Pharmacokinetic characterization of phosphatidylinerse liposomes in the rat

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1 The plasma decay, tissue uptake and biotransformation of radiolabelled phosphatidylinerse (PS) liposomes have been investigated in rats following bolus i.v. injection (2 mg kg⁻¹).
2 PS plasma concentration showed a biexponential decay with half-lives of 0.85 and 40 min. The following interpretation of the biphasic decay is proposed: (1) The rapid initial decline is due to the irreversible uptake of PS liposomes by the mononuclear phagocyte system, as demonstrated by the almost exclusive accumulation of PS in liver and spleen. (2) The slow decay phase reflects the elimination of that fraction of PS that has been internalized and the subsequent reincorporation into plasma lipoproteins (HDL). A kinetic model has been developed to describe these phenomena and a good agreement has been observed between experimental data and theoretical values.
3 Evidence has been obtained that a large fraction of PS is hydrolyzed at the injection site, probably by phospholipase A₂ and other hydrolytic enzymes released by platelets. Hydrolysis at the injection site has also been observed following intraperitoneal and intramuscular injections.
4 As shown by the comparative analysis of the biotransformation products found in tissues after administration of either [³H]-glycerol-PS or [¹⁴C]-serine-PS, parenterally administered PS follows two distinct metabolic pathways: (1) decarboxylation to phosphatidyethanolamine and (2) extensive hydrolytic degradation with release of the individual components of the molecule. These pathways probably reflect the two main mechanisms of PS uptake, incorporation into the plasma membrane and internalization by endocytosis, respectively.

Introduction

Our initial studies (Bruni et al., 1976; Bigon et al., 1979a,b) have shown that parenteral administration of phosphatidylinerse (PS) to rodents induces complex pharmacological effects. These are believed to reflect a pathophysiological role of PS and its lysodervative in the immune and inflammatory responses (Mietto et al., 1987). Consistently, macrophages, lymphocytes and mast cells have been shown to be target cells for serine phospholipids in vivo and in vitro. The interaction of PS with macrophages appears to be mediated by the scavenger receptor (Nishikawa et al., 1990). As a consequence, cells exposing PS at the external membrane surface are internalized and eliminated by macrophages (Tanaka & Schroit, 1983; Schroit et al., 1985). PS uptake is not without consequences for macrophages as it has been demonstrated that ingestion of liposomes containing this phospholipid reduces the microbicidal activity against intracellular leishmanias (Gilbreath et al., 1986). In lymphocytes, PS is incorporated by an energy-dependent process which might involve endocytosis, internalization by the translocator for the amino-phospholipids or a decaylation-reacclaylation cycle (Mietto et al., 1989). Decreased secretion of interleukin-2 follows PS incorporation (Ponzin et al., 1989). Unlike macrophages and lymphocytes, mast cells undergo activation after the interaction with PS and lysoPS. The secretion of mast cell mediators is the result of phosphoinositide C activation (Bellini et al., 1988; 1990). The in vivo disposition of phospholipid liposomes supports the possibility of an action of PS on the immune system. Several studies have indeed shown that parenteral administration of these lipid structures is followed by their accumulation in organs (liver, spleen, lungs) which are rich in immunocompetent cells (reviewed by Poste, 1983). However, the pharmacokinetic properties of liposomes have been thus far investigated in relation to their use as drug carriers. For this reason only the disposition of long-lived liposomes, such as those containing phosphatidylycholine (PC), sphingomyelin (SPH) or cholesterol as basic components, has been extensively studied (Gregoriadis & Neerunj, 1974; Allen & Everest, 1983; Gabizon & Papahadjopoulos, 1988 and references therein). In spite of their intrinsic pharmacological activity, liposomes made up exclusively of PS have not yet been characterized from a pharmacokinetic point of view. The purpose of this study is to provide essential kinetic data pertaining to the intravenous administration of PS liposomes in the rat.

Methods

Phosphatidylinerse

Conventional preparations of bovine brain PS (Bigon et al., 1979a) were further purified on DEAE-cellulose to remove a small amount of phosphatidylinositol (Rouser et al., 1969). The phospholipid was stored at −20°C as a solution in chloroform-methanol (2:1, v/v) at a concentration of 10–20 mg ml⁻¹, quantified by periodic phosphorus determinations. Phosphatidyl-[¹⁴C]-serine (160 mCi mmol⁻¹) contained 4% of hydroxysoluble radioactivity and 1% of lysoPS (Orlando et al., 1980). Experiments were also done with a preparation of [³H]-glycerol-PS (13 mCi mmol⁻¹), kindly donated by Dr P. Orlando, Catholic University, Rome. The radiochemical purity was 96%. The desired amounts of unlabelled and labelled PS were mixed in organic solvent and taken to dryness under a stream of nitrogen. Hydration of PS in the form of multimellar vesicles was obtained by the addition of 50 mM Tris HCl, pH 7.8 (50 mM HEPES KOH or 10 mM phosphate buffer were equivalent). The liposome size was decreased by 4 cycles of sonication (30s each, tip probe sonicator). The average size of the vesicles was 140 nm, when tested by dynamic light scattering using a nano sizer apparatus (Coulter Electronics). PS vesicles were then used immediately to avoid aggregation.

Experimental protocol

Male albino rats (Charles River, Sprague-Dawley of 250–350 g) were used for this study. In most experiments the PS
dispersion was injected into the exposed jugular vein as a bolus dose of 2 mg kg$^{-1}$, after pentobarbitone anaesthesia (45 mg kg$^{-1}$, i.p.). In some experiments PS was also injected into the tail vein without anaesthesia. Heparinized blood samples (0.2 ml) were removed by heart puncture at 0.5, 1.5, 3, 5, 10, 15, 30, 45 and 60 min after injection. Blood sampling was started as early as 0.5 min after injection since preliminary experiments revealed that the disappearance of PS from plasma was extremely rapid (about 75% removed from plasma within 30 s) and the initial decay phase would have been largely missed if sampling were started at a later time.

The organs were sampled, weighed and extracted 60 min after PS injection. Since at this time only a minimal amount of PS was present in the circulation no attempts were made to remove the blood from tissues completely. Correction of tissue radioactivity levels using the blood background correction factors of Hwang et al. (1980) showed that the contribution to tissue radioactivity from residual blood content was in fact negligible.

Phospholipids were extracted from plasma and tissues immediately after sampling, by 20 volumes of chloroform-methanol (2:1, v/v). The filtered lipid extract was washed with 0.2 volumes of 10 mm CaCl$_2$ and resolved by two dimensional thin layer chromatography (Punzi et al., 1986). The radioactivity of the samples was determined in a Packard Tricarb 2200 CA liquid scintillation system, using the computerized automatic external standard for quench correction.

**Pharmacokinetic analysis**

The plasma decay of PS liposomes after bolus i.v. injection was analyzed by means of the kinetic model depicted in Figure 1. The basic assumption of the model is that exit of PS liposomes out of the plasma compartment is a unidirectional process. With the possible exception of very small unilamellar vesicles (less than 60 nm in diameter) phospholipid liposomes are unable to cross either continuous or fenestrated capillaries (Poste, 1983; Hwang & Beaumier, 1988). Their extravasation is limited to tissues containing sinusoidal capillaries and appears predominantly due to endocytosis by the local macrophages, although fusion with cell membranes, deacetylation-reacylation or interaction with the transporter for the amino phospholipids may also be operative for PS (Bruni & Palatini, 1982; Mietto et al., 1989). After internalization by endocytosis, phospholipid vesicles are degraded by lysosomal enzymes, whereas the other pathways result in the incorporation of liposomal phospholipids into the phospholipid pool of the cellular membrane. A significant incorporation of liposomal phospholipids occurs also in plasma by high density lipoproteins (HDL) (see, e.g. Scherphof et al., 1978; Vidal et al., 1984; William & Tall, 1988). When cholesterol is not included in liposomes (Damen et al., 1981), or the concentration of HDL phospholipid is in excess over that of liposomes (Bienvenue et al., 1985) phospholipid transfer from liposomes to HDL is an irreversible process, the rate of which depends solely on the liposomal phospholipid concentration. Phospholipids incorporated into HDL are then removed from plasma by reactions involving the uptake of the whole lipoprotein or the independent movement of the lipid moiety (Eisenberg, 1984). A metabolic elimination route from the plasma compartment is also included in the model, since PS was found to be hydrolyzed in the plasma of rats to lysoPS and hydrolysoluble compounds (mainly serine). The model shown in Figure 1 is described by the following differential equations:

\[
\frac{d[F]}{dt} = -K[F] \tag{1}
\]

where \( K = k_1 + k_3 + k_4 \), and F designates free PS (intact liposomes).

\[
\frac{d[B]}{dt} = k_1[F] - k_2[B] \tag{2}
\]

where B indicates PS bound to HDL.

The solution for equation 1 is:

\[
[F] = C_0 e^{-Kt} \tag{3}
\]

where \( C_0 \) is the zero-time concentration of PS. Substituting equation 3 into equation 2 and solving for \( [B] \) yields

\[
[B] = \frac{C_0 k_1}{K - k_2} (e^{-kt} - e^{-Kt}) \tag{4}
\]

The total concentration of PS in plasma, \( C = [F] + [B] \), is given by

\[
C = C_0 \left( \frac{K - k_1}{K - k_2} e^{-kt} + \frac{k_1}{K - k_2} e^{-Kt} \right) \tag{5}
\]

PS plasma concentration-time data were fitted to equation 5 by a weighted \((1/\text{C}^2)\), computer-assisted, non-linear least squares regression analysis.

**Results**

**Kinetics of phosphatidylserine**

Figure 2 illustrates the time course of the plasma concentrations of PS (a) and its degradation products (b) following bolus intravenous injection. The concentration of PS showed a biphasic decline with a very rapid initial decay (Figure 2a). Assuming a plasma volume of 40 ml kg$^{-1}$ (Hwang et al., 1980) it could be calculated that 93% of the injected phospholipid was removed from plasma within 3 min. Separate controls showed that there was not significant PS uptake by the blood cells, thereby making it unlikely that the rapid decline was due to adsorption of PS vesicles to the erythrocyte surface or uptake by circulating monocytes. The decay curve of the total liposoluble radioactivity was virtually superimposable to that of PS (results not shown), indicating no phospholipid loss during the chromatographic procedure. The main kinetic parameters of PS, calculated according to the model shown in Figure 1, are given in Table 1. The zero-time concentration, \( C_0 \), was lower than the expected value of 175.1 ± 15.7 x 10$^9$ d.p.m. ml$^{-1}$ for a compound that is not reversibly distributed outside the plasma. The data of Figure 2b may provide an explanation for this apparent discrepancy. It can be seen that the concentrations of lysoPS and hydrolysoluble metabolites, abruptly increased at 30 s, decreased thereafter very quickly, in spite of the concurrent rapid disappearance of PS. This does not appear consistent with a continuous generation of metabolites, but is suggestive of a virtually instantaneous degrada-
In either case lysoPS constituted 5–10% of the liposoluble radioactivity present at the injection site. Since degradation of PS at the injection site results in a decrease of the dose effectively administered and, consequently, in an overestimation of clearance and volume of distribution, these parameters were not shown in Table 1.

**Tissue uptake and biotransformation**

Table 2 shows the content of liposoluble and hydrosoluble radioactivity in plasma and five major organs 1 h after i.v. administration of PS. It can be seen that the largest excess of liposoluble radioactivity was present in liver and spleen, suggesting that, at least in these organs, unmodified PS was prevalently taken up. The liver accumulated the largest fraction of radiolabelled vesicles (95% of the amount recovered). Although the absolute amount of radioactivity in the spleen was low, due to the small size of this organ, its concentration was remarkably high, as previously observed with other types of liposomes (Allen & Everest, 1983). When the composition of the liposoluble radioactivity was examined (Table 3) it was found that PS constituted the largest fraction of the labelled phospholipids, followed by phosphatidylethanolamine (PE). This provided the first indication that part of the incorporated PS underwent decarboxylation (see below). By contrast, only negligible sphingomyelin labelling was observed, suggesting a minor uptake of free [14C]-serine released in plasma from the injected PS. It is indeed known that serine is not incorporated exclusively into PS, but also into sphingolipids. Traces of lysoPS originating from PS hydrolysis and a small amount of lysoPE were also found in plasma. To exclude further a major contribution of free serine in the appearance of labelled PS or PE in tissues, control experiments were done with PS containing [3H]-glycerol instead of [14C]-serine (Table 4). The accumulation of liposoluble radioactivity in liver and spleen (45–66% of the unmodified) was similar to that found after administration of phosphatidyl-[14C]-serine, confirming that it was due to uptake of the unmodified PS molecule. However, the ratio of the liposoluble to the hydrosoluble radioactivity was 7–10 times higher, indicating a decreased amount of labelled hydrosoluble compounds. The analysis of the composition of the liposoluble radioactivity (Table 5) provided an explanation for this difference. It can be seen that the fraction containing labelled neutral lipids became predominant in plasma, thus indicating that [3H]-glycerol released by hydrolytic degradation of PS was readily removed from the hydrosoluble pool by incorporation into lipoproteins. The high radioactivity in plasma neutral lipids (74–88%) caused the fraction containing labelled PS to become smaller than that detected after administration of [14C]-serine-PS. By contrast, the PS

**Table 2** Distribution of radioactivity 60 min after bolus i.v. injection of phosphatidyl-[14C]-serine (PS) after bolus i.v. administration of 2 mg kg⁻¹

<table>
<thead>
<tr>
<th>Organ</th>
<th>Liposoluble</th>
<th>Hydrosoluble</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.6 ± 1.0 (1.5)</td>
<td>1.8 ± 0.2 (0.5)</td>
<td>3</td>
</tr>
<tr>
<td>Liver</td>
<td>200.0 ± 6.9 (68.8)</td>
<td>23.1 ± 1.9 (79.9)</td>
<td>8.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>61.1 ± 6.4 (0.8)</td>
<td>9.4 ± 0.8 (0.1)</td>
<td>6.5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>19.2 ± 3.0 (0.6)</td>
<td>9.3 ± 0.7 (0.3)</td>
<td>2.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>14.6 ± 3.3 (0.5)</td>
<td>3.6 ± 0.3 (0.1)</td>
<td>4</td>
</tr>
<tr>
<td>Brain</td>
<td>1.6 ± 0.2 (0.05)</td>
<td>1.4 ± 0.2 (0.04)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

PS 2 mg kg⁻¹ (14.6 ± 10³ d.p.m. kg⁻¹) was injected into the jugular vein of 6 rats (316 ± 11 g). Plasma (heart puncture) and organs were sampled, weighed and extracted 60 min later. Data are expressed as d.p.m.g⁻¹ of wet tissue (or ml of plasma) and as percentage of the administered dose that is eventually recovered in the whole organ (figures in parentheses). To calculate the recovery, the following values were used: plasma 4% of body weight; brain 0.45%; liver, 5%; spleen, 0.2%; kidneys, 0.47%; lungs, 0.5%. Data from our determinations and the Tables of Charles River (1975). Mean ± s.e.mean from 6 rats.

**Figure 2** Plasma decay curves after bolus intravenous injection of 2 mg kg⁻¹ of phosphatidyl-[14C]-serine (3.5 × 10³ d.p.m.mg⁻¹ of PS). PS liposomes were injected into the jugular vein of anaesthetized rats (45 mg kg⁻¹ of pentobarbitone, i.p.). Each point represents the mean from three rats; vertical bars show s.d. (a) Radioactivity associated with phosphatidylserine (PS). The continuous line represents the non-linear least squares regression fit to the data, based on equation 5. (b) Total hydrosoluble radioactivity (C); radioactivity associated with lysophosphatidylserine (●). Concentration data for lysoPS are shown up to the last measurable concentration. Since the injected [14C]-serine-PS contained 4% of hydrosoluble radioactivity and 1% of radioactive lysoPS (see Methods), their initial concentrations, calculated by assuming a plasma volume of 40 ml kg⁻¹ (Hwang et al., 1980), are also indicated (points on the ordinate axis).

**Table 1** Kinetic parameters of phosphatidylserine-[14C]-serine (PS) after bolus i.v. administration of 2 mg kg⁻¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mean ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀ (d.p.m. ml⁻¹ × 10⁻¹)</td>
<td>52.90 ± 15.24</td>
</tr>
<tr>
<td>K (min⁻¹)</td>
<td>0.85 ± 0.21</td>
</tr>
<tr>
<td>k₆ (min⁻¹)</td>
<td>0.071 ± 0.002</td>
</tr>
<tr>
<td>t₁/₄ (min)</td>
<td>40.00 ± 6.10</td>
</tr>
<tr>
<td>t₉/₄ (min)</td>
<td>0.11 ± 0.02</td>
</tr>
</tbody>
</table>

Parameters were obtained by fitting plasma concentration-time data to equation 5 (see Methods). Values are expressed as mean ± s.d. for three rats.

C₀ = zero-time concentration of PS; K = k₁ + k₆; k₂, k₆, k₇, and k₈ are the rate constants for the disappearance of free PS from plasma; k₆ = rate constant for the elimination from plasma of HDL-bound PS; t₁/₄ and t₉/₄ are the corresponding half-lives; k₆ = rate constant for the incorporation of PS into HDL.
fraction increased in liver and spleen, due to a decrease in PE labelling.

From a comparison of the amounts of PE recovered after the administration of [14C]-serine-PS and [3H]-glycerol-PS (Tables 3 and 5) it can be estimated that uptake of free [14C]-serine accounts approximately for 10–13% of the liposoluble radioactivity found in spleen and liver after the administration of [14C]-serine-PS.

Discussion

In this study the disposition of intravenously injected PS vesicles has been investigated to see whether a relationship exists with the pharmacological actions observed in rodents after parenteral administration. Previous investigations on phospholipid vesicles of various composition have shown two main patterns of interaction with tissue and plasma constituents:

- The biphasic clearance from plasma is generally consistent with the classical two-compartment open model, which can be interpreted as a rapid reversible distribution phase, followed by elimination. As discussed above, the interaction of PS liposomes with cells is irreversible and the fast decay step, rather than distribution, describes the main elimination route of PS, i.e. uptake by the mononuclear phagocyte system. The slow component of the PS decay curve is half-life very similar to that of rat LDL. A linear decay with half-life of 47 min was observed when lipoproteins containing radiolabelled phospholipids were injected intravenously into rats (Stein & Stein, 1966). It is therefore suggested that the slow phase of PS decay in plasma is due to its incorporation into lipoproteins, and reflects the elimination of the lipid moiety of these plasma constituents, possibly influenced by the presence of PS, an unusual phospholipid for HDL.

- With other types of liposomes the biphasic clearance from plasma may reflect vesicle size heterogeneity, the larger vesicles being cleared faster than the smaller ones (Juliano & Stamp, 1975; Patel & Ryman, 1981). With PS-containing liposomes, however, the disappearance from plasma has been shown to be independent of the vesicle size (Juliano & Stamp, 1975). This has been attributed to the strong tendency of the smaller PS vesicles to coalesce in the presence of plasma Ca2+ to form a rather homogeneous population of larger vesicles (Juliano & Stamp, 1975; Kao & Loo, 1980). A biphasic decline of intravenously injected liposomes may also reflect the presence of two uptake pathways, one of which (uptake by the mononuclear phagocyte system) is saturable (Gregoriadis & Neerunj, 1974; Hwang & Beaumier, 1988). However, evidence of saturation has been obtained at doses (around

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**Table 3** Composition of liposoluble radioactivity extracted 60 min after i.v. administration of phosphatidyl-[14C]-serine

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Plasma</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of liposoluble d.p.m.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>39.3 ± 1.7</td>
<td>63.7 ± 4.2</td>
<td>68.6 ± 3.8</td>
<td>61.1 ± 3.3</td>
<td>66.3 ± 3.7</td>
</tr>
<tr>
<td>PE</td>
<td>12.2 ± 0.5</td>
<td>21.3 ± 1.2</td>
<td>14.8 ± 2.9</td>
<td>26.6 ± 2.2</td>
<td>16.7 ± 1.8</td>
</tr>
<tr>
<td>PC</td>
<td>2.1 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>SPH</td>
<td>1.3 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>lysoPS</td>
<td>3.6 ± 0.9</td>
<td>1.5 ± 0.5</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>lysoPE</td>
<td>11.8 ± 1.3</td>
<td>2.6 ± 0.6</td>
<td>2.3 ± 0.4</td>
<td>3.2 ± 0.7</td>
<td>63.2 ± 1.2</td>
</tr>
<tr>
<td>lysoPC</td>
<td>3.3 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Neutral lipid (front)</td>
<td>6.8 ± 1.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

The liposoluble radioactivity of Table 2 was resolved by two dimensional thin layer chromatography. The amount of radioactivity not accounted for by the indicated lipids was distributed at the origin or in unidentified spots. PS denotes phosphatidylethanolamine, PC phosphatidylcholine, SPH sphingomyelin. ND, not detected. Means ± S.E.M. from 6 rats.

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**Table 4** Distribution of radioactivity 60 min after bolus i.v. injection of [3H]-glycerol-phosphatidylserine

<table>
<thead>
<tr>
<th>Organ</th>
<th>d.p.m. g⁻¹ wet wt or ml⁻¹ × 10⁻³</th>
<th>Liposoluble</th>
<th>Hydrosoluble</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, rat 1</td>
<td>5.8 (0.5)</td>
<td>1.4 (0.1)</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>rat 2</td>
<td>7.4 (0.7)</td>
<td>1.4 (0.1)</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Liver, rat 1</td>
<td>593.2 (66.4)</td>
<td>64.4 (0.7)</td>
<td>2.6</td>
<td>92.1</td>
</tr>
<tr>
<td>rat 2</td>
<td>407.9 (45.6)</td>
<td>67.0 (0.7)</td>
<td>0.6</td>
<td>60.7</td>
</tr>
<tr>
<td>Spleen, rat 1</td>
<td>131.1 (0.8)</td>
<td>1.9 (0.01)</td>
<td>0.1</td>
<td>70.5</td>
</tr>
<tr>
<td>rat 2</td>
<td>116.3 (0.7)</td>
<td>2.4 (0.1)</td>
<td>0.2</td>
<td>49.1</td>
</tr>
</tbody>
</table>

Two rats of 260 g were injected with 2 mg kg⁻¹ of phosphatidylserine labelled with [3H]-glycerol (44 × 10⁶ d.p.m. kg⁻¹). Plasma, liver and spleen were analyzed 60 min after the injection. Data are expressed as d.p.m. g⁻¹ of wet tissue (or ml of plasma) and as percentage of the administered dose that is recovered in the whole organ (figures in parentheses).

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**Table 5** Composition of liposoluble radioactivity extracted 60 min after i.v. administration of [3H]-glycerol-phosphatidylserine

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Plasma</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>5.5</td>
<td>15.3</td>
<td>85.6</td>
</tr>
<tr>
<td>PE</td>
<td>1.0</td>
<td>0.7</td>
<td>7.6</td>
</tr>
<tr>
<td>PC</td>
<td>1.0</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Neutral lipids (front)</td>
<td>87.6</td>
<td>73.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 3. Negligible radioactivity was found to be associated with other phospholipids.
100 mg kg⁻¹ far higher than those employed in this study (2 mg kg⁻¹). In the case of PS, saturation at this dose would imply a very low capacity uptake by the macrophage scavenger receptor that is expected to mediate the endocytosis of vesicles containing this phospholipid (Nishikawa et al., 1990).

The combined use of [³H]-glycerol-PS and [¹⁴C]-serine-PS enabled us to outline the metabolic fate of injected PS. Excessive hydrolysis occurs at the injection site yielding, in addition to hydrosoluble metabolites, the pharmacologically active compound lysPS. The production of lysPS is most likely due to the phospholipase A₂ released by the platelet aggregates at the site of the perfused vessel. Rat platelets are known to be rich in phospholipase A₂ acting on PS (Horigome et al., 1987). Formation of lysPS at the injection site, although in small amounts, is also observed after intramuscular or intraperitoneal injection. These observations indicate that there might be a variable contribution of lysPS to the pharmacological actions of PS, depending on the route of administration.

After incorporation into tissues, PS follows two metabolic pathways which reflect the two main mechanisms of uptake. One involves decarboxylation to PE of a minor fraction (8% in the liver) of the incorporated PS. Since this is the physiological pathway of PS metabolism, it most probably involves that fraction of PS that mixes with the phospholipid pool of the plasma membrane. The other, predominant, metabolic pathway proceeds to the complete hydrolysis of PS to the individual components of the molecule as testified by the detection in tissues of labelled glycerol and serine. This sequence is to be expected if the phospholipid vesicle is internalized by endocytosis and hydrolyzed after fusion of the endocytic vacuole with lysosomes (Dijkstra et al., 1985). Consistently, PS uptake is predominant in organs (liver and spleen) where endocytosis by the local macrophage population is especially active.

References


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