Pharmacokinetics and biodistribution of amikacin encapsulated in carrier erythrocytes

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Objectives: To study the changes in the pharmacokinetics and tissue distribution of the aminoglycoside amikacin in rats using amikacin carrier erythrocytes as a delivery system.

Methods: Amikacin-loaded erythrocytes were obtained using a hypotonic dialysis method. The pharmacokinetic and tissue distribution of amikacin were studied in three groups of rats receiving intravenous amikacin in saline solution, amikacin-loaded erythrocytes and amikacin-loaded erythrocytes treated with glutaraldehyde. Pharmacokinetic analysis was accomplished using model-independent methods.

Results: Administration of the antibiotic using carrier erythrocytes elicited a sustained release effect, with an increase in the plasma half-life and in the area under the curve of the antibiotic. The tissue pharmacokinetics of amikacin using carrier erythrocytes in comparison with a control group revealed an accumulation of the antibiotic in specific tissues such as the liver and spleen, a similar pharmacokinetics in the lung and moderate changes in the pharmacokinetics in the kidney. Studies of tissue concentrations after the injection of glutaraldehyde-treated loaded erythrocytes demonstrated important changes in organs of the reticulo-endothelial system (RES) in comparison with the results observed for standard carrier erythrocytes, higher levels being observed in the liver whereas spleen levels decreased.

Conclusions: The administration of amikacin in loaded erythrocytes in rats leads to significant changes in the pharmacokinetic behaviour of the antibiotic, a greater accumulation being observed in RES organs such as liver and spleen. This shows that loaded erythrocytes are potentially useful for the delivery of antibiotics in phagocytic cells located in the RES.

Keywords: aminoglycosides, tissue distribution, loaded erythrocytes

Introduction

Carrier erythrocytes constitute potential biocompatible vectors for different bioactive substances, including drugs and proteins. The use of erythrocytes as biological carriers offers an alternative to other carrier systems, such as liposomes or nanoparticles, used for the encapsulation of different drugs, enzyme systems and peptides with therapeutic activity.1,2

Currently, several methods are used to encapsulate drugs and other substances in erythrocytes. Among the different methods of encapsulation available, osmosis-based methods are those most widely used for the preparation of carrier erythrocytes; in particular, hypotonic dialysis methods based on the encapsulation of drugs under conditions of reduced osmotic pressure.3

The main advantages of carrier erythrocytes are that they act as a true drug delivery system, with changes in the kinetic properties of the substances encapsulated, and that they achieve a selective distribution to different organs and tissues, especially the phagocytic cells of the reticulo-endothelial system (RES).4-6

Some pathogens are able to live and multiply inside phagocytic cells, especially macrophages, causing intracellular infections.7 Aminoglycoside antibiotics such as amikacin are potentially active against intracellular infections caused by Gram-negative microorganisms, but the penetration capacity of the free drug into phagocytic cells is low, and these cells then act as a reservoir for facultative intracellular pathogens.8 This observation has prompted research into aminoglycoside antibiotics delivery systems such as liposomes9 and nanoparticles.10
The selective accumulation of therapeutic agents such as antibiotics in phagocytic cells (e.g. macrophages) by the use of carrier erythrocytes allows selective transport of antibiotics to the mononuclear phagocytic system. In vitro and in vivo research studies in the field of antibiotics with this kind of carrier are limited. Some studies have been performed on drug encapsulation and pharmacokinetics with gentamicin-loaded carrier erythrocytes.11

In previous studies, we developed an optimized method of hypotonic dialysis for the encapsulation of amikacin in rat erythrocytes.12 The aim of the present work was to study the pharmacokinetics and tissue distribution of amikacin in the rat model using amikacin-loaded erythrocytes administered by intravenous (iv) route.

Materials and methods

Encapsulation of amikacin in erythrocytes

The encapsulation of amikacin in rat erythrocytes was accomplished using a previously described hypotonic dialysis method.12 Briefly, fresh blood was obtained from male Wistar rats by retro-orbital puncture, using K2-EDTA (1.5 mg/mL) as anticoagulant. Plasma was removed after centrifugation of the blood (600 g, 5 min, 4°C); packed erythrocytes were washed twice with isotonic Hanks–PBS buffer (pH 7.4) and then resuspended in the same buffer to provide a suspension of 70% hematocrit. A 1 mL aliquot of the cell suspension containing amikacin (0.043 mM) was placed in a dialysis bag (Medicell, molecular size cutoff, 12–14 kDa). The dialysis membrane was previously activated by immersion in the dialysis buffer. Dialysis was performed against 50 mL of hypotonic buffer (15 mM NaH2PO4·2H2O, 15 mM NaHCO3, 20 mM glucose, 2 mM ATP, 3 mM glutathione reduced and 5 mM NaCl, pH 7.4) for 45 min at 4°C. The hypotonic dialysis phase was conducted using a hypotonic buffer with a mean osmolality ~90 mOsm/kg. Resealing was achieved by subsequent dialysis against 20 mL of a hypertonic (500 mOsm/kg) buffer (250 mM NaCl, 12.5 mM sodium pyruvate, 12.5 mM inosine, 12.5 mM glucose, 12.5 mM NaH2PO4·2H2O and 0.63 mM adenine, pH 7.4) for 15 min at 37°C. Resealed erythrocytes were washed twice with isotonic Hanks–PBS buffer (pH 7.4). The osmolality of the buffers employed for hypotonic dialysis and resealing was measured using an OSMOSTAT OM-6020 osmometer.

In an additional study aimed at increasing RES targeting, some experiments with cross-linking reagents were performed. With this goal, erythrocytes from another group of rats were processed as usual for amikacin encapsulation, and after resealing, the cells were suspended (1/5 v/v) in isotonic saline with a 0.1% glutaraldehyde solution. After incubation for 10 min at 22°C, they were washed twice and pharmacokinetic experiments were carried out.

Pharmacokinetic experiments

The housing and experimental treatment of the animals were in accordance with current Spanish and European Union legislation and complied with the ‘Principles of Laboratory Animal Care’.

For the study of amikacin pharmacokinetics, 66 Wistar male rats with a weight of 260.98 ± 27.12 g were used. They were divided into three groups, each group receiving a different kind of iv injection via the femoral artery. Group A (control group, n = 30) received a bolus of free antibiotic in saline solution (7.5 mg/kg dose), group B (n = 30) received an injection of 1 mL of amikacin-loaded erythrocytes and group C (n = 6) received an injection of 1 mL of amikacin-loaded erythrocytes treated with glutaraldehyde. The mean content of amikacin in the carrier erythrocytes was 0.68 ± 0.28 mg/mL.

Before sacrifice, the animals received 100 IU of sodium heparin via the femoral artery. Rats were sacrificed at fixed times under anaesthesia with sodium thiopental (all assays were carried out in triplicate), and blood, lungs, spleen, liver and kidney (renal cortex and medulla) were removed for the analysis of amikacin contents. Blood was collected and the plasma was separated by centrifugation.

In groups A and B, rats were sacrificed at the following times: 0.083, 0.25, 0.5, 1, 2, 4, 6, 12, 24 and 48 h. In group C, rats were sacrificed 6 and 12 h after administration.

Amikacin quantification

Amikacin concentrations in loaded erythrocytes, plasma and tissues were determined using an HPLC technique based on pre-column derivatization with o-phthaldialdehyde (OPA) and fluorescence detection.13 Treatment of the samples, prior to their chromatographic separation, was as follows: 75 μL of isotonic buffer plus 25 μL of sample were added to 100 μL of TCA (20%). This mixture was vortexed for 30 s and then centrifuged for 5 min at 5000 g. Following this, the supernatant was removed, 100 μL of 1 M NaOH plus 1 mL of phosphate buffer, pH 11, was added and the mixture was stirred for 30 s. Then, 2 mL of methylene chloride was added, the mixture was shaken for 10 s, centrifuged for 5 min and the aqueous phase was collected. One milliliter of OPA reagent was added to the latter. The mixture was shaken, 500 mg of anhydrous sodium carbonate was added and shaking was continued for 30 s. Finally, 500 μL of isopropanol was added to extract the derivatized amikacin, after centrifuging for 5 min at 5000 g, and 20 μL of the isopropanol extract was injected into the chromatograph. Chromatographic analysis was performed using a Shimadzu LC 10 AD chromato graph using an ion-pair technique and a LiChroCART® 55–4 (Purospher RP-18e, 3 μm) column. The mobile phase was methanol (64%) and aqueous EDTA solution (2.2 g/L, 36%). Flow rate was 1 mL/min. Fluorescence detection was accomplished at excitation and emission wavelengths of 360 and 435 nm, respectively.

The technique was exact (RSD recuperation < 10%) and accurate (RSD area < 15%) for the concentration ranges used.

Pharmacokinetic analyses

The tissue distribution of amikacin administered as an aqueous solution or encapsulated in carrier erythrocytes was characterized by means of numerical deconvolution and conventional model-independent analysis.

Considering that drug transference between plasma and tissue is due only to passive processes, and that only the free drug fraction can be transferred, the rate of tissue uptake of a drug in non-steady-state situations can be calculated according to Equation 1 [Eqn (1)].14

\[
\frac{dC_T}{dt} = \frac{k}{V_T} (f_p C_p - f_T C_T)
\]
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where $C_p$ and $C_T$ are the plasma and tissue concentrations, respectively; $f_p$ and $f_T$ are the free fractions of drug in plasma and tissue, respectively; $k$ is an intercompartmental transfer rate constant and $V_T$ the distribution volume of the tissue compartment.

Thus, the following simplified equation can be obtained:

$$\frac{dC_T}{dt} = K(PC_p - C_T)$$  \hspace{1cm} (2)

where $P$ is the partition coefficient of the drug in a specific tissue, defined as the tissue-to-plasma concentration ratio ($C_T/C_p$) at steady-state or the free fraction ratio ($f_p/f_T$) of the drug, and $K$ is a constant defined as follows:

$$K = \frac{Kf_T}{V_T}$$  \hspace{1cm} (3)

Considering a linear and time-invariant system, and integrating Eqn (3) using Laplace transforms, one obtains:

$$C_T(s) = \frac{PK}{s + K}C_p(s)$$ \hspace{1cm} (4)

where $s$ is the Laplace operator.

Laplace anti-transformation of Eqn (4) generates the following convolution integral:

$$C_T = \int_0^K PKe^{-K(t-\tau)}C_p(\tau)d\tau$$ \hspace{1cm} (5)

$$C_T = PKe^{-Kt}C_p$$ \hspace{1cm} (6)

where "*' indicates the convolution operation.

Deconvolution "//" between $C_T$ and $C_p$ yields a unit disposition function (UDF) corrected by $PK$. The UDF represents the intratissue disposition of a unit amount of drug instantaneously injected into a tissue without recirculation.\(^\text{15}\)

$$\text{UDF} = C_T//C_p$$ \hspace{1cm} (7)

Estimation of UDF was accomplished using numerical deconvolution, carried out using polyexponential functions and the PCDCON programme.\(^\text{16}\)

Additionally, model-independent pharmacokinetic parameters in plasma and tissues, such as the area under the curve (AUC$_{0-\infty}$), the apparent plasma or tissue terminal half-life ($t_{1/2\alpha}$), the mean residence time (MRT) and the mean transit time (MTT), were also calculated. MTT in tissues was calculated as the difference between the MRT in a specific tissue and the plasma MRT.\(^\text{17,18}\)

Results

Figures 1 and 2 show the amikacin levels in plasma and different tissues standardized by the mean dose after the administration of amikacin as a solution and in amikacin-loaded erythrocytes. Non-parametric statistical analysis of the concentrations revealed statistically significant differences in liver and spleen ($P < 0.001$) and in renal cortex ($P < 0.05$).

Table 1 shows a comparison between the two groups of the amikacin-estimated AUC$_{0-\infty}$, $t_{1/2\alpha}$, MRT and MTT in plasma and in different tissues after administration of the antibiotic in aqueous solution and when incorporated in carrier erythrocytes.

The modifications in the tissue pharmacokinetics and mean transit time were reflected in the profile of the UDF obtained by numerical deconvolution and are shown in Figure 3.

Figure 4 shows the amikacin concentrations observed in spleen and liver in rats receiving glutaraldehyde-treated carrier erythrocytes in comparison with the 6 and 12 h concentrations for the control and carrier erythrocyte groups.

Discussion

The administration of amikacin in autologous loaded erythrocytes in rats demonstrated that the encapsulation in erythrocytes significantly changed the pharmacokinetic behaviour of amikacin both in plasma and in specific tissues. These changes involved a prolonged plasma half-life and an increase in the area under the curve of plasma concentrations of the amikacin encapsulated in erythrocytes in comparison with the free antibiotic.

The encapsulation of many drugs, including antibiotics, in carrier erythrocytes is able to give rise to a sustained release of the drug that affects the in vivo pharmacokinetic behaviour of the loaded drugs.\(^\text{20}\) Previous in vitro studies addressing the release of amikacin from carrier erythrocytes in autologous plasma have shown that most of the encapsulated amikacin remains retained in erythrocytes for long periods of time\(^\text{12}\) and this allows the prediction of an in vivo sustained release of the antibiotic from the erythrocytes, together with possible RES targeting when the loaded erythrocytes are phagocytosed. The sustained release of the antibiotic to the plasma can be accounted for in terms of the notion that amikacin is a polar drug and, once entrapped inside the erythrocyte, it does not undergo processes of diffusion across the cell membrane owing to its polar nature. Thus, release must occur through a process of cell lysis, as happens with other aminoglycosides.\(^\text{11}\)

The tissue pharmacokinetics of amikacin incorporated in carrier erythrocytes in comparison with the control group showed a higher accumulation of the antibiotic in the liver and in the spleen, a similar pharmacokinetics in the lung and moderate changes in the pharmacokinetics in renal cortex and medulla, as shown in Table 1. A dramatic increase in amikacin levels in liver and spleen can be seen in Figures 1 and 2. A previous study examining the pharmacokinetics of gentamicin in rats using carrier erythrocytes also revealed changes in the pharmacokinetics of the antibiotic incorporated in this biological delivery system. Such changes involved a higher half-life and mean retention time and a higher accumulation of the antibiotic in liver and spleen.\(^\text{20}\) These are two important organs of the immune system. The liver contains many phagocytic cells, which capture bacteria from the blood when it passes through

Statistical analyses

The statistical curve-to-curve comparison between normalized plasma and tissue levels of amikacin in different groups of rats was carried out with non-parametric analysis (Kruskal–Wallis test), using the SPSS 14.0.1 statistical software.\(^\text{19}\)
this organ. The spleen also contains phagocytic cells, both lymphocytes and monocytes. Therefore, the increased levels of amikacin observed in the liver and spleen of the animals treated with amikacin-loaded erythrocytes in comparison with the control group suggest a higher degree of phagocytosis of carrier erythrocytes by the phagocytic cells of these organs.

One of the main limiting factors regarding the use of aminoglycosides in clinical practice is their nephrotoxicity, because these drugs are excreted through the renal route without previously having undergone any type of metabolism. After glomerular filtration, the drug is reabsorbed and accumulated in tubular cells, causing toxicity through the inhibition of lysosomal phospholipases. The pharmacokinetic profile estimated in this study did not point to striking changes in renal tissues. This points to the absence of a drug accumulation in the renal tissue, in turn suggesting no increases in the nephrotoxicity of amikacin when it is administered in the form of carrier erythrocytes. Moreover, a lower apparent half-life and a shorter mean transit time were observed in renal cortex and medulla in the group receiving carrier erythrocytes. This suggests a potential decrease in nephrotoxicity, owing to the relationship between the toxic effects of aminoglycosides and their accumulation in the deep renal compartment, and as a result, nephrotoxicity seems to depend on the time of exposure to the drug and on the concentrations achieved in the kidney.21,22

The UDF in different tissues obtained by numerical deconvolution reflects the tissue distribution kinetics of the drug after the first passage through the tissue. In organs such as the liver and spleen, the UDF of amikacin was modified when the drug was administered in the form of carrier erythrocytes, exhibiting slow delivery kinetics in this kind of tissues, with an increase in the MTT (Figure 3).

It is also possible to observe a modification in renal cortex and medulla UDF in the carrier erythrocytes group, with a decrease in the MTT in comparison with the control group. This confirms a lower degree of exposure of renal tissue to the aminoglycoside when it is administered in carrier erythrocytes, as mentioned above.

Experiments with glutaraldehyde-treated erythrocytes demonstrated important differences in tissue levels in comparison with

Figure 1. Mean amikacin plasma and tissue levels standardized by the mean dose in the control group.
the control and carrier erythrocyte groups. This was especially significant in the case of RES organs, such as the liver, where higher concentrations than in the carrier erythrocyte group were observed. In contrast, lower antibiotic levels were observed in the spleen, as reported by DeLoach et al. According to the authors, low glutaraldehyde concentrations induce mild cell damages, detected in the spleen, whereas higher concentrations induce important changes in cellular membranes, these changes being responsible for massive removal by the liver due to the much greater blood flow of this organ.

Table 1. Pharmacokinetic parameters of amikacin in groups A (control group) and B (carrier erythrocytes)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$\lambda$ (h$^{-1}$)</th>
<th>$t_{1/2}\lambda$ (h)</th>
<th>AUC$_{0-\infty}$ (µg·kg·h/g·mg)$^b$</th>
<th>MRT (h)</th>
<th>MTT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.93E-01</td>
<td>8.05E-02</td>
<td>0.87</td>
<td>8.61</td>
<td>3.84</td>
</tr>
<tr>
<td>Liver</td>
<td>9.40E-03</td>
<td>2.77E-03</td>
<td>73.72</td>
<td>250.18</td>
<td>29.70</td>
</tr>
<tr>
<td>Spleen*</td>
<td>1.95E-02</td>
<td>1.23E-02</td>
<td>35.54</td>
<td>56.34</td>
<td>18.00</td>
</tr>
<tr>
<td>Lung</td>
<td>3.50E-02</td>
<td>3.35E-02</td>
<td>19.80</td>
<td>20.69</td>
<td>14.87</td>
</tr>
<tr>
<td>Renal cortex*</td>
<td>5.30E-03</td>
<td>9.10E-03</td>
<td>130.78</td>
<td>76.15</td>
<td>2051.77</td>
</tr>
<tr>
<td>Renal medulla</td>
<td>3.73E-02</td>
<td>5.60E-02</td>
<td>18.58</td>
<td>12.38</td>
<td>76.89</td>
</tr>
</tbody>
</table>

$^a$Statistically significant differences between drug concentrations of the control and carrier erythrocyte groups ($P < 0.05$).

$^b$Statistically significant differences between drug concentrations of the control and carrier erythrocyte groups ($P < 0.001$).

*Terminal phase slope.

Plasma units: (µg·kg·h/mL·mg).
The selective uptake of carrier erythrocytes by phagocytic cells\(^2\) and the higher accumulation of the drug in RES organs such as the liver and spleen when amikacin is incorporated into erythrocytes show that carrier erythrocytes can be used as alternatives to other delivery systems, such as liposomes, and that they are potentially useful for intracellular infections caused by aminoglycoside-sensitive germs.

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**Transparency declarations**

None to declare.
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References


