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Postmortem Stability of Lung Surfactant Phospholipids

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ABSTRACT: The postmortem stability of the main phospholipids of lung surfactant-phosphatidyl choline (PC), phosphatydal ethanolamine (PE), phosphatydal inositol (PI), phosphatydal serine (PS) and sphyngomyelin (S) in three different deaths; one caused by fresh-water drowning, one by salt-water drowning, and one from a sodium-pentobarbital overdose has been studied. The drug overdose was considered the control because there was no surfactant involvement. The results show the stability of these kinds of lipids in the first 24 h, with a progressive decrease from 48 h on until 96 h, with a significant correlation to the time of $P < 0.01$ in most cases.

KEYWORDS: pathology and biology, phospholipids, surfactant, drowning, postmortem stability

In daily forensic practice there are some causes of death—those due to asphyxial syndromes, pulmonary edema, intoxications, for example, where a systematic and detailed study of the lungs must be performed.

It has been clearly stated that the diagnosis of death by drowning may be one of the most difficult challenges in forensic pathology because findings are often minimal, ambiguous, or even completely negative [*1*]. Fortunately, there is a large number of analytical procedures that can be performed [*2*] when autopsy findings are not clear enough. However, there are still some special cases—primarily those of criminal origin—where laboratory results can not help to establish the exact cause of death: was it truly drowning? Was it drowning in fresh or salt water?

Pulmonary surfactant is a highly surface-active material with a very complex composition that maintains alveolar stability at low lung volumes. Surfactant consists mainly of phospholipids (PLs), but also contains specific proteins and carbohydrates. From these lipids, phosphatydal choline (PC) accounts for about 52% of the total phospholipids in pulmonary tissue, and phosphatydal ethanolamine (PE) 20% [*3*].

In a previous paper [*4*], we have demonstrated the usefulness of some surfactant phospholipids as markers available, not only to distinguish between drowning and postmortem immersion, but also between fresh- and salt-water drownings.

Their variations have also been studied in experimental cases of asphyxia [*5*], and the reported results are quite similar to ours.

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Although there are some publications that deal with postmortem degradation of lipids [*6–10*], there is no exact reference to the postmortem stability of phospholipids in pulmonary tissue, which should be known before determining their application in the forensic field.

Materials and Methods

We used 75 Wistar albino female rats (body weight, 300 ± 30 g), and divided them into three groups.

1. *Control Series (Group I)*: 25 animals killed using an overdose of sodium pentobarbital (150 mg/kg, i.p.).
2. *Fresh-water series (Group II)*: 25 animals killed by drowning in fresh (tap) water, according to a technique previously described [*11*].
3. *Salt-water series (Group III)*: 25 animals killed by drowning in salt water [*11*], which had been artificially prepared [*12*].

Once dead, five animals from each group were immediately autopsied, and the lungs and trachea removed. The other 60 cadavers were kept in supine position at ambient weather conditions (averages: temperature: 24.1 ± 0.5 OC; humidity: 50.2 ± 1.3%; atmospheric pressure: 712.2 ± 1.8 mmHg), and 5 from each group were autopsied in the following 4 days: 24, 48, 72, and 96 h after death.

The main phospholipids in pulmonary tissue, PC, PE, phosphatydal inositol (PI), phosphatydal serine (PS), and sphyngomyelin (S), were extracted with chloroform:methanol (2:1 ratio) from 0.6 g lung-tissue pieces [*13*]; the final extracts were concentrated under nitrogen stream, divided into aliquots and frozen at −40°C until used.

Surfactant phospholipids were separated into their component species with chromatographic procedures in high-performance thin layer chromatography (HPTLC) silica-gel 60 plates, from E. Merck, with chlorophorm:methanol:petroleum ether: acetic acid:boric acid (40:20:30:10:1.8, v:v:v:v:w), according to a method widely used [*14*]. The solvent system was freshly prepared and the chromatograms were developed to within 1 cm of the top of the plate in tanks lined with solvent saturated filters. Lipids were made visible by brief exposure to iodine vapor, and identified by using appropriate standards (Sigma, St. Louis, MO). Their phosphorus contents were then quantified directly in the gel using a reaction based [*15*] on the acid digestion of the gel with 70% perchloric acid. Amidol and ammonium molybdate were then added, and the resulting blue color was quantified at 830 nm by spectrophotometry.

To develop the calculations, phosphorus was assumed to be $\frac{1}{25}$ of the total weight of any phospholipid [*16*]. The results for all the five different phospholipids are expressed as a percentage of the lipid in the total phospholipid composition of a given sample.

To test their postmortem stability, statistical analysis was carried out using a linear regression studies and ANOVA-1 tests.

Results and Discussion

Results are expressed in the following three tables, each one corresponding to a different cause of death.

The expressed results confirm our previous studies [*4*] about the usefulness of some surfactant phospholipids to establish the diagnosis of drowning, as could be observed by comparing the different profiles of the three series at time "0." These differences are also observed in phospholipids obtained from rats autopsied 24, 48, 72, and 96 h after death.

Regarding the postmortem degradation of the different lipids, we have observed that

TABLE 1—*Surfactant phospholipids composition (%) in Series I (Control).*

Phospholipid %	Time postmortem (h) 0	24	48	72	96
PE	23.8 ± 2.0	23.6 ± 1.8	19.2 ± 1.1	12.6 ± 0.8	8.8 ± 0.5
PS	5.6 ± 0.4	5.5 ± 0.4	5.3 ± 0.4	3.5 ± 0.4	3.1 ± 0.3
PI	4.6 ± 0.4	4.3 ± 0.4	3.8 ± 0.4	3.3 ± 0.3	1.9 ± 0.2
PC	54.3 ± 2.9	51.6 ± 2.1	44.5 ± 3.8	33.5 ± 2.9	24.6 ± 1.9
S	5.5 ± 1.1	5.8 ± 0.5	6.2 ± 0.4	4.4 ± 0.4	4.6 ± 0.7
TOTAL	93.8	91.8	79.0	57.3	43.0

TABLE 2—*Surfactant phospholipids composition (%) in Series II (Fresh Water Drowning).*

Phospholipid %	Time postmortem (h) 0	24	48	72	96
PE	21.8 ± 1.8	21.7 ± 1.6	15.2 ± 0.8	15.1 ± 0.8	11.0 ± 0.7
PS	4.9 ± 0.2	4.9 ± 0.5	3.4 ± 0.3	3.2 ± 0.5	2.7 ± 0.4
PI	3.9 ± 0.5	3.5 ± 0.4	2.4 ± 0.3	1.3 ± 0.3	N.D.
PC	44.1 ± 3.3	45.3 ± 3.4	37.6 ± 2.3	29.9 ± 1.6	20.6 ± 2.5
S	6.9 ± 0.6	6.4 ± 0.5	5.5 ± 0.5	4.8 ± 0.3	4.2 ± 0.4
TOTAL	81.6	81.8	64.1	54.3	38.5

TABLE 3—*Surfactant phospholipids composition (%) in Series III (Salt Water Drowning).*

Phospholipid %	Time postmortem (h) 0	24	48	72	96
PE	24.0 ± 1.6	24.3 ± 2.2	17.0 ± 0.9	12.5 ± 0.7	10.6 ± 0.8
PS	4.7 ± 0.7	4.5 ± 0.4	4.4 ± 0.3	3.0 ± 0.6	2.1 ± 0.4
PI	3.5 ± 0.4	3.6 ± 0.3	3.0 ± 0.2	1.4 ± 0.3	N.D.
PC	58.5 ± 2.2	59.9 ± 3.1	51.6 ± 2.1	44.8 ± 2.0	31.9 ± 2.2
S	6.3 ± 0.4	6.2 ± 0.4	5.5 ± 0.4	4.9 ± 0.8	4.5 ± 0.6
TOTAL	97.0	98.5	81.5	66.6	49.1

there is a clear stability in the first 24 h for all the phospholipids in all the three groups studied. Little variations between 0 and 24 h have no statistical significance and cannot be attached to biologic processes. Between 24 and 48 h start the processes of degradation, diminishing the levels (%) of the different lipids. Similar results were formerly published by Oliveira de Sa [*6*] working with guinea-pig brain phospholipids, and by others working with phospholipids extracted from blood in cases of fatal strangulations [*7*], aqueous humor [*8*], and subcutaneous fats [*9*].

After 48 h of postmortem interval, degradation is clearly stabilized, and we can find a significant diminishing of all lipids for all series.

The progressive degradation in all the PLs points out that they are affected by the same degradant factors. In our conditions, these factors start their influence from 24 h on, but not in the first 24 h. According to these data, we have to think in autolytic and putrefactive processes as causes of degradation. In the first 24 h, lipases show a high stability and phospholipids tend to keep their values [*6*], but after this time, the already mentioned processes (autolysis, putrefaction and the diminution of pH) produce a degradation of the phospholipidic molecules, what are conversed into more simple molecules, usually free acid fats and closely related ones. According to a previous paper [*7*] we also

believe that degradation is enhanced by phospholipases from thrombocytes and granulocytes, since red cells has not this kind of enzymes.

After the analysis of postmortem stability and rate of degradation, we have not found differences among the three series. It means that neither fresh nor salt water seem to affect—in our artificial conditions—the previously mentioned factors that produce degradation of the PLs.

Because levels at 0 h are the same as at 24 h, we have calculated the linear regression equation in all the lipids and series between 24 and 96 h, as shown in Table 4.

All the lipids—except Sphyngomielin in Group I—show a progressive degradation from 24 to 96 h, time of our last determination.

With regard to the very high correlation indexes, we should consider that this is an experimental design, carried out in specially selected laboratory rats of closely related families, with the same age and sex. The variation coefficient (VC)—that may be studied in rats of the first series at 0 h—is around 8% for all the lipids (except sphyngomyelin, 20%). We used a series of rats with great homogeneity, an uncommon situation in forensic-science specimens, where VC ranging between 10 and 40% are accepted as usuals in healthy human adults [*14*]. All these facts should be taken into account before their extrapolation to human samples.

The very best results are offered by PC, with "r" close to 0.99 in the three series. This is logical if we consider that PC supposes about 54% of all the PL and has little VC. On the contrary, those who are scarcely present in lung surfactant, as PI and PS, has a more heterogeneous rate of degradation in our experimental conditions, perhaps due to lack of sensitivity in the techniques used.

In conclusion, we have confirmed the great postmortem stability of the lung surfactant phospholipids, whose levels have been found to be similar in the first 24 h, and we have shown that they decrease progressively and according to linear correlation equations until the time of our last determination, 96 h after death. In consequence, we think that PIs are very appropriate biochemical substances to perform studies of forensic interest, even some days after death.

TABLE 4—*Linear regression equation expressing the degradation of the PL along the time (between 24 and 96 h).*

Phosphatidyl-Ethanolamine			
I	$Y = 28.8 - 0.21X$	$r = -0.985$	$P < 0.01$
II	$Y = 23.9 - 0.14X$	$r = -0.939$	$P < 0.05$
III	$Y = 27.5 - 0.19X$	$r = -0.967$	$P < 0.05$
Phosphatidyl-Serine			
I	$Y = 6.6 - 0.04X$	$r = -0.948$	$P < 0.05$
II	$Y = 5.3 - 0.03X$	$r = -0.927$	$P < 0.05$
III	$Y = 5.7 - 0.04X$	$r = -0.959$	$P < 0.05$
Phosphatidyl-Inositol			
I	$Y = 5.3 - 0.03X$	$r = -0.961$	$P < 0.05$
II	$Y = 5.1 - 0.05X$	$r = -0.988$	$P < 0.01$
III	$Y = 5.1 - 0.05X$	$r = -0.986$	$P < 0.02$
Phosphatidyl-Choline			
I	$Y = 61.6 - 0.38X$	$r = -0.991$	$P < 0.01$
II	$Y = 53.8 - 0.34X$	$r = -0.993$	$P < 0.01$
III	$Y = 69.8 - 0.38X$	$r = -0.990$	$P < 0.01$
Sphingomyelin			
I[a]	—	—	—
II	$Y = 7.1 - 0.03X$	$r = -0.986$	$P < 0.01$
III	$Y = 6.7 - 0.024X$	$r = -0.983$	$P < 0.01$

[a]There is no correlation since values at 48 h are higher than at 24 h, as expressed in Table 1).

Death by drowning—both in fresh and salt water—do not seem to significantly affect either the postmortem stability or the rate of degradation.

In order to confirm these experimental results, we are performing studies in phospholipids from human specimens obtained from deaths due to different causes.

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