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Formulation and in vivo evaluation of sodium alendronate spray-dried microparticles intended for lung delivery

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Abstract

Spray-dried powders for lung delivery of sodium alendronate (SA) were prepared from hydroalcoholic solutions. Formulations display geometric particle size below to 12 μm and spherical shape associated to a hollow structure. The addition of leucine and ammonium bicarbonate leads to porous particles with rough surfaces. The tapped density ranges from 0.016 to 0.062 g/cm^3 , decreasing with the increase of the leucine concentration. For all formulations, the calculated aerodynamic diameters are lower than 5 μm . The *in vitro* aerodynamic evaluation shows that all powders present a high emitted fraction of 100%, a fine particle fraction ranging from 34.4% to 62.0% and an alveolar fraction ranging from 23.7% to 42.6%. An optimized sample was evaluated regarding sodium alendronate acute pulmonary toxicity and lung bioavailability. The bronchoalveolar lavage study shows that the intratracheal administration of sodium alendronate dry powder and sodium alendronate aqueous solution do not induce significant increases of lung toxicity indicators as compared with the positive control. Moreover, the intratracheal administration of sodium alendronate dry powder results in a $6.23 \pm 0.83\%$ bioavailability, a 3.5-fold increase as compared to oral bioavailability. Finally, these results suggest that sodium alendronate pulmonary delivery could be a new and promising administration route.

Keywords: Sodium Alendronate, Lung Toxicity, Spray Drying.

1- Introduction

Sodium alendronate belongs to a class of drugs called bisphosphonates known for their effectiveness in the treatment of bone diseases [1-2]. The pharmacological action of sodium alendronate is based on its affinity for the hydroxyapatite crystals of bone and selective inhibition of osteoclastic bone resorption during the remodeling cycle [1, 3]. As a potent antiresorptive drug, sodium alendronate was approved by the FDA in 1995 for the oral treatment of Paget's disease and postmenopausal osteoporosis [4]. The clinical dose of sodium alendronate is about 0.2 mg/Kg orally (10 mg tablet daily). However, oral administration of sodium alendronate has been reported to be associated with gastrointestinal intolerance [5]. Gastric and esophageal lesions such as erosions and ulcers have been observed as a result of a topic irritant effect of the drug [6-7]. Patients presenting gastrointestinal problems or esophageal abnormalities such as achalasia have to avoid treatment with sodium alendronate [7]. In addition, oral bioavailability of sodium alendronate is very low, around 1.7% [1, 8]. Intake together with meals and beverages other than water further reduces its bioavailability. High hydrophilicity and complexation with divalent cations, such as Ca^{++} , are the reasons for poor sodium alendronate absorption [1].

Studies on alternative routes of administration have been conducted to overcome the adverse gastrointestinal effects and the poor bioavailability of sodium alendronate. However, intravenous administration of sodium alendronate presents the risk of nephrotoxicity [9]. Indeed, after intravenous injection in rats an accumulation of sodium alendronate in non-calcified tissues was observed [8]. Subcutaneous and intramuscular administrations are associated with local tissue damage and irritation at the site of injection [1, 10]. As an alternative to the peroral and parenteral administrations, Sutton et al. studied the nasal absorption of sodium alendronate in dogs and rats [11]. Although the intranasal administration showed a greater absorption than the peroral route, no formulations concerning the nasal delivery of sodium alendronate have been reported in the literature.

In recent years, the pulmonary route has gained attention as a potential route of administration for the treatment of local and systemic diseases [12-16]. Lung is an attractive target for drug delivery due to its large absorptive surface area, thin pulmonary epithelium and rich blood supply [15]. A large number of drugs which are poorly absorbed from enteral route are well absorbed from the lung [17]. In addition, inhaled drugs bypass the GI tract avoiding hepatic first-pass metabolism and dietary complications [14]. Considering the advantages of pulmonary delivery, the development of a formulation of sodium alendronate intended for inhalation would be another interesting strategy to circumvent the disadvantages presented by other routes. Reducing the incidence of GI irritation and improving the absorption may increase the patient convenience and compliance to sodium alendronate therapy.

In this study, we report the development of spray-dried powders for the lung delivery of sodium alendronate. The influence of leucine concentration, a dispersibility enhancer, on the physical properties of the powders, including particle size, morphology, density and *in vitro* deposition, was investigated in order to determine an optimized formulation to deliver sodium alendronate into the lungs via inhalation. Lung toxicity and bioavailability of the optimized formulation was also assessed.

2- Materials and methods

2-1 Materials

Sodium alendronate (SA) was provided by Chemos (Germany), ammonium bicarbonate (AB), DL-leucine (Leu), *o*-phthalaldehyde (OPA) and 2-mercaptoethanol were purchased from Sigma-Aldrich (France). Ethanol 96.2% was obtained from Carlo Erba Réactifs (France). 9-Fluorenylmethyl chloroformate (Fmoc) was purchased from Sigma-Aldrich (Brazil). Acetonitrile and methanol were HPLC grade and obtained from Tedia (Brazil). All other chemicals and solvents were of analytical grade.

2-2 Preparation of the spray-dried powders

Sodium alendronate and leucine at different concentrations (Table 1) were dissolved into 200 mL of water. Ammonium bicarbonate (2.5 g) was dissolved into 100 mL of water and then added to the first solution. The resultant aqueous solution was mixed with 200 mL ethanol prior to atomization to form a 40% ethanolic solution with final solid content of 2 g/L. The ammonium bicarbonate, a volatile pore forming agent, was omitted in the final concentration of solids since this compound sublimates during the drying process.

The prepared solutions were spray-dried using a BÜCHI Mini Spray Dryer B-191 (Büchi, France) equipped with a 0.7 mm two-fluid nozzle, using the following operational conditions: inlet temperature of 160 °C, aspiration at 100%, feed rate of 15 mL/min and atomizing air flow rate of 500 L/h. Powders samples were stored in a dessicator at room temperature until analysis. The outlet temperature was around 70 °C.

2-3 Granulometry and tap density

The volume median geometric diameter (d) was measured by light diffraction using Mastersizer 2000 equipped with a Sirocco dry disperser (Malvern Instruments, France) at dispersing pressures of 1, 2 and 3 bar. Each sample was measured in triplicate.

Tap densities of the samples were determined using a Pharma test PT-TD1 (Copley Scientific, Switzerland). The powder density (ρ) was measured after 2500 taps because preliminary results have shown that there was no change in the volume after this number of taps. Measurements were performed in duplicate.

The theoretical aerodynamic diameter of the particles (d_{aer}) was calculated from the particle size and tap density using the following equation [12, 18]:

$$d_{aer} = d \cdot \left(\frac{\rho}{\rho_1} \right)^{\frac{1}{2}}$$

where $\rho_1 = 1 \text{ g/cm}^3$.

2-4 Scanning Electron Microscopy

The morphology of the particles was evaluated by scanning electron microscopy (SEM) using a LEO 1530 (LEO Electron Microscopy Inc, Thornwood, NY) operating between 1 kV and 3 kV with a filament current of about 0.5 mA. Samples were deposited on carbon conductive double-sided tape (Euromedex, France) and dusted to remove the excess. They were sputter-coated with a layer of palladium-platinum alloy of about 4nm (Cressington sputter-coater 208HR) before analysis.

2-5 Aerodynamic properties

The aerodynamic properties of the powders were assessed using a multi-stage liquid impinger (MSLI, Apparatus C, European Pharmacopoeia 2000, Copley Scientific, Switzerland) through a dry-powder, breath-activated inhaler device (Aerolizer[®], Novartis). Twenty milliliters of water were added into each of the four stages of the impinger. A size 4 capsule containing the sample was placed into the inhaler device. The capsule was punctured and the powder was delivered at a flow rate of 60 L/min for 4 s to simulate an inspiration. Depending on the sample, this process was repeated at maximum 10 times to ensure adequate quantities of powder on each stage for dosing. Particles were separated between the stages based on their aerodynamic diameters. The cut-off diameters were comprised between 3.1-6.8 μm , 1.7-3.1 μm and < 1.7 μm for stages 3, 4 and filter, respectively. The amount of powder deposited on the impinger stages, as well as the amount retained by the filter was assayed spectrophotometrically by derivatization of the samples using the o-phthalaldehyde reagent which forms a UV-absorbing derivative with sodium alendronate at 333 nm [19]. Stock solutions of each sample were prepared by dissolving 50 mg of the powder in 50 mL of 0.2 M NaOH. Calibration curves were prepared in the range of 10-60 $\mu\text{g}\cdot\text{mL}^{-1}$ by adding aliquots of the stock solutions in the 50 mL volumetric flasks. Four milliliters of the derivatizing agent were added to each flask and the volume was completed to 50 mL with 0.2 M NaOH. The absorbance values were measured after 30 min at 333 nm. The solvent of each stage was collected, the filter was rinsed with water and the respective volumes were transferred to 50 mL volumetric flasks. Then, 4 mL of derivatizing reagent were added and the volume was completed with 0.2 M NaOH. After 30 minutes, the absorbance was measured at 333 nm. The fine particle fraction (FPF) corresponds to the percentage of powder with an aerodynamic diameter lower than 5 μm , which expresses the fraction of powder reaching the deep lung. The alveolar fraction (AF) was calculated in percent by dividing the dose collected on the fourth stage and the filter by the initial mass in the capsule. The emitted fraction (EF) defined as the percent of total loaded powder mass exiting the capsule was determined gravimetrically and calculated using the following equation:

$$EF = \left(\frac{m_{\text{full}} - m_{\text{empty}}}{m_{\text{powder}}} \right) \cdot 100$$

where m_{full} and m_{empty} are the masses of the capsule before and after simulating the inhalation and m_{powder} the mass of the powder.

2-6 In vivo lung toxicity evaluation

Male Wistar rats weighing 280-350 g were used (Biotério Central, UFRGS, Brazil). The protocol has been approved by the Ethical Committee of the *Universidade Federal do Rio Grande do Sul* (protocol number 2006637). After arrival, animals were kept in separate cages at maximum 5 animals per cage, and maintained on a diet of tap water and standard laboratory rat food until the beginning of the experiments.

On the day of the experiments, animals were divided into four groups of six, and were intraperitoneally anesthetized with urethane (1.25 g/Kg) before the experiments. The trachea was exposed and an incision was made between the fifth and sixth tracheal rings caudal to thyroid cartilage. The rats were maintained at an angle of 70° and animal body temperature was maintained at 37°C with a 40 W incandescent heat lamp. The following treatments were administered intratracheally: (i) insufflation of 10

mL air (negative control group); (ii) instillation of 0.1% sodium dodecyl sulfate aqueous solution (100 µL, positive control group); instillation of 5.0 g/mL sodium alendronate aqueous solution (2.0 mg/Kg); and insufflations of sodium alendronate dry powder (Sample 4, 2.0 mg/Kg). In the case of insufflation, the dry powder was put in the body of a needle and dispersed in the incision of rat trachea by releasing air compressed in a syringe. The amount of dry powder administered was calculated by subtracting the needle weight after administration from before administration. In the case of instillation, the solution was injected into the lungs through the needle of a calibrated syringe. Four hours after treatment administration, animals were sacrificed and bronchoalveolar lavage was performed by cannulating the trachea and infusing lungs 3 times with 3 mL physiological saline. The bronchoalveolar lavage fluid (BALF) was centrifuged (1000 rpm, 10 min, 4°C) and the supernatant was analyzed for lactate dehydrogenase activity (LDH) and total protein content using commercial kits (Roche Diagnóstica®). Preliminary studies have shown that the 4 hours time point was relevant to assess membrane toxicity (data not shown). Statistical analysis was performed using one way analysis of variance (ANOVA). When the differences in the means were significant, Tukey test was used to compare the treatments. The significance level was set at $P < 0.01$.

2-7 Lung bioavailability evaluation

Four male mixed-breed dogs weighing 6-12 Kg were used (Biotério Central, UFSM, Brazil). The protocol has been approved by the Ethical Committee of the *Universidade Federal de Santa Maria* (protocol number 23081.003008/2010-51). After arrival, animals were kept in individual cages and maintained on a diet of tap water and standard laboratory dog food until the beginning of the experiments.

All the animals were fasted for 18 h before the experiments but they were allowed free access to water. On the day of the experiment, dogs were intravenously administered with a saline solution of sodium alendronate (2.0 mg/Kg) via the jugular vein. For the intratracheal administration, the same animals were previously anaesthetized with an intravenous injection of propofol (4 mg/Kg) and a tube was inserted through the throat in order to make an insufflation of the sodium alendronate dry powder (Sample 4, 2.0 mg/Kg) directly into the trachea, using the same device described above. After the administrations, dogs were returned to cages for 36-hr urine collection via catheter inserted in the urethra. Total urinary excretion of sodium alendronate following i.v. and intratracheal administrations was assayed by an HPLC method with fluorescence detection [20]. Briefly, sodium alendronate was extracted by using diethylamine solid phase extraction (Bond Elut®-DEA, Varian, Brazil). The sample was precipitated with sodium hydroxide and derivatized with FMOC in sodium carbonate buffer at pH 11.9. Separation was performed on a Gemini C18 column (150 x 4.6 mm, 5 µm particles, Phenomenex, Brazil), using a gradient method starting with mobile phase acetonitrile/methanol-citrate/pyrophosphate buffer (28:72, v/v). The fluorometric detector was operated at 260 nm (excitation) and 310 nm (emission). Sodium alendronate bioavailability was calculated using the following equation:

$$F = \frac{D_{iv}}{D_{it}} \cdot \frac{A_{it}}{A_{iv}} \cdot 100$$

where D_{iv} and D_{it} are the doses and A_{iv} and A_{it} are the total milligrams of sodium alendronate excreted in the 36-hr urine following intravenous and intratracheal administration, respectively. Comparisons were performed using one way analysis of

variance (ANOVA) and Student's *t* test and the differences were considered significant at $P < 0.05$.

3- Results and discussion

Aqueous ethanol spray-drying feedstocks containing sodium alendronate in the presence of leucine as a dispersibility enhancer and ammonium bicarbonate as a pore-forming agent were prepared with ethanol concentration of 40% v/v. It has been accepted that the inclusion of a nonaqueous solvent in the feedstock is advantageous because generally both the outlet temperature and the yield increase whereas the moisture content and particle size decrease [21]. Since sodium alendronate presents high water solubility, solutions with ethanol concentration higher than 40% are not stable due to the precipitation of the drug.

Previous studies have demonstrated that leucine is a useful additive for dry powders designed for pulmonary delivery due to the improvement of the aerosolization properties [22-27]. The effect of leucine on the aerodynamic properties is often explained by its distinct hydrophilic and lipophilic regions, which leads to an accumulation of leucine molecules at the air-water interface [27-29]. In this study, a total of six samples were prepared with leucine concentration ranging between 5% and 20%, except for sample 1 which was prepared with sodium alendronate only and sample 2 which was prepared without leucine.

The volume median geometric diameter of all samples varies from 4.8 to 11.7 μm (Table 1). Regarding the literature, a large number of factors and process conditions can influence particle size such as nozzle diameter, inlet temperature, air flow rate, feed rate, feedstock concentration, non-aqueous solvent and excipient materials [27]. In this work, we observed that an increase of leucine concentration in the formulations led to an increase of particle diameter, despite the fact that all formulations were prepared with the same solid content (2 g/L) and using the same operating conditions. Increasing the concentration of leucine above 10% results in doubling particle size. This effect is totally different from the one observed with similar formulations, in which a reduction of particle size with increasing leucine concentration was observed [26]. The slight hydrophobicity of leucine, which has a tendency to stand at the air/liquid interface reducing the size of the droplets produced at the spray-drying nozzle, was used to explain particle size reduction. However, the study of Gervelas et al. used high ethanol concentration (70% v/v), whereas here ethanol volume fraction was only 40% v/v. Spray-drying solutions with different ethanol water ratios present distinct surface tension, vapor pressure and viscosity, which will influence the size of the droplet produced during atomization, and thus the size of resultant dried particle. Taking into account that leucine presents a low water solubility, (22 mg/mL), and even a lower one in ethanol [30-31], leucine surface activity probably differs for each solvent mixture as previously suggested by others [25]. Moreover, sodium alendronate can interact with leucine affecting its surfactant-like properties as well as changing its initial saturation in the droplet.

Sample	SA (g/L)	Leu (g/L)	AB (g/L)	d (μm)	ρ (g/cm^3)	d_{aer} (μm)
1	2.0	0.0	0.0	7.7 ± 1.0	0.101	2.29 ± 0.26
2	2.0	0.0	5.0	4.9 ± 0.8	0.062	1.10 ± 0.06
3	1.9	0.1	5.0	4.8 ± 0.3	0.041	0.93 ± 0.10
4	1.8	0.2	5.0	5.4 ± 0.4	0.042	1.06 ± 0.06
5	1.7	0.3	5.0	8.0 ± 0.2	0.024	1.22 ± 0.09
6	1.6	0.4	5.0	11.7 ± 1.4	0.016	1.42 ± 0.14

Table 1. Composition and characteristics of sodium alendronate spray-dried powders. SA=sodium alendronate, Leu=leucine, AB=ammonium bicarbonate, d =volume median geometric diameter (dispersing pressure of 1 bar), ρ =tap density, d_{aer} =aerodynamic diameter. Data are represented as mean \pm S.D.

The tap density data are showed in Table 1. Sodium alendronate spray-dried alone (sample 1) presents a tap density of $0.101 \text{ g}/\text{cm}^3$. When ammonium bicarbonate is added in the formulation, the tap density value decreases down to $0.062 \text{ g}/\text{cm}^3$, proving the pore forming properties of this excipient (sample 2). This is further confirmed by SEM images of samples 1 and 2: particles of sample 2 are obviously more porous than particles of sample 1 (Figure 1). When ammonium bicarbonate is added in the formulation, the tap density values range between $0.016 \text{ g}/\text{cm}^3$ and $0.062 \text{ g}/\text{cm}^3$ diminishing with the raise of leucine concentration. A low density is much more desirable for powders designed for pulmonary delivery because it improves dispersibility and delivery efficiency [32]. It has been demonstrated recently that the addition of ammonium bicarbonate to formulations intended for inhalation reduces their density by formation of porous particles [26]. A sodium alendronate formulation prepared with 20% leucine, but without ammonium bicarbonate was spray-dried under the same operating conditions and showed a tap density of $0.584 \text{ g}/\text{cm}^3$ (data not shown) demonstrating the influence of ammonium bicarbonate on powder density. The laser diffraction and tap density data were used to calculate the theoretical aerodynamic diameter (d_{aer}). For all formulations the d_{aer} are lower than $5 \mu\text{m}$ (Table 1), suggesting that the powders are suitable for deposition in the deep lungs.

Figure 1 shows the scanning electron micrographs of the dry powders. As a general rule, all samples are spherical in shape and possess a hollow structure. Particles of sodium alendronate (sample 1) present a smooth surface without pores. In contrast, with the addition of leucine and ammonium bicarbonate, particles surface becomes rougher and porous confirming the tendency of leucine to enhance surface roughness and the ability of ammonium bicarbonate to form large pores or voids. It was previously demonstrated that an increase of surface roughness reduces the cohesion between particles and therefore improves powder aerosolization properties [23, 25-26].

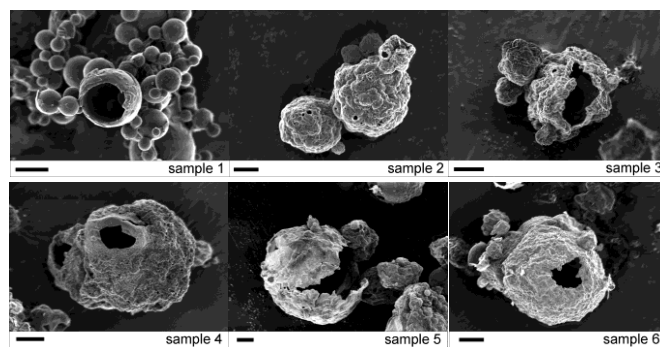


Figure 1. SEM images of typical particles from the different formulations of sodium alendronate spray-dried powders. Scale bars represent 2 μm on all images.

However, the formulations present a tendency to form aggregates which could increase the values of particle sizes. To confirm this hypothesis, the ratio between the volume median geometric diameter of particles measured at different dispersing pressures (1, 2 and 3 bars) and the volume median geometric diameter of particles measured at a dispersing pressure of 1 bar $d(\text{pressure})/d(1\text{bar})$ for each powder was calculated (Figure 2). All samples show a decrease of the $d(\text{pressure})/d(1\text{bar})$ values as the dispersing pressure increases. For samples 2, 3 and 4, results suggest the presence of a few aggregates. On the other hand, for sample 1, results show that aggregates are numerous and need higher pressure to be individualized since the volume median geometric diameter decreases by 50% at 3 bars. Similarly, samples 5 and 6 present particles that are either aggregated or very fragile and break as dispersion pressure increases.

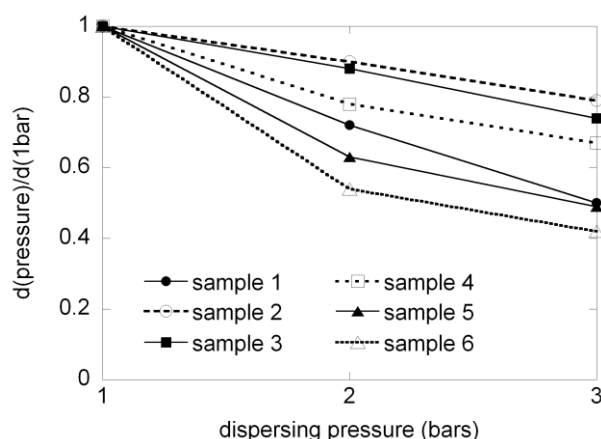


Figure 2. Evolution of the ratio of the volume median geometric diameter over the volume median geometric diameter measured at 1 bar $d(\text{pressure})/d(1\text{bar})$ as a function of the dispersing pressure.

In order to verify the *in vitro* aerosolization properties, powders were tested on a MSLI. Table 2 displays the values obtained for EF, FPF and AF for each sample. All powders present a high emitted fraction of 100%. In spite of the excellent emitted fraction, there is a considerable variation in FPF and AF values. The FPFs of the powders range from 34.4% to 62.0% and the AF values range from 23.7% to 42.6%. Since analyses were carried out at least three times with different batches, and under the same operating conditions, differences observed in FPF and AF can be related to the different proportions of leucine. Sample 4, prepared with 10% leucine, displays the best aerosolization properties with a FPF around 80% and an AF around 42%. Contrarily to previous results that demonstrated an improvement of

the aerosolization properties with an increase of leucine content [22, 25-26] samples 5 and 6 showed lower values of FPF and AF than sample 4. Doubling leucine concentration (sample 6) causes approximately a 50% reduction in the FPF and AF values. This result may be due to the presence of lot of aggregates which deposit via inertial impaction higher in the MSLI, leading to a reduced FPF and AF. Since sample 4 possesses the best aerodynamic properties, this formulation was chosen to evaluate sodium alendronate powder toxicity following pulmonary administration.

Sample	EF (%)	FPF (%)	AF (%)
1	101.5 ± 2.3	46.6 ± 3.7	30.5 ± 2.8
2	100.3 ± 1.5	53.1 ± 10.2	34.7 ± 8.3
3	100.7 ± 1.1	42.2 ± 7.2	26.2 ± 9.8
4	100.0 ± 0.4	62.0 ± 5.5	42.6 ± 8.6
5	100.1 ± 0.3	48.9 ± 1.7	39.7 ± 3.0
6	100.1 ± 0.1	34.4 ± 1.4	23.7 ± 1.8

Table 2. Aerodynamic evaluation of sodium alendronate spray-dried powders. EF=emitted fraction, FPF=fine particle fraction, AF=alveolar fraction. Data are represented as mean±S.D.

Given that sodium alendronate treatment has been associated with topical irritant effects following oral, subcutaneous and intramuscular administration [8, 10, 33], it seemed relevant to evaluate whether the pulmonary delivery of the drug could lead to lung damage. Bronchoalveolar lavage studies have proven to be useful as a rapid method to investigate damage of the airways epithelial cells by the analysis of biochemical content of BALF [34]. High protein content in the lavage fluid is associated with alterations in the permeability of alveolar-capillary barrier [34]. LDH, an indicator of cell damage or inflammation, is a cytoplasmic enzyme whose extracellular presence indicates cell death [35]. In this work, the anionic surfactant sodium dodecyl sulphate (SDS) was used as a positive control because of its harsh effect on biological membranes. The acute toxicity of sodium alendronate was compared with SDS by determining the changes in the markers of lung injury in BALF.

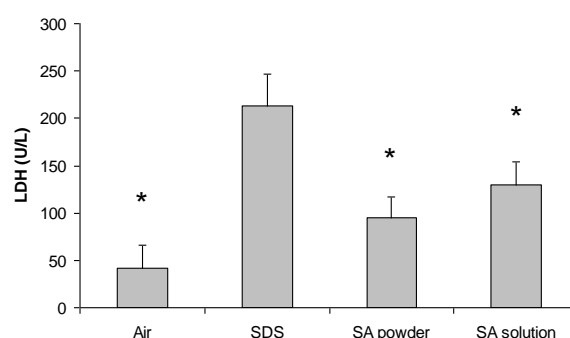


Figure 3. Lactate dehydrogenase activity (LDH) in the bronchoalveolar lavage fluid 4 hours after intratracheal administration of air, sodium dodecyl sulfate (SDS), sodium alendronate dry powder (SA powder) and sodium alendronate solution (SA solution). The asterisk (*) denotes significant differences as compared with SDS.

The LDH activities with sodium alendronate dry powder, sodium alendronate solution and negative control group are significantly lower than those with the positive control group ($P < 0.01$) (Figure 3). SDS administration produces a five-fold increase in LDH levels 4h after the treatment as compared to the negative control. It is important to note that groups treated with sodium alendronate present enzymatic activities 2.2-

fold and 1.6-fold lower than the positive control group, respectively. Comparing both treated groups, no significant difference was observed ($P = 0.143$). Regarding total protein, the positive control group shows protein levels three-fold greater than the negative control group suggesting lung injury. On the other hand, sodium alendronate treated groups show significant decrease in this marker as compared to SDS ($P < 0.01$) (Figure 4). Regardless of the formulation (dry powder or solution), no significant increase of indicators for acute lung injury seem to be induced by sodium alendronate intratracheal administration.

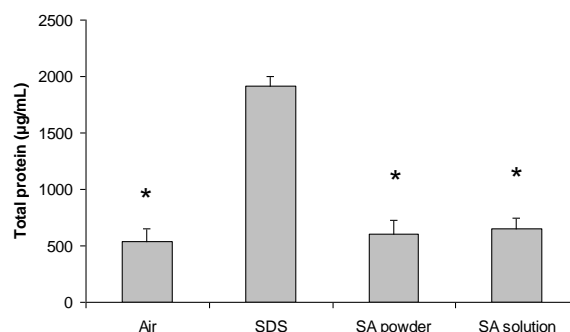


Figure 4. Total protein content in the bronchoalveolar lavage fluid 4 hours after intratracheal administration of air, sodium dodecyl sulfate (SDS), sodium alendronate dry powder (SA powder) and sodium alendronate solution (SA solution). The asterisk (*) denotes significant differences as compared with SDS.

Then, we evaluated whether the dry powder was able to promote the lung absorption of sodium alendronate. Sodium alendronate was therefore administered either intravenously as a solution, or insufflated in dog lungs as a powder (sample 4). The total dose of sodium alendronate excreted in the 36-hr urine following intravenous and intratracheal was used to calculate the lung bioavailability. The administration of the powder results in a $6.23 \pm 0.83\%$ bioavailability. The incomplete absorption could be explained by the high pharyngeal deposition and the hydrophilicity of the drug, combined with the negative charge of sodium alendronate. In contrast, when sodium alendronate was administered to dogs by oral route the bioavailability was only of $1.76 \pm 0.07\%$ [36]. Thus, the absorption of sodium alendronate by lungs was 3.5-fold greater than by oral route. This substantial improvement of sodium alendronate bioavailability arises from the good alveolar deposition of the powder. In alveoli, the epithelium is very thin around a few hundreds of nanometers and even a charged molecule such as sodium alendronate is able to diffuse into the blood stream [37]. These results confirm the pulmonary route is a promising for sodium alendronate delivery.

4 Conclusion

A dry powder prepared by spray-drying offers great promise as a formulation for the lung delivery of sodium alendronate. The optimized formulation developed in this study exhibits good *in vitro* aerosolization properties. The bronchoalveolar lavage study shows that the intratracheal administration of sodium alendronate dry powder and sodium alendronate aqueous solution does not induce significant increase of lung toxicity markers as compared with the positive control. Moreover, the pulmonary administration of sodium alendronate dry powder leads to a lung bioavailability 3.5-fold greater than oral administration. These results suggest that the pulmonary delivery of sodium alendronate could be a new and safe administration route.

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