Effect of Albumin and Lamellar Bodies on Fluorescence Polarization of Amniotic Fluid

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Using a probe and procedure developed for the Abbott TDx analyzer, we investigated the influence of albumin on fluorescence polarization of amniotic fluid. Polarization readings increased when albumin was added to either "mature" or "immature" amniotic fluids. Albumin added to a fraction rich in lamellar bodies (10,000 × g pellet) greatly increased polarization readings, but albumin added to the lamellar-body-free fraction resulted in only small increases. Adding increasing amounts of the lamellar-body-rich fraction from mature and immature pools to a constant amount of albumin decreased the polarization readings. We saw no correlation between amniotic fluid polarization and albumin concentration but found a significant correlation between polarization values and either total extractable phospholipids (r = 0.725, P < 0.001) or the ratio albumin/total extractable phospholipids (r = 0.784, P < 0.001). The concentration of albumin in amniotic fluid was variable and correlated poorly with gestational age.

Additional Keyphrases: fetal status • probes

Two new fluorescence polarization assays have recently been developed for assessing fetal lung maturity (1-3). Their major new feature is that phospholipid probes are used instead of the 1,6-diphenyl-1,3,5-hexatriene (DPH) used in the original fluorescence polarization assay for fetal pulmonary maturity (4).

Several factors determine the degree of polarization of DPH in amniotic fluid. According to Shinitzky and Barenholz (5), changes in surfactant alter the microviscosity of the lipid component of surfactant fluid, such that polarization values are lower for mature surfactant. Tait et al. (7) have discussed many of the factors affecting DPH polarization, and reasons for doubting the proposed mechanism. Others have demonstrated the influence of fatty acid composition (6, 7), and high-density lipoproteins have been proposed as an important factor (6, 8, 9). The concentration of phospholipids in amniotic fluid is almost certainly a factor in determining fluorescence polarization readings. Little has been published to document this, but several authors report that fluorescence polarization readings decrease with increasing gestational age, and it is well known that the total phospholipid concentration increases with gestation.

The value of these polarization assays has been justified because of their correlation with the lecithin/sphingomyelin (L/S) ratio and validation through clinical trials. However, these assays have been used without a full understanding of the mechanism of action of the probes and the factors affecting polarization (4, 10, 11). In addition, the new phospholipid probes might well be expected to behave differently from DPH.

Russell (2, 3) states that the fluorescent dye used in his assay partitions between lung surfactant and protein, resulting in higher polarization values when bound to protein than when bound to surfactant. We have investigated the influence of albumin on fluorescence polarization and intensity in amniotic fluid, because the concentrations of protein in amniotic fluid do not remain constant throughout gestation (12, 13). We also studied the correlation between polarization and total phospholipid in amniotic fluid.

Materials and Methods

Equipment and reagents. We used a standard-model TDx analyzer (Abbott Laboratories, Irving, TX) with software supplied by Abbott for determining net fluorescence polarization (mPol units) and net fluorescence intensity (arbitrary units) of amniotic fluid. Reagents were supplied and packaged by Abbott in unit-dose samples. Between-run CVs for centrifuged amniotic fluid (150 × g, 5 min) at 303 and 222 mPol (n = 7) were respectively 0.4% and 0.5%, and at intensity readings of 13079 and 27430 were respectively 1.3% and 3.0%.

Bovine and human serum albumin were obtained from Sigma Chemical Co., St. Louis, MO. Tris was purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. All other reagents used were reagent-grade quality.

Albumin concentrations were determined by rate nephelometry (ICS™ Analyzer II with ICS reagents; Beckman Instruments, Brea, CA). The between-run CVs for centrifuged amniotic fluid (150 × g, 5 min) at 3000 mg/L and 1000 mg/L (n = 7) were respectively 5.1% and 2.5%.

Phospholipid concentrations were determined by measuring the phospholipid phosphorus content of extractable phospholipids (14). The between-run CV for centrifuged amniotic fluid at 29 mg/L (n = 8) was 10.3%, and at 51, 171, and 320 mg/L (n = 10) the CVs were respectively 11.8%, 4.7%, and 4.7%.

L/S ratios were determined with the "Fetal-Tek 200" thin-layer chromatographic system (Helena Laboratories, Beaumont, TX). These specimens were centrifuged at 1000 × g for 5 min before extraction.

Experimental designs. We analyzed, in duplicate, different concentrations of human serum albumin in phosphate-buffered isotonic saline (pH 7.4; phosphate 0.1 mol/L, NaCl 8.5 g/L) for net fluorescence polarization and intensity.

We made two separate pools of amniotic fluid by combining uncentrifuged amniotic fluids that had been obtained by...
transabdominal amniocentesis. The "mature" pool comprised samples having a mature L/S ratio and positive for phosphatidylglycerol; the "immature" pool samples had an immature L/S ratio and were negative for phosphatidylglycerol. Each pool was centrifuged (150 × g, 5 min) to remove cells and debris. Various volumes of 100 g/L bovine serum albumin solution were added to aliquots of each pool, increasing the albumin content by 0.3, 0.6, 1.3, 2.5, and 5.0 g/L, and to aliquots of two unpooled amniotic fluids, increasing their respective albumin contents by 0.3, 0.6, and 1.3 g/L and 1.3, 5.0, and 10.0 g/L. Initial albumin concentrations for the "immature" pool, "mature" pool, and the two unpooled samples were 3.1, 1.0, 2.3, and 1.6 g/L, respectively. Net polarization and intensity were measured in duplicate for each concentration of added albumin.

We separated the lamellar body fractions of the pooled amniotic fluids by centrifugation at 10 000 × g for 20 min (15, 16) and removed the supernates. Pellets were resuspended to their original volume in Tris-buffered (50 mmol/L, pH 7.4) isotonic saline. Again we added 100 g/L bovine serum albumin solution to aliquots of both the supernate and resuspended pellet, for increases in albumin concentrations of 0.6, 1.3, 2.5, 5.0, and 7.5 g/L. Net polarization and intensity were measured in duplicate for each albumin-supplemented sample.

We also added various amounts of the resuspended pellet and Tris-buffered saline to a constant concentration of albumin (2.5 g/L), such that samples contained 2.5, 10.0, 40.0, and 70.0% of the resuspended lamellar body fraction for each pool. Phospholipid concentrations for the "mature" pool and "immature" pool resuspended solutions were respectively 159 and 35 mg/L. Net polarization and intensity were measured in duplicate for each pool.

We used the Abbott fetal lung maturity test to measure net polarization and intensity in 24 samples of amniotic fluid that had been centrifuged (1000 × g, 5 min) before analysis. All samples were collected by transabdominal amniocentesis except for one by vaginal drainage. Gestational ages were based on the Ballard score evaluation of the infant at birth (17). All samples were analyzed for albumin and total phospholipid phosphorus as described above.

We also measured albumin in 26 samples of uncontaminated amniotic fluid from pregnancies of known gestational ages, centrifuging the samples (1000 × g, 5 min) before analysis.

Results

As Figure 1 shows, the polarization value for the probe and buffer alone is low, but addition of small amounts of albumin increases the polarization value sharply to a maximum value, which does not change with further increases in the albumin concentration. Net intensity values follow a similar pattern, but reach a plateau at a slightly lower concentration of albumin.

Figure 2 demonstrates the change in net polarization and net intensity as albumin is added to amniotic fluid. When albumin was added to amniotic fluid samples and pools, the polarization values increased, but the changes in fluorescence intensity with increasing amounts of albumin were variable: the mature pool showed a marked decrease in fluorescence, the immature pool showed less change. Of the unpooled specimens, one showed a decreasing fluorescence as albumin increased; the other showed no change.

Figure 3 demonstrates the change in net polarization and net intensity of the lamellar body fraction and the lamellar-
The pellet from the mature pool had about twice the fluorescence intensity of the immature pool, and native unfractionated pools (Figure 2) had fluorescence values that were between the high value for the resuspended pellet and the low value for the supernate.

Figure 4 shows the effect of adding different amounts of the 10000 × g resuspended pellet to albumin (2.5 g/L); with an increasing amount of pellet both pools lowered the polarization values and increased intensity values, but the effect of the mature pool was much greater than that of the immature pool. Aliquots of the two pools with the same polarization value did not contain the same amount of phospholipid. For example, the immature pool aliquot containing 70% of the resuspended lamellar body fraction had a polarization of 284 and contained 24.5 mg of phospholipid per liter, but the mature pool at the same polarization value contained approximately 14.6 mg of phospholipid per liter.

Correlation of polarization with the L/S ratio in amniotic fluid, excluding the vaginal pool sample, was significant (r = 0.894, P < 0.001). Linear regression for polarization versus L/S ratio (n = 23) was y = -40.2x + 352 mPol, S_{yx} = 18.7 mPol, r^2 = 0.80.

There was no significant correlation (linear or nonlinear) of polarization with albumin concentration, but there was a significant correlation of polarization with total extractable phospholipids (r = 0.725, P < 0.001) and with the ratio albumin/total extractable phospholipids (r = 0.784, P < 0.001). Regression analysis for polarization versus albumin/phospholipid ratio (n = 22) was y = 182 mPol + 66.5 log x, S_{yx} = 24.7 mPol, r^2 = 0.615, and for extractable phospholipids y = 321 mPol - 65 log x, S_{yx} = 27.5 mPol, r^2 = 0.525. The vaginal pool sample, because of albumin contamination, and one other sample, because of phosphorus contamination, were excluded. Extractable phospholipid concentrations ranged from 19 to 350 mg/L.

Figure 5 shows albumin concentration vs gestational age (n = 26). Albumin concentrations ranged from 0.65 to 3.16 g/L (mean 1.45, SD 0.60 g/L), and tended to be lower near term.

Discussion

Using the Abbott fetal lung maturity assay, we have shown the influence of albumin on net fluorescence polarization of amniotic fluid. A change in albumin concentration alters the polarization value, with mature samples, which have more surfactant material, being affected more than immature samples (Figure 2). Thus, the addition of albumin to the 10000 × g supernate of mature and immature pools...
resulted in markedly less change in polarization than when albumin was added to the lamellar body fraction (Figure 3).

As explained by Russell (3), the polarization value of a sample is affected by binding of the probe to proteins and by the phospholipid composition. Likewise, the fluorescence intensity readings will be affected by the presence of albumin and, when phospholipids are present, will result in lower readings with increased concentrations of protein. If albumin concentrations in amniotic fluid varied little from patient to patient and remained stable throughout gestation, it would be unnecessary to account for albumin. However, our data (Figure 5) show that albumin concentrations are variable, and that they tend to decrease with gestation.

Even when the albumin concentration is constant, our data indicate that the concentration of phospholipid is not the only important variable (Figure 4). When more surfactant was present, the polarization values were much lower; however, equivalent concentrations of phospholipid in the two pools did not result in the same polarization value. Using artificial amniotic fluid matrices, Russell (3) has shown that polarization values are affected not only by albumin concentration, but also by the proportion of dipalmitoylphosphatidylcholine and dioleoylphosphatidylcholine. Dohnal et al. (7) have demonstrated, using DPH, that fluorescence polarization values decrease as the mole fraction of phospholipids with unsaturated fatty acids increase. This may help explain why equivalent concentrations of phospholipids from the two different pools did not have the same polarization and intensity values. It may also help explain the wide scatter we found correlating polarization with albumin/total phospholipid ratio.

Our correlation of polarization with the L/S ratio was similar to that seen by others (3, 10) but, as explained by Dohnal et al. (7), this may actually be ascribable to the increased concentration of unsaturated fatty acid chains on the phospholipids rather than to an increase in lecithin concentration.

As would be expected from knowing the effect of adding albumin to amniotic fluid, we found a slightly better correlation for polarization with the albumin/extractable phospholipid ratio than for phospholipids alone. It remains to be demonstrated how the probe interacts with albumin and whether other compounds such as bilirubin, free fatty acids, or drugs may compete for binding sites. The use of this assay and others involving similar fluorescent probes will be clinically more useful as we learn about the factors affecting polarization readings. Nevertheless, this assay holds considerable promise because it is so easily performed.

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References