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Surface activity, lipid profiles and their implications in cervical cancer

Preetha A, Banerjee R, Huilgol N[a]
School of Biosciences and Bioengineering, Indian Institute of Technology, Mumbai-400076, India. [a]Division of Radiation Oncology, Nanavati Hospital, Mumbai, India.

For correspondence:
R Banerjee,
E-mail:
rinti@cc.iitb.ac.in

ABSTRACT

Background: The profiles of lipids in normal and cancerous tissues may differ revealing information about cancer development and progression. Lipids being surface active, changes in lipid profiles can manifest as altered surface activity profiles. Langmuir monolayers offer a convenient model for evaluating surface activity of biological membranes.

Aims: The aims of this study were to quantify phospholipids and their effects on surface activity of normal and cancerous human cervical tissues as well as to evaluate the role of phosphatidylcholine (PC) and sphingomyelin (SM) in cervical cancer using Langmuir monolayers.

Methods and Materials: Lipid quantification was done using thin layer chromatography and phosphorus assay. Surface activity was evaluated using Langmuir monolayers. Monolayers were formed on the surface of deionized water by spreading tissue organic phase corresponding to 1 mg of tissue and studying their surface pressure-area isotherms at body temperature. The PC and SM contents of cancerous human cervical tissues were higher than those of the normal human cervical tissues. Role of PC and SM were evaluated by adding varying amounts of these lipids to normal cervical pooled organic phase. Statistical analysis: Student's t-test ($p < 0.05$) and one-way analysis of variance (ANOVA) was used.

Results: Our results reveals that the phosphatidylglycerol level in cancerous cervical tissue was nearly five folds higher than that in normal cervical tissue. Also PC and sphingomyelin SM were found to be the major phospholipid components in cancerous and normal cervical tissues respectively. The addition of either 1.5 µg DPPC or 0.5 µg SM /mg of tissue to the normal organic phase changed its surface activity profile to that of the cancerous tissues. Statistically significant surface activity parameters showed that PC and SM have remarkable roles in shifting the normal cervical lipophilic surface activity towards that of cancerous lipophilic component.

Conclusion: The Langmuir monolayer technique was sensitive to detect changes in tensiometric profiles of cervical cancers and these could be modulated by alterations in phosphatidylcholine and sphingomyelin levels. Therapeutic strategies may be designed to modulate these tensiometric profiles and lipid constituents of cancerous tissues.

Key words: Cervical cancer, Phospholipids, Surface tension

INTRODUCTION

A number of changes in the biochemical characteristics of malignant tissues have been reported. Various studies of tumour phospholipid metabolism have shown that the individual phospholipids and their degradation product levels in normal and tumour tissues are different. For example, in cultured colon cancer tissues, the phosphatidylcholine (PC) and phosphoglycerol (PGly) levels showed an elevation from the normal tissue whereas the glycerolphosphoglycerol (GPG), sphingomyelin (SM) and phosphatidylglycerol (PG) levels were decreased from normal.[1,2] The phosphocholine level in colon cancer tissues was depressed whereas these were elevated in brain, breast and liver cancer tissues compared to their normal tissues. As far as glycerol derivatives are concerned, the glycerophosphocholine (GPC) level in brain tumour tissue and glycerophosphoethanolamine (GPE) level in colon cancer tissues showed increase from their respective controls where as GPC in liver cancer tissue was shown to be significantly depressed from control value. The levels of individual phospholipids and their derivatives were significantly different in normal and cancerous tissues. Apart from the differences in phospholipids in cancerous and normal tissues, changes in phospholipid metabolism have also been correlated with the severity of various cancers.[3] However, no generalization of the lipid profiles in cancer and normal tissues is possible. The changes are specific to the type of cancer evaluated. Experiments to date suggest that not only biosynthetic pathways but also oncogene-induced activation of specific phospholipases probably contribute to the altered phospholipid profiles in tumour cells. The relative contributions

given by the different biosynthetic pathways to build up the profile alteration may vary according to the nature of the cells, the phase of cell growth and malignancy grade.[4]

The main function of phospholipids in mammalian cells is to maintain the structure of cellular membranes. Changes in lipid profiles of membranes alter fluidity, which in turn affects permeability of membranes. Alterations in membrane lipid levels can also influence cell proliferation and viability.[5] Phospholipids are well known for their amphiphilic nature and are surface active. Here we propose that interfacial tensiometric tools that monitor surface activity of phospholipid mixtures and organic extracts of tissues can be used as an extremely sensitive technique to reveal even subtle changes in the levels of phospholipids. Quantifying the phospholipid levels in normal and cancerous tissues and studying their interfacial properties may be of benefit in understanding changes in membrane properties and modulating these properties for therapeutic benefits. The aim of this study was to quantify the levels of different phospholipids in normal and cancerous cervical tissues and also to correlate the lipid levels with interfacial properties of the normal and cancerous tissues. The quantification of phospholipids was done by thin layer chromatographic technique followed by phosphorus assay. Pure lipids and their mixtures of known compositions were used to evaluate the potential of interfacial tensiometry in detecting lipid profile differences. We then studied the interfacial properties of cancerous and normal cervical lipid extracts and compared them with the lipid profiles of these tissues. We also added phosphatidylcholine and sphingomyelin to normal cervical lipid extract to modulate the lipid profiles of the tissues and studied their effects on tensiometric profiles. Thus the role of phosphatidylcholine and sphingomyelin in cervical cancer was evaluated in this study by using Langmuir monolayer technique.

MATERIALS AND METHODS

HPLC grade methanol, isopropyl alcohol and chloroform were purchased from Loba chemie, Mumbai., AR grade sodium chloride, potassium chloride, triethylamine malachite green, concentrated hydrochloric acid, polyvinyl alcohol, perchloric acid, ammonium molybdate methanol, acetone and potassium dihydrogenphosphate were purchased from SRL, Mumbai. Dipalmitoylphosphatidylcholine (DPPC), phosphatidylethanolamine (PE) and sphingomyelin (SM), all 99 % pure, were obtained from Sigma-Aldrich Co. (St. Louis, USA). Pre coated thin layer plates LK5 (silica gel) were obtained from E.Merck (India) Ltd. Mumbai. High purity water purified by a Milli Q Plus water purifier system (Milli pore, USA), with a resistivity of 18.2 MΩcm, was used in all experiments.

All cancerous samples were collected prior to start of radiotherapy and were of stage III squamous cell carcinoma. Normal controls of cervical tissues were obtained from hysterectomy patients having non-cervical disorders. All the normal cases were reported to be free from malignancy by histopathological analysis.

All tissue samples were washed thoroughly with normal saline, dried on tissue paper and weighed. The weighed samples were processed by using liquid nitrogen and dissolved in measured volumes of normal saline to obtain a tissue homogenate of known concentration. The organic phases containing lipophilic components of tissue homogenates were separated by using Bligh–Dyer extraction procedure.[6] The separated organic and aqueous phases were stored at –10 °C till experimentation. The organic phases of cancerous tissues were pooled to obtain pooled cancerous organic phase. Similarly organic phases of normal cervical tissues were separately pooled to obtain pooled normal organic phase.

The separation of phospholipids from the pooled organic phases of the cancerous and normal tissues was done by modified touchstone's thin layer chromatographic method. The mobile phase used was 30: 9: 25: 6: 18 (by volume) chloroform: methanol: isopropyl alcohol: 0.25 % potassium chloride: triethylamine. The silica gel plates (20 x 20 cm) were washed by using the mobile phase and heated in an oven at 150 °C for 30 minutes in order to reactivate. Desired volumes of pooled organic phases and standard phospholipids were applied to the activated plates by using 5 μl Hamilton micro syringe and air dried for 10 minutes for the evaporation of solvent. The developing chamber with the mobile phase was allowed to saturate for 10 minutes before development of plates. The spotted plates were placed in the developing chamber for 150 minutes and then air dried for 10 minutes and placed in a 180 °C oven for 5 minutes. The visualization of developed chromatogram was done by iodine vapor. About 30 mg iodine crystals were placed in a chamber and left to stand for 10 minutes. In iodine vapor, the phospholipids were yellow on a white background. On comparison with the separation of the standard, which was a mixture of all the phospholipids studied, the visualized spots were identified. Individual spots were scraped out carefully for estimation by phosphorus assay.

The phospholipid content in each spot was quantified separately by malachite green phosphorus assay procedure. Briefly, the scraped spot was digested with perchloricacid to obtain inorganic phosphorus from the phospholipids. To this, a solution containing malachite green (0.08 % in deionized water), ammonium molybdate (5.72 % in 6 N hydrochloric acid) and polyvinyl alcohol (2.32 % in boiling deionized water) in the ratio 2:1:1 (by volume) was added to develop the desired colour and then the resultant solution was centrifuged at 100 x g for 5 minutes to separate

the silica gel. The optical density of a known volume of the supernatant solution was measured at 640 nm in a UV-Visible spectrometer (Lambda 25, Perkin Elmer) using the photometric mode. The concentration of phosphorus in our lipid was determined from the calibration curve obtained by the above method using standard solutions of potassium dihydrogenphosphate having varying phosphorus concentrations. The amount of phosphorus in the lipid was multiplied by a factor 25 to obtain a measure of the total amount of phospholipid. The accuracy of this malachite green procedure was confirmed by quantification of a known weight of pure phosphatidylcholine and the error was found to be 1.5 %.

Monolayer studies were performed by using a computer controlled Langmuir Blodgett film balance (KSV Mini trough model, KSV Instruments, Finland). This technique consists of spreading an insoluble monolayer on the surface of an aqueous sub phase, which is filled in a specially designed thermostated trough. The trough is placed on an antivibration table, which is enclosed by an environmental chamber. The Teflon coated trough is equipped with two delrin barriers (for monolayer compression and expansion) and the entire trough is surrounded by a water jacket, providing temperature control. A wilhelmy plate balance with a platinum plate is used for sensing the surface pressure. On movement of the barriers, the monolayers formed can be compressed and expanded and changes in the orientation and packing of the molecules in the monolayer lead to changes in surface pressure which is sensed with the wilhelmy plate and a force transducer. [Figure 1] depicts the schematic representation of Langmuir Blodgett trough along with a standard surface pressure area isotherm showing the different molecular orientations on compression of the monolayer.

Before each monolayer experiment, the trough and barriers were thoroughly cleaned by organic solvents (methanol and acetone) and deionized water in sequence several times. Highly pure deionized water, having resistivity 18.2 MÙcm, was the subphase in all experiments. The temperature of the subphase was maintained at 37 ± 1 °C with the help of an external circulating water bath. The surface was cleaned with the help of an aspirator and a zero reading of the surface pressure ensured the cleanliness. Appropriate volumes of lipid/lipid mixture/cervical tissue lipid extract solutions in chloroform was spread as tiny droplets on the surface of the subphase using a Hamilton syringe. Thirty minutes wait time was given for the evaporation of the organic solvents. The plots of surface pressure versus area change are called isotherms. The surface pressure-area isotherms were recorded by continuous compression and expansion of the monolayer for three cycles (1 cycle = 1 compression + 1 expansion) with a barrier speed of 120 mm/min. The maximum relative area change during compression was 86.5 %.

Pure phospholipids DPPC, PE and SM as well as DPPC: PE

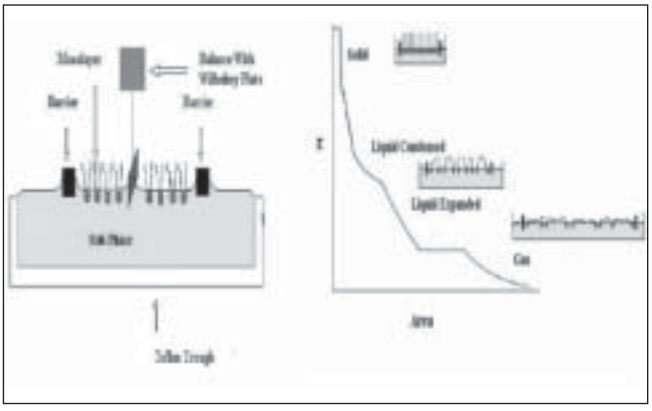

Figure 1: Schematic representation of Langmuir Blodgett trough and Surface pressure (π)- Area isotherm

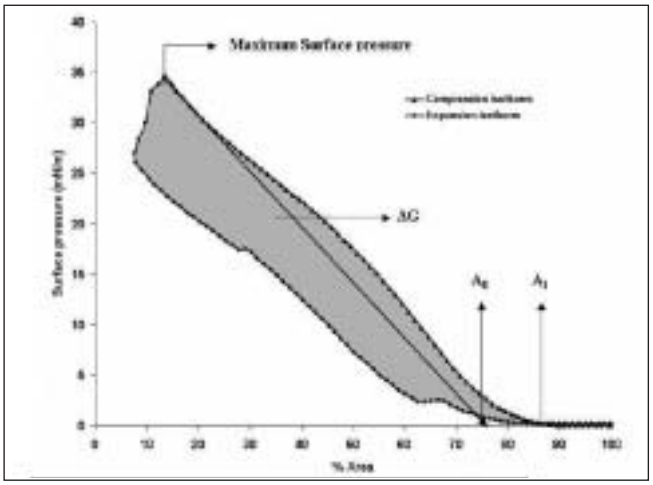

Figure 2: Schematic representation of tensiometric parameters A_0 and A_1 corresponding to the compression isotherm are shown in the figure and the shaded portion represents the hysteresis area (ΔG)

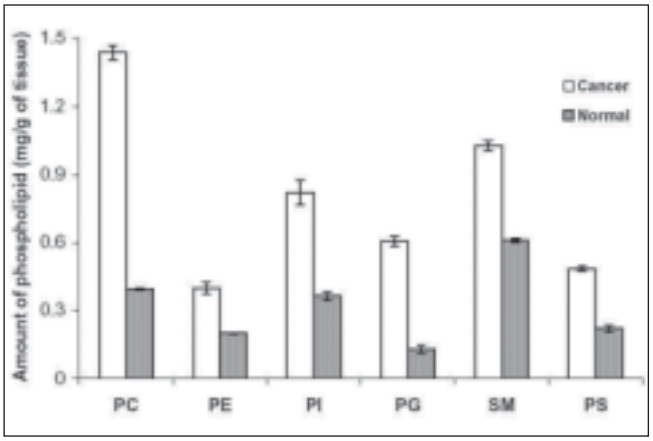

Figure 3: Phospholipid profiles of cancerous and normal human cervical tissues. Data are expressed as mean ± standard deviation. Each bar is a mean of three experiments. PC – phosphatidylcholine, PE - phosphatidylethanolamine, PI - phosphatidylinositol, PG - phosphatidylglycerol, SM – sphingomyelin and PS - phosphatidylserine

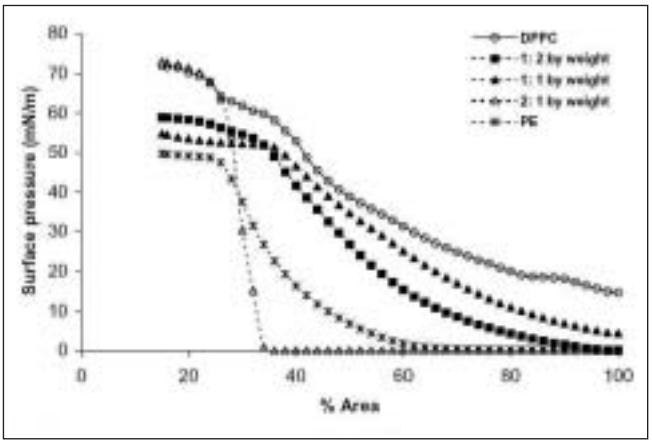

Figure 4: Surface pressure-area isotherms of DPPC: PE mixtures Only first compression isotherms recorded at 37 ºC are shown here. Each curve is a mean of three trials

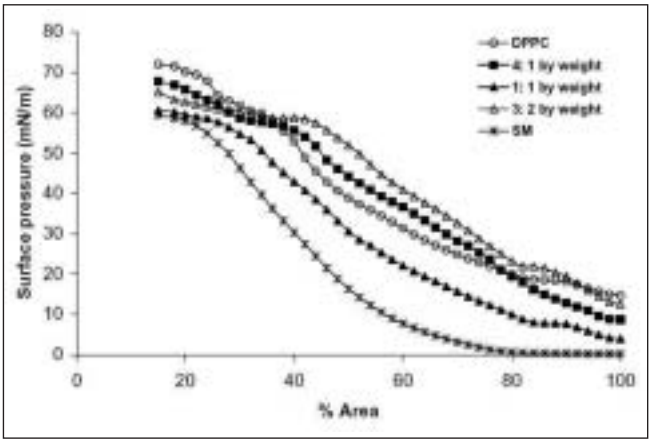

Figure 5: Surface pressure-area isotherms of DPPC: SM mixtures Only first compression isotherms recorded at 37 ºC are shown here. Each curve is a mean of three trials

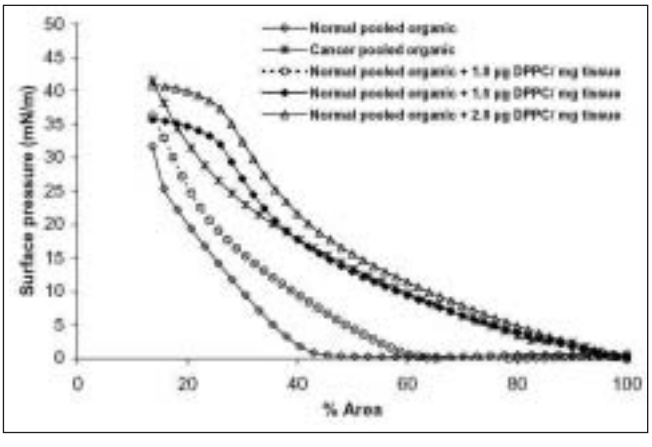

Figure 6: Effect of addition of DPPC to normal cervical organic phase Only first compression isotherms recorded at 37 ºC are shown here. Each curve is a mean of three trials

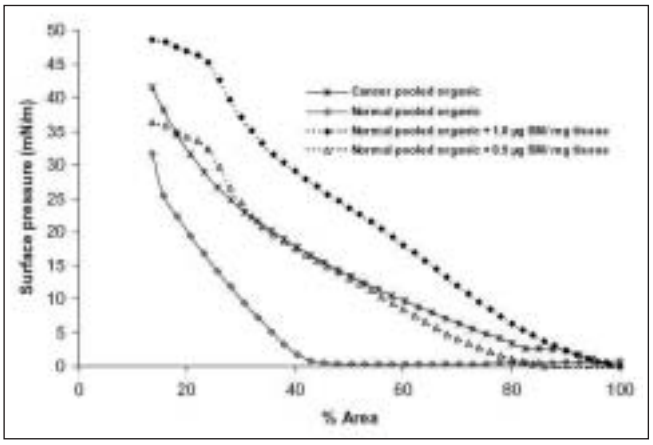

Figure 7: Effect of addition of SM to normal cervical organic phase Only first compression isotherms recorded at 37 ºC are shown here. Each curve is a mean of three trials

and DPPC: SM mixtures were characterized in this study. Lipid mixtures were prepared from their chloroform solutions (0.5 μg/ml) by mixing appropriate volumes. The ratios of DPPC: SM in our mixtures were 4:1, 1:4, 3:2, 2:3, and 1:1 (w/w) and those of DPPC: PE were 1:2, 1:1 and 2:1 (w/w). Monolayers of lipid/lipid mixtures with mean molecular area 100 $Å^2$ were used in our study. These experiments were performed to evaluate whether slight changes in composition of lipids could alter tensiometric profiles of the mixtures.

Based on the phospholipid quantification all the six phospholipids namely phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS) and sphingomyelin (SM), quantified in this study were lower in normal cervical tissues than in cancerous cervical tissues. Cancerous tissues had PC as the major phospholipid component whereas in normal cervical tissue the major component was SM. Also the surface activity of normal cervical organic phase was lower than that of the cancerous organic phase.[7] To elucidate the role of PC and SM in cervical cancer we evaluated the effects of variable amounts of phosphatidylcholine and sphingomyelin addition to normal cervical organic phase. The normal cervical tissues had a PC content which was lower than the cancerous by 1 ìg/mg of tissue and an SM content 0.5 μg/mg of tissue lower than the cancerous tissue. According to the above mentioned data we added 1, 1.5 & 2 μg of PC and 0.25 & 0.5 μg of SM to the normal organic phase corresponding to 1 mg of tissue and studied the modulation of tensiometric profile by PC and SM. In the case of cervical lipid extract monolayers; volumes corresponding to 1 mg of tissue were spread on to the sub phase to form a monolayer.

From the surface pressure-area isotherms obtained, the following parameters were calculated. The minimum surface tension (γ_{min}) was calculated as $\gamma_{min} = \gamma_s - \pi_{max}$ where π_{max} is the maximum surface pressure and $\tilde{a}_s$ is the surface tension of the subphase. Limiting area (A_0) lift off area (A_1) are quantities, which indicate molecular packing and interaction of molecules in the monolayer. The limiting area was determined by extrapolating the final steep linear region of the isotherm at end compression to the % area axis. The lift off area was obtained by extrapolating the area at which an increase in surface pressure from the baseline value was observed to the % area axis. The hysteresis area (ÄG), which is indicative of energy trapped in a monolayer [8] is the difference between the free energy of compression and free energy of expansion, and was calculated from the area under the corresponding surface pressure-area isotherms. A schematic representation of the tensiometric parameters is shown in Figure 2.

Ethics

Human biopsy specimens of cervical cancerous tissues and normal cervical tissues (n = 8, in each group) were obtained from the Radiation Oncology Division of Nanavati Hospital, Mumbai. The use of human tissue biopsies was approved by the ethical committee of the hospital and was in accordance with ethical standards as formulated in Helsinki declaration.

Statistics

All experiments were repeated thrice in this study and for each trial the tensiometric parameters were calculated as explained earlier. The data are expressed as mean ± standard deviation. Statistical comparison for differences was performed by student's t-test and the null hypothesis was rejected for $p < 0.05$. In the case of pure lipids and their mixtures statistical comparisons were done by one-way analysis of variance (ANOVA).

RESULTS

Figure 3 represents the phospholipid profiles of normal and cancerous cervical tissues. All the six phospholipids quantified in this study were found to be higher in cancerous cervical tissues compared to normal tissues. The PC, PE, PI, PG, SM and PS levels in cancerous cervical tissues were 3.6, 2.0, 2.3, 4.7, 1.7 and 2.2 times higher than those of normal cervical tissues respectively. In cancerous cervical tissues PC was the major phospholipid component contributing 30.1 % (weight %) of total phospholipids whereas in normal cervical tissue the major component was SM (31.7 % by weight of total phospholipids). Thus all the six phospholipid levels were significantly different in cancerous and normal cervical tissues.

In order to evaluate the potential of interfacial tensiometry in differentiating changes in lipid profiles, we evaluated surface activity profiles of pure lipids and their mixtures. DPPC: PE and DPPC: SM mixtures were evaluated in our study. Figure 4 depicts the surface pressure-area isotherms of DPPC, PE and their mixtures in different ratios. It can be noted from figure 2 that the tensiometric profiles of DPPC: PE mixtures depend on their composition and were different from those of the pure lipids. Isotherms of DPPC and 1: 1 DPPC: PE mixture showed rise in surface pressure value at the start of compression where as isotherms of PE, 2: 1 DPPC: PE mixture and 1: 2 DPPC: PE mixture showed horizontal regions till 70 %, 35 % and 95 % areas respectively prior to a rise in surface pressure. All the isotherms in figure 2 showed horizontal region below 25 % area. Unlike other isotherms, 2: 1 DPPC: PE mixture isotherm showed a sudden increase of surface pressure value below 35 % area. These isotherms depict the sensitivity of the Langmuir technique in detecting the graded changes in the amounts of PC and PE in the monolayers.

Figure 5 represents the surface activities of DPPC, SM and their mixtures in different ratios. Similar to DPPC: PE mixtures, here also the tensiometric profiles depend on the composition of the mixtures. For example, the minimum surface tensions of DPPC, SM, 1: 1, 4: 1 and 3: 2 DPPC: SM mixtures were 0 ± 0.2, 12.5 ± 0.1, 11.5 ± 0.1, 4.3 ± 0.2 and 6.9 ± 0.1 mN/m respectively and were significantly different as evidenced by one way ANOVA. All isotherms in figure 5, except that of SM, showed increase in surface pressure right from the beginning of compression whereas SM showed a horizontal region above 80 % area. In these mixtures the magnitudes of collapse pressures were directly proportional to the amount of DPPC in the mixture.

After establishing the potential of interfacial tensiometry to fingerprint different lipid profiles, by our model lipid mixture experiments, we evaluated the effect of addition of extra PC and SM to the pooled organic phase of normal cervical tissues. This was done to compensate the decrease in the levels of these lipids in normal tissues as compared to cancerous cervix. Figure 6 depicts the effect of DPPC addition to normal cervical organic phase. It was clear from figure 6 that addition of 2 μg of DPPC to normal organic phase corresponding to 1 mg of tissue produced an isotherm having same maximum surface pressure value as that of cancerous organic phase but the position of isotherm was slightly above that of cancerous one. Similarly addition of 1 μg DPPC to normal cervical organic phase produced an isotherm, which was below that of cancerous organic phase isotherm and 1.5 μg DPPC added isotherm was merging with that of cancerous one till 40 % area but reached a maximum surface pressure value slightly less than that of cancerous organic phase. On addition of 1.5 and 2 μg DPPC, the surface pressure started increasing right from the beginning of compression like the cancerous organic phase whereas in the normal organic phase and 1 μg DPPC added isotherms horizontal regions were observed till compres-

sion to 45 and 60 % areas respectively.

Figure 7 represents the effect of addition of varying amounts of sphingomyelin to normal cervical organic phase. Addition of 1 and 0.5 μg SM/ mg tissue produced horizontal regions in the respective isotherms below 30 % area of compression. Though 1 μg SM added isotherm of normal organic phase showed a rise in surface pressure right from the beginning of compression like that of cancerous isotherm, its position was far away from that of cancerous isotherm. But 0.5 μg SM added isotherm of normal organic phase was close to that of cancerous one and reached a similar low value of maximum surface pressure at end compression. The changes of tensiometric parameters of normal cervical organic phase due to addition of DPPC and SM are summarized in [Table 1]. A gradual shift in profiles was observed with the tensiometric profile of normal cervix changing towards that of the cancerous cervix on addition of 1.5 – 2 μg of PC/ mg tissue and 0.5 μg SM/ mg tissue.

DISCUSSION

We quantified the phospholipids in cancerous and normal human cervical tissues and established significantly different phospholipid profiles in them. Our phospholipid quantification revealed PC and SM as the major phospholipids in cancerous and normal human cervical tissues respectively. These results go hand in hand with the reported literature [9]. Also based on the phospholipid profiles of cervical tissues, we evaluated the role of PC and SM in cervical cancer through a monolayer study.

By using pure lipids and their mixtures we proved that the Langmuir monolayer technique could track the subtle changes in lipid composition. Monolayers of two sets of lipid mixtures namely DPPC/PE and DPPC/SM were evaluated in this study. The isotherms of both sets of mixtures were able to fingerprint the difference in composition. Similar to this, the surface activity of 2: 3 PC: PE mixture was reported to be different from those of the pure components by Banerjee *et al.* [10]. Rfler *et al.* also reported different surface pressure-area isotherms for mixtures of dilauroylphosphatidylcholine and dilauroylphosphatidyle- thanolamine in different ratios at 295 K.[11] The horizontal regions in surface pressure-area isotherms mostly represent phase transitions in the monolayers during compression. In both DPPC: PE and DPPC: SM systems all the mixtures as well as pure components showed liquid expanded (LE) to liquid condensed (LC) phase transition at the maximum compressed end of the isotherm. The gaseous to LE transitions were observed in PE, SM, 2: 1 and 1: 2 DPPC: PE mixtures at the maximum expanded end of the isotherm. Our results show that the Langmuir monolayer technique is a potential tool to track minute lipid composition changes as may be expected in cancerous tissues.

Our earlier results had revealed lower surface activity of the normal cervical organic phase compared to that of cancerous cervical lipid extract [7]. In the present study we added DPPC and SM to the normal cervical organic phase and compared the tensiometric profile with that of cervical cancerous organic phase monolayers to evaluate the role of PC and SM in cervical cancer. Addition of DPPC and SM to the normal organic phase made it more surface active and a phase transition was observed which might correspond to LE to LC transitions in the monolayer [12]. Addition of 2 and 1.5 μg DPPC and 1 μg SM removed the gaseous to LE transition in the normal organic phase monolayer. Monolayers having lower minimum surface tension are more closely packed and in the gel state as compared to more fluid monolayers having higher minimum surface tension [12]. Thus data in Table 1 indicates that DPPC and SM addition to normal pooled organic phase made the monolayers more rigid. The rigidity of the monolayers was found to be directly proportional to the amount of lipid added. A similar phenomenon of increase in rigidity of films by addition of cholesterol was reported in oleic acid /cholesterol mixed films by Romao *et al.* [13].

Also Table 1 revealed that the tensiometric parameters of 1.5 μg DPPC and 0.5 μg SM added normal organic phase monolayers were closer to those of cancerous organic phase. The achievement of a tensiometric profile close to that of cancerous profile by 1.5 μg DPPC and 0.5 μg SM added to the organic phase of normal cervical tissues once again indicates the remarkable role of these two lipids in cancerous tissue organic phase. In cancers changes in lipid levels are reported. For example, in cultured human colon cancer cells the PC level was reported to be 1.1 times higher than that of normal human colon cells [1]. Similarly the SM level

Table 1: Effect of DPPC and SM on surface activity of normal cervical organic phase monolayers

Monolayers	γ_{min} (mN/m)	A_1 (% area)	A_0 (% area)	ΔG (μJ)
Normal organic	40.6 ± 0.9	48.7 ± 1.3	32.9 ± 1.1	21.4 ± 1.4
Cancer organic	30.7 ± 1.2*	101.0 ± 1.4*	41.7 ± 0.7*	43.1 ± 2.1*
Normal organic + 2.0 µg DPPC	31.4 ± 0.1*	100.3 ± 2.1*	55.8 ± 1.8†*	89.6 ± 1.4†*
Normal organic + 1.5 µg DPPC	36.6 ± 0.2†*	102.0 ± 1.9*	42.5 ± 1.2*	44.6 ± 2.4*
Normal organic + 1.0 µg DPPC	36.2 ± 0.6†*	62.2 ± 1.6†*	36.0 ± 0.6†*	38.9 ± 1.9†*
Normal organic + 1.0 µg SM	23.7 ± 0.3†*	99.4 ± 1.3*	59.5 ± 1.5†*	74.8 ± 2.2†*
Normal organic + 0.5 µg SM	36.0 ± 0.1†*	86.6 ± 2.2†*	50.4 ± 1.6†*	42.8 ± 3.8*

*Values expressed as mean ± standard deviations and in all cases n = 3. * represents significantly different value compared to normal organic value and * represents significantly different value compared to cancer organic value. DPPC – Dipalmitoylphosphatidylcholine and SM - Sphingomyelin*

was shown to be elevated in human astrocytoma compared to the normal brain tissues [9]. Lipid composition determines the structure, function and integrity of biological membranes, and PC and SM particularly play a role in stabilizing the bilayer structure [14]. Our monolayer studies revealed that a small change in PC or SM level of the monolayer could markedly affect the monolayer fluidity and also PC and SM acts as monolayer rigidifiers. The changes in membrane fluidity, due to PC and SM levels, in turn might affect the permeability of the membranes in the cancerous state.

In conclusion, our study revealed that the phospholipid profiles of normal and cancerous cervical tissues were significantly different. Also phosphatidylcholine was found to be the major component of cancerous cervical tissue whereas in normal tissues, the main lipid was sphingomyelin. The difference in tensiometric profiles of normal and cancerous cervix could perhaps be due to changes in levels of these lipids. On addition of DPPC and SM to normal cervical monolayers there was a shift in the tensiometric profile towards that of the cancerous one. Also DPPC and SM behaved like rigidifying agents. Addition of 1.5 μg DPPC and 0.5 μg SM to normal organic phase corresponding to 1 mg of tissue produced tensiometric profiles closer to that of cancerous organic phase. Overall, the contributions and the interactions of specific constituents can be evaluated precisely by the Langmuir technique to obtain a better understanding of the complex interactions between the tissue components in cervical cancer. This can lead to development of strategies towards altering the lipid content or fluidizing the rigid cancerous tissue and modulating the interfacial properties for therapeutic benefit. The increased rigidity manifests as a lower surface tension and can reduce the penetration of drugs through such membranes. The role of fluidizers in reversing these rigidifying effects and improving drug penetration in cancerous tissues is postulated.

In this study phosphatidylcholine and sphingomyelin are shown to be the major phospholipids in cancerous and normal cervical tissues respectively with a significant elevation in all the phospholipid levels of cancerous ones in comparison with the normals. Dietary lipid levels may perhaps be related to these changes in lipid profiles and steps may be taken to try and modulate their levels. Addition of small amounts of PC and SM can markedly and adversely affect the fluidity of the cell membranes thus leading to change in membrane penetration properties. The rigidifying effect of these lipids in cervical cancer implies that there is scope of an improved drug penetration on use of fluidizing agents. Further, this also has implication in the development of drug carriers based on lipids. These can be optimized to contain fluidizing lipids that will alter the effect of the rigidifiers and improve drug penetration to cancer cells.

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