{26}Al-labelled AH adjuvant, two rabbits received a similar i.m. injection of ^{26}Al-labelled AP adjuvant, one rabbit received an equivalent i.v. injection (0.3 ml of ^{26}Al-labelled aluminium citrate followed by 0.1 ml of sterile 0.9% NaCl to wash the syringe) of ^{26}Al-labelled aluminium citrate, and one rabbit received an equivalent i.m. dose of AP adjuvant containing no ^{26}Al as a cross-contamination monitor. All rabbits received a total of 0.85 mg aluminium.

The rabbits were killed 28 days after the injections by sodium pentobarbital overdose. This study was approved by the Purdue University Animal Care and Use Committee and performed in accordance with all federal regulations.

Sample collection

One milliliter of whole blood was collected at 0, 1, 2, 4, 6, 10, and 12 h and at 1, 2, 4, 6, 8, 12, 16 and 21 days. Three milliliters of blood were collected at 28 days. The samples were collected in 3 ml vials with premeasured ethylenediaminetetra-acetic acid and refrigerated immediately.

Urine was collected for 24 h before dosing and for the following intervals: 0–5, 5–9 and 9–24 h, 1–2, 2–4, 4–6, 6–8, 11–12, 15–16, 20–21 and 27–28 days. Urine was collected in screened pans placed under the cages. The pans were filled with 2 l of water at the beginning of each collection period. At the end of the collecting period, the pans were agitated and 40 ml aliquots were placed in 50 ml polypropylene centrifuge tubes and immediately refrigerated. The total volume of liquid in the pans when the aliquot was collected was recorded.

Tissue samples were collected after the rabbits were killed on day 28. Whole brain, heart, left kidney, liver, mesenteric lymph node and spleen tissues were collected and frozen in comercial plastic freezer bags. Bone (femur) samples were also collected, but these samples were lost during chemical preparation. The brain sample for one of the AP-dosed rabbits was also lost during chemical preparation.

Sample preparation

Blood and urine samples were prepared for AMS analysis by the addition of 1–100 mg ^{27}Al carrier from Al_3Cl (ICP 10000 p.p.m. ^{27}Al standard). The samples were then repeatedly digested in nitric acid (70%) at 80°C in a porcelain crucible and allowed to evaporate to dryness. After two digestions in nitric acid, the samples were ashed at 800°C to yield Al_2O_3 powder. This Al_2O_3 powder was then mixed with silver powder in a 1:3 ratio by mass and analysed by AMS.

Tissues were prepared by first dissolving the tissue in 20–200 ml (depending on tissue size) of nitric acid (70%) in polyethylene bottles. Aliquots of the dissolved tissue were then prepared as described above except that hydrogen peroxide (30%) was used as well as nitric acid in the wet digestion.

Data analysis

Since AMS measures relative amounts of ^{26}Al and ^{27}Al in samples, the actual recovery percentage of aluminium during sample preparation is irrelevant provided that the carrier ^{27}Al is homogenized with the ^{26}Al native to the sample. In order to test the reproducibility of the carrier addition, sample digestion, and AMS analyses, ten samples were separately prepared in triplicate. The results for each of these samples agreed

within 10% (standard error of the mean) or within the AMS precision.

Cross-contamination of ^{26}Al between the animals was monitored by the measurement of samples from the rabbit receiving no ^{26}Al dose. Data was rejected if the ^{26}Al concentration in a given sample was not at least five times higher than the equivalent sample from the cross-contamination monitor. Also, the ^{26}Al concentration in blood, urine and tissue samples from the cross-contamination monitor rabbit was subtracted from the ^{26}Al concentration in equivalent samples of the other rabbits.

Cross-contamination of ^{26}Al between samples during chemical preparation was monitored with the preparation of chemistry blanks. In no case did these blanks indicate more than a 1% cross-contamination during chemical preparation. Chemistry blanks are samples that are prepared alongside experimental samples. These blanks undergo the same preparation procedure in order to monitor any possible cross-contaminatin of ^{26}Al between samples during the chemical preparation of experimental samples.

All AMS analyses were conducted at the Purdue Rare Isotope Measurement Laboratory, PRIME Lab[9]. Although all samples were analysed for ^{26}Al content, data is reported in terms of aluminium arising from the ^{26}Al-labelled adjuvants or ^{26}Al-labelled aluminium citrate. The result for the 4 h blood sample for rabbit 1 was rejected and not included in any analysis due to an error in the recording of data for that sample.

RESULTS

Figure 1 shows the time profile for the aluminium blood concentration of the four rabbits receiving the ^{26}Al-labelled adjuvants. The blood level curve of both adjuvants exhibit an absorption phase and an elimination phase, as is typical of i.m. administration. It is noteworthy that ^{26}Al was found in the blood at the first sampling point (1 h) for both adjuvants. Thus dissolution of the adjuvants in interstitial fluid begins upon administration. The aluminium concentration produced by AH adjuvant at 1 h was similar to the concentrations found from 2 to 28 days.

The mean area under the blood concentration versus time curve (AUC) from days 0 to 28, determined using the trapezoid rule, was 1.6×10^{-3} mg h g^{-1} for the i.v. dose of ^{26}Al-labelled aluminium citrate ($n = 1$); 8.1×10^{-4} mg h g^{-1} for the ^{26}Al-labelled AP adjuvant ($n = 2$); and 2.7×10^{-4} mg h g^{-1} for the ^{26}Al-labelled AH adjuvant ($n = 2$). Thus, three times as much aluminium was absorbed from the AP adjuvant as from the AH adjuvant within 28 days. However, during the first 48 h (*Figure 1* insert), the AUC of the AH adjuvant was 1.4 times the AUC of the AP adjuvant. These data also indicate that 17% of the AH adjuvant and 51% of the AP adjuvant were absorbed within 28 days based on the AUC of the i.v. dose of ^{26}Al-labelled aluminium citrate. The blood concentration of aluminium for each of the rabbits receiving an adjuvant had not reached a terminal elimination phase by day 28.

Cumulative urinary excretion of aluminium (*Figure 2*) indicates that the body is able to eliminate the aluminium absorbed from the adjuvants. The cumulative amount of aluminium eliminated in the urine during the 28 days of the study was 6% of the AH adjuvant dose and 22% of the AP adjuvant dose. Aluminium from both adjuvants was still being excreted at a steady rate at day 28.

The pharmacokinetic parameters determined from the blood and urine data are presented in *Table 1*.

Distribution of aluminium in tissues 28 days after administration of AH and AP adjuvants is shown in *Figure 3*. For each tissue, the concentration of aluminium was greater in the rabbits which received AP adjuvant. The average aluminium tissue concentration was 2.9 times greater for AP adjuvant than for AH adjuvant.

DISCUSSION

It is noteworthy that the aluminium concentration produced by AH adjuvant at the first sampling point

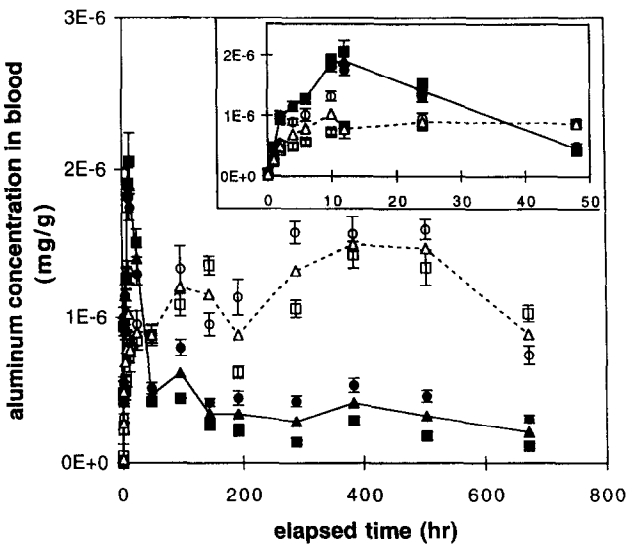

Figure 1 Blood concentration profile after i.m. administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean

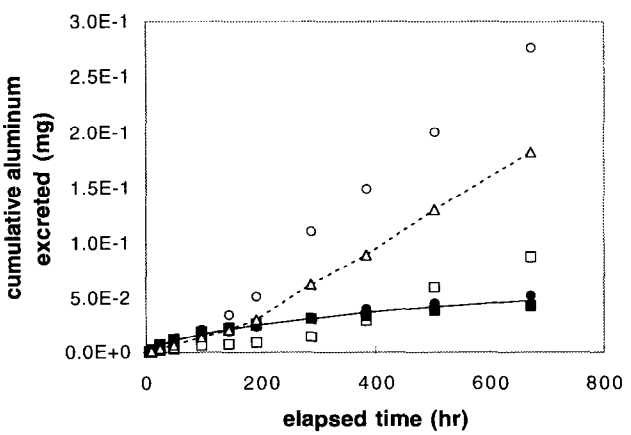

Figure 2 Cumulative urinary excretion of aluminium after i.m. administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean. Error bars of <5% are not shown

(1 h) was similar to the 2–28 day concentrations. This indicates that dissolution of aluminium-containing adjuvants in interstitial fluid begins quickly after i.m. administration. It is surprising that the aluminium concentrations were greater during the first 24 h for crystalline AH adjuvant than for the amorphous AP adjuvant. This suggests that the initial rate of dissolution from the edges of the fibrous AH adjuvant particles is greater than from the platy AP adjuvant particles.

The rapid appearance of aluminium in the blood may have implications for theories regarding the mechanism of adjuvant action of aluminium-containing adjuvants. The most widely accepted theory is the repository effect[10], whereby the antigen adsorbed by the aluminium-containing adjuvant is slowly released after i.m. administration. The rapid appearance of aluminium as seen in the insert of *Figure 1* challenges the repository mechanism as it is likely that the adsorbed antigen would be quickly desorbed as a result of the fast initial dissolution of the substrate.

After 2 days, the absorption rate for AP adjuvant was considerably more than the AH adjuvant which confirms the difference in *in vitro* dissolution rates in simulated interstitial fluid[3]. The blood concentration of aluminium was fairly steady from days 2 to 28 indicating a relatively constant absorption rate for each adjuvant even 28 days after i.m. administration. No terminal phase had been reached for the blood concentration of aluminium so it is difficult to determine the mean residence time of each adjuvant, It is clear, however, that AP adjuvant will be eliminated before AH adjuvant because the long term absorption rate of the AP adjuvant is greater.

Table 1 Pharmacokinetic parameters after i.m. injection of ^{26}Al-containing aluminium hydroxide and aluminium phosphate adjuvants

Adjuvant	AUC for 0–28 days (mg h g^{-1})	% Absorbed in 28 days	Cumulative aluminium in urine after 28 days (%)
Aluminium hydroxide			
Rabbit 1	2.0×10^{-4}	13	5.0
Rabbit 2	3.5×10^{-4}	22	6.2
Average	2.7×10^{-4}	17	5.6
Aluminium phosphate			
Rabbit 3	2.7×10^{-4}	47	10
Rabbit 4	8.7×10^{-4}	55	33
Average	8.1×10^{-4}	51	22

The measured increase in the plasma concentration of aluminium from the i.v. dose was *ca* 600 ng ml^{-1}, which is considerably more than the increase of 2 ng ml^{-1} from the i.m. dose. Since it has been shown that the pharmacokinetics of aluminium depend on the concentration in the blood[11], the pharmacokinetics of the i.v. bolus dose were probably somewhat different from those of the i.m. dose. Thus the AUC from the i.v. dose may not provide a completely accurate baseline for determining the fraction of the aluminium absorbed from the i.m. administration of the AH and AP adjuvants. However, this does not affect the relative comparison of the AH and AP adjuvants.

The two rabbits which received AH adjuvant exhibited very similar pharmacokinetic characteristics. The blood level data for the two rabbits receiving AP adjuvant were also very similar. However, the cumulative urinary excretion of aluminium differed by a factor of three between the two rabbits which received AP adjuvant. This difference is probably due to intersubject variability in the elimination of aluminium[12]. In spite of this intersubject variation, the cumulative urinary excretion of aluminium after 28 days in each rabbit receiving AP adjuvant was greater than the cumulative urinary excretion of aluminium in the rabbits receiving AH adjuvant.

The normal plasma aluminium concentration in rabbits is 30 ng ml^{-1}[13]. The maximum increase in the plasma aluminium concentration from the 0.85 mg aluminium doses of either adjuvant was *ca* 2 ng ml^{-1}. This small increase would have been masked by the aluminium background if ^{26}Al-labelled adjuvants were not used. If the same dose of these adjuvants was administered i.m. to adult humans, an increase in the plasma aluminium concentration of *ca* 0.04 ng ml^{-1}

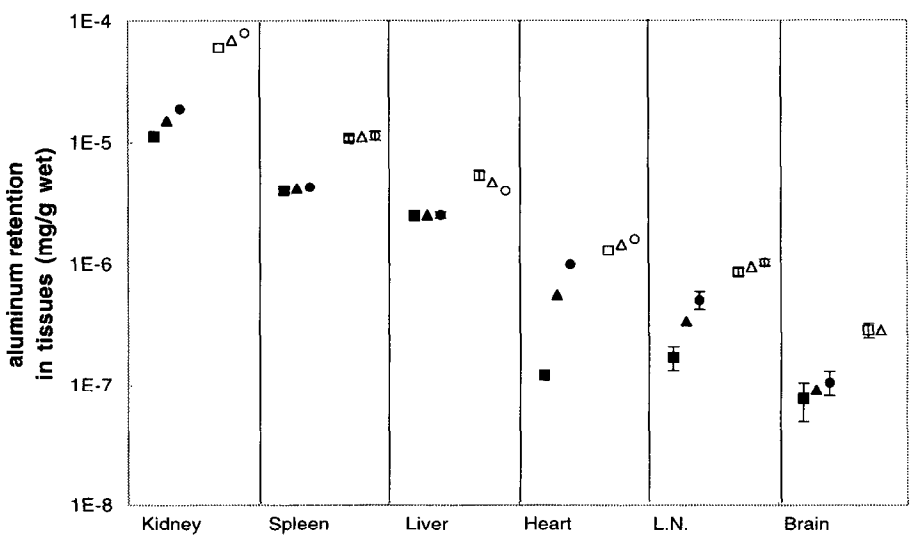

Figure 3 Aluminium tissue concentration 28 days after administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean. L.N., lymph node. Error bars of <5% are not shown

could be expected based on the larger blood volume of humans and assuming the same rate of dissolution in interstitial fluid. This represents a 0.8% increase in plasma aluminium concentration based on a normal value of 5 ng ml [14]. This small change explains the safety of aluminium-containing adjuvants and emphasizes the utility of AMS for studying aluminium concentration *in vivo*.

The relative tissue distribution was the same for both adjuvants (kidney > spleen > liver > heart > lymph node > brain). This distribution pattern is typical of results obtained when ^{26}Al was given by other routes of administration[15]. Since the concentration of aluminium was 2.9 times greater on average in each tissue (*Figure 3*) for the rabbits which received AP adjuvant, the tissue data is consistent with the ratio of 3.0 which was observed for the AUC of AP adjuvant compared to AH adjuvant. Thus, the relative ^{26}Al tissue concentrations can be inferred from the ^{26}Al blood concentrations.

Since the adjuvants are being dissolved by interstitial fluid which flows directly into the lymphatic system, one may expect the aluminium concentration to be quite high in the lymph tissue that was collected. However, the i.m. doses were given in the hind quarter where the nearest lymph node is difficult to isolate. For this reason, the mesenteric lymph node, located in the abdominal cavity, was removed. Thus the aluminium from the dissolved adjuvants does not flow directly to the lymph tissue that was collected and measured.

Dissolution, absorption, distribution and elimination of aluminium-containing adjuvants after i.m. administration has been demonstrated by the use of ^{26}Al-labelled adjuvants. The two adjuvants studied exhibited significantly different dissolution rates in interstitial fluid which were reflected in different blood, urinary excretion and tissue profiles. Human studies using ^{26}Al-labelled adjuvants can be performed since the radiation exposure to ^{26}Al is negligible. There was 1.6 Bq ^{26}Al used in each rabbit. In humans, *ca* 74 Bq ^{26}Al would need to be used resulting in a maximum whole body exposure to radiation of *ca* 15 μSv year^{-1} compared to the natural background exposure of 3000 μSv year^{-1} [9].

The application of AMS to the *in vivo* performance of vaccines should lead to a fuller understanding of the mechanism of adjuvant action of aluminium-containing adjuvants. The ability to label an aluminium-containing compound with ^{26}Al, as demonstrated in this study, may prove useful in studying the *in vivo* absorption, distribution, metabolism and elimination profiles of other aluminium-containing compounds.

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