Effect of Anti-Leishmania Drugs on the Structural and Elastic Properties of Ultradeformable Lipid Membranes

Ma. Florencia Peralta,† Hannah Smith,‡ Diamond Moody,‡ Stephanie Tristem-Nagle,‡* and Dolores C. Carrer†

†Instituto de Investigación Médica M. y M. Ferreyra-INIMEC, CONICET-Universidad Nacional de Córdoba, Córdoba 5000, Argentina
‡Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States

ABSTRACT: Drugs for treating Leishmaniasis, a parasitic tropical orphan disease, currently have several limitations on their use, which topical treatments could alleviate. Topical treatment requires penetration of drugs deep into the skin, which is aided by encapsulation within ultradeformable liposomes. Penetrability depends on the flexibility of the lipid membrane, which may be affected by the drugs. We have studied the biophysical effects of four anti-Leishmania drugs (miltefosine (Milt), amphotericin B (AmpB), indole (Ind), and imiquimod (Imiq)) on a soy phosphatidylcholine/sodium cholate membrane. Using diffuse X-ray scattering techniques, we determined bending modulus ($K_C$) and chain order parameter ($S_{X-avg}$) of the membrane at several drug concentrations. Form factor scattering data allowed construction of electron density profiles, which yielded bilayer thickness and area per lipid. Results show that AmpB had the largest effect on $K_C$ and $S_{X-avg}$ causing the bilayer to lose integrity at high concentrations. Imiq and Ind induced slight membrane stiffening, whereas Milt had little effect. Imiq also notably decreased chain order at high concentrations. These results will aid in the design of new topical treatments, where Milt, Ind, and Imiq could be used at any concentration without affecting liposome integrity or physical properties, whereas AmpB should not be used at high concentrations.

1. INTRODUCTION

Cutaneous Leishmaniasis (CL) is a parasitic disease endemic in 88 countries, with an annual incidence of 0.7–1.2 million cases.¹ It is listed by the World Health Organization as one of the six most important orphan diseases because of the increasing number of new cases reported each year, the scarcity of affordable adequate treatment, and the lack of interest of big pharmaceutical companies.² CL produces lesions in the form of ulcers, nodules, papules, and plaques on exposed body parts, which can be unique or multiple (diffuse CL). In America, 5–10% of the patients with CL develop Mucocutaneous Leishmaniasis, which destroys the mucosa of the nose, mouth, and pharynx.³ In immunocompromised patients, CL can develop into the most severe visceral form of the disease, which can be fatal.⁴

Most existing anti-Leishmania drugs are used systemically, but have serious side effects that limit their use, including nephro- and hepatotoxicity or damage to the pancreas and bone marrow.⁵ The purpose of treating CL is to prevent the progression into more serious, even fatal forms, and to diminish the disfiguring scars it causes. A topical treatment has the great advantages of fewer adverse effects, better patient compliance, lower costs, and greater feasibility of use in a rural environment, where the presence of trained medical personnel is scarce. Thus, the development of formulations for topical use is critical.

Since Leishmania parasites localize in the dermis below the lesion in the skin, the drugs must be able to penetrate through the stratum corneum and epidermis to reach the dermis and to be effective. The use of ultraflexible liposomes is advantageous, since they have been proven to function as transepidermal penetration enhancers.⁶ Also, due to their physical properties, they can be useful for dissolving and carrying hydrophobic, hydrophilic, and amphiphilic drugs. This is important since most of the anti-Leishmania drugs have poor water solubilities and/or form unstable solutions. Ideal lipid mixtures for transepidermal drug penetration enhancement should be in the fluid phase at skin temperature (35 °C). The flexibility of the liposomal membrane has been shown to be an important modulator of penetration into the skin.⁷ To increase membrane flexibility, a detergent is normally incorporated in the lipid mixture.

In this work, we have used X-ray diffuse scattering of fully hydrated, oriented multilayers on a solid substrate to study the effect of the addition of four different anti-Leishmania drugs on the elasticity and structure of membranes composed of soy phosphatidylcholine (SoyPC) and sodium cholate (NaChol). This mixture has been shown to be highly flexible and to act...
efficiently as a transdermal drug penetration enhancer. The four drugs are indole (Ind), imiquimod (Imiq), amphotericin B (AmpB), and miltefosine (Milt), of which AmpB and Milt are currently in clinical use for parenteral and oral treatments, respectively.

Since AmpB is insoluble in water, the first formulations were composed of AmpB and deoxycholate (Fungizone). However, this drug is very toxic and its side effects include, in the acute stage, vomiting, rigor, fever, hypertension or hypotension, and hypoxia. Its principal chronic adverse effect is nephrotoxicity. A liposomal formulation that significantly reduces the toxicity has been developed for intravenous use (Ambisome). Serious side effects are however still produced by this formulation (anemia, nephrotoxicity, and gastrointestinal hemorrhage). The lipid mixture used in Ambisome contains this formulation (anemia, nephrotoxicity, and gastrointestinal hemorrhage).12 The lipid mixture used in Ambisome contains..12

Phosphatidylglycerol imparts a net negative charge to the liposomal membrane, diminishing the probability of interliposomal fusion. Mixed with long saturated lipids, cholesterol also induces an increase in liposomal fluidity at temperatures below the phase transition of the lipid mixture and increases the interaction of amphotericin B with the bilayer (AmpB has a high affinity for sterols, in particular ergosterol, as well as cholesterol). Clearance of liposomes from circulating blood is dependent on many factors, but in general, smaller liposomes have longer lifetimes, which is why preventing aggregation and fusion is important when formulating liposomes to be used intravenously.

In our case, however, the liposomes are not designed to survive in the circulating blood but to act as penetration enhancers through the skin. Thus, we used a lipid mixture designed to be as flexible as possible, since as mentioned above, the flexibility of the membrane is crucial for skin penetration. Ultraflexible liposomes can be prepared by mixing low-melting-temperature lipids with an "edge activator," usually a detergent. The presence of charge is useful to prevent aggregation and fusion of the liposomes. For these reasons, we chose to work with a mixture of SoyPC (mainly unsaturated lipids, low melting temperature) and NaChol, a negatively charged bile salt that acts as a detergent and has been proven to increase membrane flexibility when added to liposomes. To this lipid mixture, we added drugs with potential therapeutic use against Leishmaniasis in increasing quantities, AmpB in the first case.

A commercial imiquimod cream (Aldara), designed for topical treatment of genital warts, has been used to treat CL in mice. It reduced the size of the lesions and the parasitic charge; however, the infections were not completely cleared. This may be because Imiq activates macrophages close to the skin surface but cannot penetrate more deeply, where parasites are actively proliferating. Other groups improved the effect of Glucatime, the common meglumine antimonite treatment, with application of Aldara. However, the use of the highly toxic antimotionals is still necessary. In this scenario, we propose the use of liposomes to enhance Imiq penetration through the skin, avoiding the combination with other drugs.

There are currently two commercial miltefosine formulations, one for oral administration (Impavido) and other for topical administration (Miltex). The oral formulation is used for CL treatment, with serious side effects like teratogenicity, vomiting, and diarrhea. The topical formulation is used for malignant cutaneous lesions in breast cancer. These formulations have been tried for topical treatment of CL in mice and humans with an incomplete effect on parasitic charge. This lack of effectiveness may be due to an inability of the formulation to induce Milt penetration into the skin.

The addition of drugs to SoyPC/NaChol can change the properties of the membranes used as a drug vehicle, which could alter their ability to penetrate the skin. Our X-ray diffuse scattering results allow us to obtain the bending modulus (Kc), the hydrocarbon chain order parameter (Sx), and electron density profiles (EDPs) of the mixtures. These yield information about membrane stiffness, membrane chain order, thickness of the membrane, and area/lipid. We have thus obtained a global picture of both the membrane structure and flexibility. We expect this information to be useful for the formulation of a liposomal-based topical treatment for CL.

2. MATERIALS AND METHODS

2.1. Reagents. The naturally occurring lyophilized lipid soy l-α-phosphatidylcholine (purity > 99%) and Milt (MAPCHO-16, n-hexadecyl-phosphocholine) were purchased from Avanti Polar Lipids (Alabama, AL) and used as received. Thin-layer chromatography showed that the SoyPC ran as a single spot with <0.1% lysolecithin before experiments. All experimental procedures were designed to minimize lipid oxidation, but this was not specifically tested. The X-ray experiments caused ~1% lysolecithin formation. NaChol, Ind, Imiq, AmpB, and high-performance liquid chromatography-grade organic solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. The chemical structures of SoyPC and NaChol are shown in Figure 1. The configuration of SoyPC in Figure 1 is only one of the many possible structures: 18:2 represents ~63% of fatty acids.

2.2. Sample Preparation. Membrane mimics were prepared at the Cornell High Energy Synchrotron Source (CHESS) by first dissolving lyophilized SoyPC, NaChol (6:1 w/w, 3:3:1 mole ratio), and the drugs in organic solvent stock solutions. SoyPC was dissolved in chloroform (200 mg/mL), NaChol in methanol (100 mg/mL), Milt and Ind in trifluoroethanol (3 and 1 mg/mL, respectively), and AmpB...
and Imiq in hexafluoro-isopropanol (5 and 2 mg/mL, respectively). Four different lipid/drug mole ratios were tested for each drug (200:1, 20:1, 10:1, and 5:1) corresponding to 0.005, 0.05, 0.1, and 0.2 mol fractions. Appropriate volumes of lipid and drug stock solutions were mixed to obtain a final volume of 200 μL, containing 3.43 mg of SoyPC and 0.57 mg of NaChol. This mixture was used to prepare multilamellar stacked samples for X-ray scattering by plating onto the surface of a 1.5 × 3 cm² silicon wafer using the rock and roll procedure in a hood. The samples were then dried under −30 in. Hg vacuum for at least 2 h and trimmed to a 0.5 × 3 cm² strip centered on the silicon wafer. Previous NMR experiments have indicated that 2 h of evaporation removes samples’ solvent to at least 100:1 mole ratio lipid/solvent. Dried, 10 μm thick oriented multilayer samples were X-ray treated in a hydration chamber, which permits full hydration through the vapor. All samples achieved full hydration in less than 1 h, as evidenced by a static bilayer lamellar repeat D-spacing in the stack. The D-spacing includes the bilayer thickness plus water layer between the bilayers.

Aqueous samples for densimetry were prepared and analyzed according to ref 22. The concentration of SoyPC/NaChol (6:1 w/w) was 38 mg/mL, which was weighed to 0.1 mg using an analytical balance (Mettler AE163).

2.3. X-ray Scattering. Low-angle X-ray scattering (LAXS) data from the oriented, fully hydrated samples were obtained at the G1 line at CHESS using previously described methods (X-ray wavelength = 1.096 Å, S-distance (sample-to-detector distance) = 396.6 mm). Wide-angle X-ray scattering (WAXS) data were also obtained at CHESS (S-distance = 179.3 mm).

The elastic properties of the oriented, hydrated membranes were studied by determining the bending modulus Kc from the q₁ dependence of the second and third lobes of X-ray diffuse scattering and applying liquid crystal theory using the NFit program according to ref 23. The bending modulus is synonymous with the curvature-elastic modulus Kc. All measurements were carried out in the fluid phase at 37 °C. Values of Kc are averages taken at three different sample positions for each drug concentration. Electron density profiles (EDPs) of the samples were calculated by first obtaining the continuous form factor from the square root of the diffuse scattering intensity divided by the structure factor of the interacting stacked bilayers. Then, by measuring the molecular volumes (Table 2), the scattering density profile program allows calculating EDPs and membrane structural parameters, such as molecular area and membrane bilayer thickness. Hydrocarbon chain order parameters were estimated by measuring the angular dependence of the interchain WAXS signal according to the model developed by Mills et al. using a Matlab program, also based on liquid crystal theory. SX-ray is a chain order parameter that differs from the more commonly used NMR-derived order parameter by a constant factor of 1.35. The values of SX-ray are the averages of two different sample positions for each drug concentration. All results were plotted using OriginPro 2016.

3. RESULTS

3.1. LAXS Diffuse Scattering. LAXS images were taken of oriented, fully hydrated stacks of controls and drug-containing lipid membranes at 200:1, 20:1, 10:1, and 5:1 mole ratios,
Figure 3. Two-dimensional CCD images of WAXS data collected at 37 °C at CHESS. (A) SoyPC control, (B) SoyPC/NaChol:AmpB (200:1 mole ratio), (C) SoyPC/NaChol:Milt (200:1 mole ratio), (D) SoyPC/NaChol control, (E) SoyPC/NaChol:Imiq (200:1 mole ratio), and (F) SoyPC/NaChol:Ind (200:1 mole ratio). In all images, the beam stop (dark rectangle on the bottom left) covers the beam and the first two lamellar orders. The thin, white vertical line is the X-ray reflectivity from the underlying silicon wafer. The 200:1 mole ratio corresponds to 0.005 mol fraction.

Table 1. Bending Moduli (\(K_C\)) and Chain Order Parameters (\(S_{X\text{e}xy}\))

<table>
<thead>
<tr>
<th></th>
<th>SoyPC</th>
<th>SoyPC/NaChol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_C (\times 10^{-22} J))</td>
<td>4.17 ± 0.33</td>
<td>2.26 ± 0.60</td>
</tr>
<tr>
<td>(S_{X\text{e}xy})</td>
<td>0.250 ± 0.033</td>
<td>0.089 ± 0.015</td>
</tr>
</tbody>
</table>

The Journal of Physical Chemistry B

DOI: 10.1021/acs.jpcb.8b04001

NaChol was used as the control for the drug studies since it represents the “empty” liposome vehicles.

Drugs caused additional changes in membrane elastic properties and structure, as exhibited by \(K_C\) (Figure 4A) and \(S_{X\text{e}xy}\) (Figure 4B) of the SoyPC/NaChol membrane at different drug concentrations. AmpB showed the most dramatic changes in both \(K_C\) and \(S_{X\text{e}xy}\) parameters. First, by significantly stiffening the membrane by a factor of \(~2\) at the lowest concentration of 0.005 mol fraction, AmpB caused extreme softening at higher concentrations. Similarly, AmpB ordered chains at 0.005 mol fraction and disordered chains at every higher mole fraction. Because of sample misorientation, the \(S_{X\text{e}xy}\) data were not analyzable at higher concentrations. Imiq caused slight stiffening at all concentrations. However, chain ordering was increased only at 0.005 mol fraction and then decreased at higher concentrations. While Ind showed \(K_C\) behavior similar to Imiq, \(S_{X\text{e}xy}\) showed slight chain ordering at all concentrations. Milt had a small effect on both \(K_C\) and \(S_{X\text{e}xy}\) parameters, showing a slight decrease in \(K_C\) and a slight increase in \(S_{X\text{e}xy}\) with values very similar to those induced by Ind.

3.4. Structural Results. To create absolute electron density profiles (EDPs), we first measured the volumes of the lipids and detergent separately and then the mixture using the Anton-Paar 5000M densimeter as in ref22. These volumes
are shown in Table 2. We used the headgroup volume of 331 Å³ for PC lipids.28

<table>
<thead>
<tr>
<th>sample</th>
<th>volume (Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoyPC</td>
<td>1289</td>
</tr>
<tr>
<td>NaChol</td>
<td>563</td>
</tr>
<tr>
<td>SoyPC/NaChol</td>
<td>1120</td>
</tr>
</tbody>
</table>

Figure 5 shows the EDPs for SoyPC and SoyPC/NaChol, with the structural results summarized in Table 3. As shown, the area which represents the SoyPC increases slightly with the addition of NaChol to SoyPC. While the apparent headgroup-to-headgroup thickness $D_{\text{HH}}$ decreases by ~8 Å, a more accurate measure of the membrane thickness is $2D_{\text{C}}$, the hydrocarbon thickness, which decreases by ~3 Å. Thus, the addition of NaChol to SoyPC membranes induces a larger area/lipid and a thinning of the membrane.

Similar EDPs were constructed for SoyPC/NaChol membranes containing drugs (data not shown). The structural results from these EDPs are summarized in Figure 6a (Ind), Figure 6b (Imiq), Figure 6c (AmpB), and Figure 6d (Milt).

Figure 4. Effect of drugs on (A) bending modulus ($K_C$) and (B) chain order parameter ($S_{X-ray}$) of SoyPC/NaChol membranes. See legend for drug details. The mole ratios correspond to 200:1, 20:1, 10:1, and 5:1.

4. DISCUSSION

4.1. Elasticity and Structural Results. The intent of studying the interactions of anti-Leishmania drugs with liposomal carriers is to determine the utility of each as a topically administered formulation. To understand how the lipid/drug mixtures behave in the skin, it is important to focus on how each drug modifies the SoyPC/NaChol membrane structure and flexibility, since these parameters will have an impact on liposome–skin interaction. In particular, flexibility of the membrane correlates well with the ability of liposomes to function as drug carriers/penetration enhancers.29 We used LAXS and WAXS data to determine the values of $K_C$ and $S_{X-ray}$, which indicate the membrane stiffness and chain order of the bilayer, respectively. As shown in Table 1, the value of $K_C$ for SoyPC ($4.2 \times 10^{-20}$ J) is lower than that of many monounsaturated lipids (see https://www.cmu.edu/biolphys/jfstn with summary table on left). This could be caused by the duo-unsaturation in ~63% of fatty acid chains, since ref 30 found di18:2PC to have a $K_C$ value of $4.4 \times 10^{-20}$ J using micropipette aspiration. The addition of NaChol significantly decreased both $K_C$ and $S_{X-ray}$, signifying that the membrane was softer and more bendable (factor of ~2) and less ordered (factor of ~3) when containing this detergent. This softening result is in agreement with a previous NMR investigation of lipids containing a nonionic surfactant.31 From the electron density profiles, NaChol also increased the area/unit cell ($A_{\text{UC}}$) (~33%) and decreased the bilayer thickness ($2D_{\text{C}}$) (~11%) (Table 3). The unit cell contains SoyPC plus NaChol (107.5 Å² total area). The area of SoyPC that we obtained (80.6 Å²) was large compared to the area of many other PC lipids. One previously studied lipid, the branched-chain diphytanoylPC, had a similarly large area of 80.5 Å² and a low $K_C$ of $5.2 \times 10^{-20}$ J.32 The larger area in SoyPC could have resulted from the two double bonds in both chains in 63% of components of SoyPC, which caused a thinning of the bilayer (23.7 Å compared to 27.1 Å for POPC).21

Vesicles composed of a low-melting-temperature lipid and a detergent have been shown to be efficient enhancers for transepidermal/transdermal penetration of different drugs.19,33 There are controversial results about how liposomes penetrate the skin: some evidence shows that liposomes break down upon contact with the stratum corneum, i.e., they act as penetration enhancers, but do not penetrate the skin intact.34

whereas other authors confirm that ultradeformable lipid vesicles can penetrate unfragmented.29

4.2. Indole. Using NMR spectroscopy, a previous experiment found that Ind remained primarily in the headgroup region and upper chain region, independently of the chosen lipid.35 Our data indicate that the stiffness and chain order of the membrane increased only slightly from control for all Ind concentrations. This stability makes Ind an ideal choice for drug delivery because it suggests that the addition of Ind will not disturb the drug vehicle. Thus, the liposome should retain the high flexibility required. This agrees with findings of other experiments that show that the addition of Ind does not significantly alter the physical properties of the vehicle or the liposome diameter.36

4.3. Imiquimod. Imiq presents many challenges to skin penetration due to its water insolubility. Other studies have shown that the retention rates in the epidermis and dermis combined remain below 0.5% when many diverse solutions saturated with Imiq are topically applied.37 Accumulation of Imiq in skin was found to relate directly to penetration of its solvent vehicle. One study showed that significantly higher drug penetration was seen in both the dermis and epidermis for Imiq encapsulated in SoyPC/NaChol, resulting in a combined retention rate of around 10%, a rate which was also seen with Imiq in N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid buffer.38 In both cases, more drug is found in the epidermis than in the dermis. Using SoyPC alone, the penetration rate drops to around 3%, with almost no Imiq found in the dermis.36 We suggest that since Imiq causes only slightly stiffer membranes as well as disordered chains, it would also be a good choice for topical drug delivery in SoyPC/NaChol liposomes.

4.4. Amphotericin B. Kiryakova et al. found that the $K_C$ values of (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine) SOPC membranes and SOPC membranes with 40 mol % cholesterol both decrease with the addition of AmpB.38 They used the thermally induced shape fluctuation method to analyze their data, so their method for obtaining $K_C$ values was different from ours. Generally, absolute $K_C$ values obtained with the shape analysis are ~1.5 times greater than the values obtained using diffuse X-ray scattering.39 We note in Figure 7 that our $K_C$ values are much lower than Kiryakova’s values, which is also attributable to the fact that our samples contain SoyPC and a detergent rather than pure SOPC or pure SOPC plus cholesterol. The important point is that in both studies, the effect of AmpB on $K_C$ is being studied. We found that the $K_C$ value increased for our lowest drug concentration (200:1 mole ratio, 0.005 mol fraction) and then decreased with the addition of more drug. For their SOPC membrane, the $K_C$ value decreased by ~17%, and for the SOPC/cholesterol membrane, the $K_C$ value decreased by ~16% both with 3 mol % of AmpB. We found that the $K_C$ value decreased for the range of 0.5–5 mol % by ~49%. So, the general trend, although not the absolute $K_C$ values of our results, agrees with that observed by Kiryakova et al.38:

In addition, our results show a parallel behavior between the bending modulus and the order parameter: AmpB first stiffened the SoyPC/NaChol membrane at 200:1 mole ratio and then decreased with the addition of more drug. For their SOPC membrane, the $K_C$ value decreased by ~17%, and for the SOPC/cholesterol membrane, the $K_C$ value decreased by ~16% both with 3 mol % of AmpB. We found that the $K_C$ value decreased for the range of 0.5–5 mol % by ~49%. So, the general trend, although not the absolute $K_C$ values of our results, agrees with that observed by Kiryakova et al.38:

Table 3. Structural Results for SoyPC and SoyPC/NaChol Controls

<table>
<thead>
<tr>
<th>sample</th>
<th>area (Å²)</th>
<th>$2D_C$ (Å)</th>
<th>$D_{HH}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoyPC</td>
<td>80.6 ± 0.4</td>
<td>23.7 ± 1.4</td>
<td>34.9 ± 0.1</td>
</tr>
<tr>
<td>SoyPC/NaChol</td>
<td>82.4 ± 1.4</td>
<td>21.0 ± 0.4</td>
<td>27.1 ± 0.5</td>
</tr>
<tr>
<td>SoyPC/NaChol b</td>
<td>107.5 ± 1.8</td>
<td>21.0 ± 0.4</td>
<td>27.1 ± 0.5</td>
</tr>
</tbody>
</table>

*a* Area represents the area/lipid in the presence of NaChol.

*b* Area represents the lipid + NaChol area (unit cell).

Figure 6. Summary of structural parameters as a function of drug concentration. (A) Ind, (B) Imiq, (C) AmpB, and (D) Milt added to SoyPC/NaChol. The left axis shows area/unit cell (red), and the right axes show $D_{HH}$ (blue) and $2D_C$ (cyan). Area per unit cell is in units of Å², and $D_{HH}$ and $2D_C$ are in units of Å.
In our model the lipid membrane is too small to yield a clear positional result. Their findings are in agreement with our results, showing that the bilayer fluidity was not significantly changed with the addition of Milt.

Our results show that Milt had a small effect on $K_C$ as well as on $S_{X-ray}$. Molecular dynamics studies have shown that Milt has a high propensity to permeate bilayers with multiple double bonds. This allows for passive transport and minimal disruption of the physical properties of the bilayer, which agrees with our results that the lipid bilayer stiffness is only slightly affected when Milt is added. Like Ind, this is advantageous for drug delivery because high Milt concentrations will not disturb the liposomes.

4.5. Miltefosine. A previous experiment measured the fluidity of unilamellar vesicles of DPPC using electron paramagnetic resonance spectroscopy and fluorescence methods. They found that adding very high mole ratios of Milt did not dramatically change the fluidity of the vesicle in the fluid phase. An effect was not observed until 25 mol %, where there was a small decrease in the bilayer fluidity (3:1 mole ratio). Their findings are in agreement with our $S_{X-ray}$ findings, showing that the bilayer fluidity was not significantly changed with the addition of Milt.

Our results show that Milt had a small effect on $K_C$ as well as on $S_{X-ray}$. Molecular dynamics studies have shown that Milt has a high propensity to permeate bilayers with multiple double bonds. This allows for passive transport and minimal disruption of the physical properties of the bilayer, which agrees with our results that the lipid bilayer stiffness is only slightly affected when Milt is added. Like Ind, this is advantageous for drug delivery because high Milt concentrations will not disturb the liposomes.

5. CONCLUSIONS

Relatively few labs are able to determine both the bending modulus and chain order parameter of a membrane. Through our international collaboration, we were able to exploit the pioneering technique of diffuse X-ray scattering to gain insight into the structure of the lipid membrane in the presence of anti-Leishmania drugs. We suggest that the drug-induced changes in membrane structure and elastic properties will aid in understanding liposomes designed to act as transepithelial penetration enhancers by correlating with their efficacy in CL treatment. One invaluable consequence of such correlations is the possibility of estimating a drug’s effectiveness based on its known properties before beginning extensive research and trials. The most relevant area of application for the purposes of this paper is the penetration of drugs in a lipid vehicle through the skin. SoyPC/NaChol is useful as a vehicle in topical drug delivery because of its low bending modulus. Drugs incorporated into the liposomes can affect this flexibility, as our present work shows. This effect is very likely to affect the efficacy of SoyPC/NaChol as a drug carrier/penetration enhancer. The shelf stability of the formulations as regards size, lamellarity, and solubility is also extremely important for their usefulness. Major increases in lipid area and/or substantial decreases in chain order imply loss of lipid vehicle structural integrity, possibly decreasing their effectiveness and shelf stability. We suggest that Ind and Milt, which had few disruptive effects on chain order and/or lipid area, should be pursued as potential options for topical liposomal treatment of Leishmaniasis. However, at appropriate concentrations, Imiq and AmpB also show potential for liposomal delivery. Although this study is focused on topical treatments for Leishmaniasis, knowledge of how these four drugs impact structure of ultraflexible liposomes may have broader implications for a range of diseases and oral, intravenous, or other methods of treatment administration.

AUTHOR INFORMATION

Corresponding Author
E-mail: stn@cmu.edu. Tel: 412-268-3174.

ORCID
Ma. Florencia Peralta: 0000-0001-8468-6828
Stephanie Tristram-Nagle: 0000-0003-2271-7056

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was financed by grants from Bunge y Born Foundation and SeCyT-UNC to D.C.C. and from the Samuel and Emma Winters Foundation to S.T.-N. M.F.P. is a Ph.D. Fellow of CONICET, and D.C.C. is a Career Researcher of CONICET. The authors thank Dr. John F. Nagle and Dr. Fernando Dupuy for helping with equipment setup and use of computer programs. This work is based on research conducted at the Cornell High Energy Synchrotron Source (CHES), which is supported by the National Science Foundation under award DMR-1332208.

REFERENCES