Analysis of aluminum-26 labeled aluminum chlorohydrate

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Abstract

A small quantity of aluminum chlorohydrate (ACH), the active ingredient in antiperspirants, was labeled with the radioisotope 26Al. This labeled drug will be used in future studies to measure the absorption of aluminum from antiperspirant use. The purpose of this study was to demonstrate that the technique used to make the 26Al-labeled ACH resulted in a uniformly labeled complex, and therefore any measurement of 26Al would be indicative of total aluminum. The labeled ACH was fractionated using gel filtration chromatography into 72 evenly spaced samples. The fractions were then measured for 26Al and total aluminum content using accelerator mass spectrometry (AMS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). Results indicate that the ACH is uniformly labeled. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

Antiperspirants and deodorants are used by over 90% of the US population, and aluminum chlorohydrate, or a similar aluminum salt, is the active ingredient in all antiperspirants on the market in the United States. Aluminum chlorohydrate (ACH) is a soluble aluminum complex [1]. It is believed that ACH acts as an antiperspirant by precipitating inside the eccrine sweat glands to produce insoluble aluminum hydroxide which then plugs the gland [2,3]. The fate of the aluminum applied to the skin as ACH is currently unknown. However, using accelerator mass spectrometry (AMS) [4] to analyze 26Al, the amount of aluminum from ACH which is absorbed into the blood via the skin or inhalation can be measured with extreme sensitivity [5].

The healthy body has an efficient mechanism to remove aluminum which is absorbed into the blood. However, individuals with reduced kidney function have difficulty removing aluminum from their blood. In patients with renal failure, prolonged high levels of aluminum in the blood can cause anemia, bone disease, and dementia [6]. There have also been reports which associate aluminum with Alzheimer’s disease [7] and Down’s syndrome [8]. It has been reported that aluminum can be absorbed through the skin when aluminum chloride is applied to the skin of mice [9]. One epidemiological study has found a possible correlation between the use of aluminum-containing antiperspirants and Alzheimer’s disease [10], but the author of that study admits that the results are inconclusive. Therefore, since the absorption and metabolism of aluminum from antiperspirants is currently unknown and it may be possible for aluminum to be absorbed through the skin, there is a pressing need to determine the bioavailability of aluminum which is applied in various formulations of antiperspirants such as aerosol, roll-on, or solid.

There are many soluble aluminum salts that can be used as antiperspirants which differ only in the relative amounts of aluminum, chloride, and zinc (11). Only ACH may be used in aerosol formulations [12]. ACH is also desirable because it has a pH of about 4.5 which is less acidic than other aluminum salts and therefore does not irritate the skin or damage clothing as much as plain aluminum chloride. The empirical formula for ACH is Al₂(OH)₃·Cl·2H₂O. However, when dissolved, the basic unit of ACH is more closely given by Al₂(OH)₃(OH)²⁺·7H₂O with seven dissociated Cl⁻ ions [13]. Commercial ACH has been measured to consist of this Al₂(OH)₃ unit along with other monomer, dimer, and polymer units of various sizes [2,14]. The acidity of this complex is therefore buffered by the many hydroxyls present. The
physical structure of the ACH (Al₃⁺) complex is somewhat spherical with one aluminum in tetrahedral form surrounded by 12 aluminum in octahedral form.

In practice aluminum chlorohydrate is a term often used to refer to a broad spectrum of very similar salts. For labeling purposes, the FDA OTC considers aluminum chlorohydrate to be any formulation resulting in a final Al/Cl ratio of 2:1 down to not including 1:3.1 [15]. Thus, a formulation which is labeled 'aluminum chlorohydrate' cannot consist solely of the complex Al₃(OH)₁₆(OH₂)₅(H₂O)₇ since the Al/Cl ratio of 13:7 or 1.86:1 for this specific complex does not fall within the defined range. A formulation which is labeled as aluminum chlorohydrate actually consists of a large variety of aluminum species. Some of species are Al₃(OH)₁₆(OH₂)₅(H₂O)₇, while others are aluminum complexes which contain a varying number of aluminum atoms per species. The equimolar among these many similar complexes of aluminum salts is affected by the concentration of both aluminum and chloride, pH, and age of the solution [14].

In some antiperspirant formulations, the Al/Cl ratio is less than 2:1. Two such formulations which are used are called aluminum sesquichlorohydrate (ASC) (AlCl of about 1:33:1) and aluminum dichlorohydrate (ADCH) (AlCl of about 1:1) [14,15]. Each of these three formulations, ACH, ADCH and ASCH, are very similar and contain many of the same monomer, dimer, and polymer units of aluminum, although the relative concentrations of the smaller monomers and dimers is greater than in ACH and the larger Al₃(OH)₁₆(OH₂)₅(H₂O)₇ species is less prevalent. Gel filtration chromatography (GFC) has been established as an effective technique for distinguishing these different antiperspirant formulations by separating the various aluminum species in each formulation [14].

A common technique of making ACH is to dissolve aluminum powder in an aluminum chloride solution until the desired concentration is achieved [14]. However, since it is not practical to obtain aluminum-26 powder, this method was used with all of the ²⁶Al present in the initial aluminum chloride and no ²⁶Al in the powder. This is important to the labeling of ²⁶Al-ACH due to the proposed [2] way ACH forms. The aluminum atom in tetrahedral form, which makes up the core of an Al₃(OH)₁₆(OH₂)₅(H₂O)₇ molecule, only exists in regions of high pH. When aluminum powder is added to aluminum chloride, the area of high pH will be near the surface of the aluminum powder granules. Then the surrounding octahedrals will form in regions of neutral or acidic pH, i.e. away from the aluminum powder granules.

As the Al₃⁺ species of ACH forms following these two processes, there could be a distinct difference between the predominance of aluminum-26 and aluminum-27 in the two regions of high and low pH were the processes take place. Smaller aluminum species (monomers and dimers) may also be affected in a similar fashion depending on local pH conditions and relative concentrations of ²⁶Al and ²²Al. Thus, it was possible that the labeled antiperspirant could have ²⁶Al non-uniformly distributed among the many species present. It is also conceivable that the bioavailability could be different for each species making up the antiperspirant formulation.

Therefore, as a prerequisite to measuring the absorption of aluminum from antiperspirants, it was desired that the labeled antiperspirant be tested to ensure that the ²⁶Al is distributed equally among the various complexes making up the antiperspirant.

2. Experimental

2.1. Preparing the ²⁶Al-labeled aluminum chlorohydrate

The ²⁶Al-labeled aluminum chlorohydrate was prepared by first adding a very small amount of ²⁶Al as AlCl₃ solution to a larger amount of prepared AlCl₃ solution. Then aluminum powder (all ²⁶Al) was slowly dissolved in the AlCl₃ solution until the Al/Cl ratio was 2:1. The details of this procedure were provided by a commercial pharmaceutical company and are proprietary. Using this technique, a quantity as small as approximately 10 ml of ACH could be prepared.

2.2. Fractionating the aluminum chlorohydrate

To fractionate the ACH, a preparative gel filtration column was used with Sephadex G-25 and a mobile phase of 100 mmol KCl adjusted to a pH of 3.0 using HCl. The column measured 60 cm long with a 1.5 cm diameter (I.D.), and the mobile phase was supplied by a peristaltic pump. An aliquot of the ACH was diluted to 7% using 100 mmol KCl and 0.1 ml was injected into the column with a flow rate of about 0.36 ml/min. Fractions were collected over 5 min intervals in separate vials for a total elution time of 360 min. This corresponds to a total eluted volume of 130 ml. A refractive index (RI) detector was used to match peaks to the appropriate fractions collected.

It is well known that the spectrometry of aluminum compounds is highly pH dependent. Thus, when the labeled ACH is diluted to 7% using the KCl/HCl solution of pH 3.0 and then injected into the column where the mobile phase has a pH of 3.0, the spectrometry of the ACH is probably altered. However, the industry standard is to verify the complex makeup of antiperspirants using chromatography techniques which inevitably alter the antiperspirant itself. With this in mind, the gel filtration method used here and the concentration of the injected ACH is similar to what has been reported elsewhere [16]. The method used in this study is also based upon a technique provided by the antiperspirant industry which was modified for preparative GFC in conjunction with ²⁶Al and accelerator mass spectrometry.

2.3. Analysis of aluminum-26 and total aluminum

Fractions were then diluted using 1 M HCl to a total volume of 6-15 ml depending on how much aluminum was estimated to be contained within each fraction (based on RI profile). Small aliquots of these dilutions were transferred to porcelain
crucibles and 3-7 mg of $^{27}$Al (as 10,000 ppm Al ICP standard solution) was added so that the estimated isotope ratio of $^{26}$Al/$^{27}$Al was approximately 10$^{-19}$. The crucibles were heated at 80°C until dry, the samples were redissolved in 5 ml of 8 M nitric acid and again heated at 80°C until dry. Dried samples were baked at 90°C for several hours to produce Al$_2$O$_3$ powder. The Al$_2$O$_3$ was subsequently analyzed by AMS for $^{26}$Al content.

After aliquots were removed for $^{26}$Al analysis, the diluted fractions were measured for total aluminum content using ICP-AES.

3. Results

3.1. Precision and background

Blank samples without any $^{26}$Al were prepared along with the unknown samples to check for cross-contamination. The cross-contamination was less than 1% in all batches except one where the contamination was measured at 7% between samples of relatively similar $^{26}$Al concentration. The precision of the AMS measurements was better than 5% (SD) for 97% of the data, and better than 8% (SD) for all measurements. No attempt was made during the design of this study to quantify the total recovery of $^{26}$Al or total aluminum.

3.2. Calibration of the Sephadex G-25 column

The total volume ($V_t$) of the column was calculated to be 106 ml, and the void volume ($V_v$) of the column was found to be 47 ± 3 ml by injecting three probes in the column with molecular weights in the range of 8000–5 650 000 Da.

3.3. Agreement of refractive index, $^{26}$Al, and total aluminum profiles

Fig. 1 shows the refractive index chromatogram, $^{26}$Al content, and total Al content. There are three easily distinguishable peaks which occur in all three spectra (labeled 1-3). The first peak occurs for an eluted volume of 48 ml, the second for 69 ml, and the third for 82 ml. Since the $^{26}$Al and total aluminum spectra are nearly identical, we conclude that the procedure used to label the ACH resulted in uniformly labeled antiperspirant. There is also a fourth peak from the refractive index which occurred for an eluted volume of 105 ml. This peak did not correspond to any measured increase in $^{26}$Al or total aluminum and has been reported previously [16].

As a further check, the same procedure used to make the $^{26}$Al-labeled ACH was also used to make a similar batch of ACH (without $^{26}$Al). This similar batch was then sent to a commercial pharmaceutical company where it was analyzed using a proprietary chromatography technique and was shown to have a similar profile to commercial ACH.

3.4. Consistency between $^{26}$Al and total aluminum concentration

The ratio of $^{26}$Al and total Al content in all samples measured is seen in Fig. 2 and has an average ratio of 408 ± 3 Bq/g Al (mean ± SEM) with a standard deviation of 28 Bq/g Al. This ratio is remarkably close to the calculated ratio of 413 Bq/g Al which is based on the amount of $^{26}$Al and total aluminum used to prepare the labeled ACH. This indicates that the $^{26}$Al is distributed equally among the aluminum.

Fig. 1. Chromatograms of $^{26}$Al content, total aluminum content, and refractive index for 7% ACH separated on Sephadex G-25.

Fig. 2. Ratio of $^{26}$Al to total aluminum for all fractions collected.
containing species contained in ACH. Therefore, the technique of using $^{26}$Al in only the ACl solution to produce the ACH resulted in a uniformly labeled antiperspirant.

A 14-hr chi-squared test was performed to check whether the fluctuations in the measured ratios ($^{26}$Al/$^{29}$Al) seen in Fig. 2 were caused by some physical process rather than due to statistical variations. The reduced chi-square indicated a probability of $P > 0.05 \ (P = 0.5)$ that the variations did not have a physical meaning and therefore followed a normal statistical distribution.

4. Discussion

This study shows that it is possible to use the technique given above to uniformly label antiperspirants with $^{26}$Al. Aluminum-26 labeled ACH can now be used in further studies to determine the absorption of aluminum from antiperspirants using AMS. The sensitivity of AMS [5] allows a minuscule absorbed fraction to be quantified even when a subject is exposed to a small dose of antiperspirant. Because the antiperspirant is known to be uniformly labeled, there can be no doubt that any amount of $^{26}$Al which is absorbed from the labeled ACH will correctly represent how much total aluminum is absorbed from an antiperspirant (under similar usage).

Aluminum-26 labeled ACH which is produced as described above can be used in virtually any formulation of antiperspirant and then administered to test the bioavailability of aluminum from antiperspirant via the skin, lungs, GI-tract, or olfactory system. Future work in the area should address all these routes as recently proposed [17] in an effort to fully determine the safety of antiperspirant use.

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References