Partitioning of Anti-inflammatory Steroid Drugs into Phosphatidylcholine and Phosphatidylcholine-Cholesterol Small Unilamellar Vesicles as Studied by Second-Derivative Spectrophotometry

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Received December 5, 2007; accepted February 8, 2008; published online February 14, 2008

The partition coefficients ($K_p$) of six anti-inflammatory steroid drugs, dexamethasone (DMS), betamethasone (BMS), triamcinolone acetonide (TCLA), flucinolone acetonide (FCLA), betamethasone 17,21-dipropionate (BMSDP), and clobetasol propionate (CBSP), for phosphatidylcholine (PC), and PC-cholesterol small unilamellar vesicles (SUVs) were determined by a second-derivative spectrophotometric method. The $K_p$ values were obtained with a relative standard deviation of below 10% and the following order was observed: BMS $<$ DMS $<$ TCLA $<$ FCLA $<$ BMSDP $<$ CBSP. BMSDP which has a structure that the two hydroxyl groups of BMS were esterified with propionic acid showed a largely enhanced $K_p$ value of 10.5 times that of BMS. Further, replacement of a propionate group in BMSDP with a chlorine atom resulted in the highest $K_p$ value (CBSP) within the drugs examined, i.e., the $K_p$ value of CBSP was 1.2 times that of BMSDP. The presence of 30 mol% cholesterol in the SUV bilayers reduced these $K_p$ values to approximately 35–50% of those values for the PC SUVs, although the order of the $K_p$ values remained unchanged. The order of the $K_p$ values agreed with that of the reported dermatological therapeutic potency of these drugs, although the order of their log $P$ values for n-octanol/water systems showed a discrepancy. Our results indicate that the potency of steroid drugs in dermatological treatments depends to some extent on the $K_p$ values of the drug, that is, the affinity of steroid drugs for PC bilayers influences their clinical potency, since potency is related to transdermal absorption.

Key words steroid drug; partition coefficient; liposome; second-derivative spectrophotometry; dermatological therapeutic potency

Synthetic glucocorticoids (anti-inflammatory steroid drugs) are widely used in the treatment of many kinds of inflammatory and allergic diseases. Following administration, these drugs pass through the cell membrane and exert their action by associating with glucocorticoid receptors (GRs) in cells.1 The pharmacological action of anti-inflammatory steroid drugs is influenced by ability of GRs to discern between different drug structures. The crucial factor in the therapeutic potency of anti-inflammatory steroid drugs is the binding with the GRs in cells, also, the partitioning of this class of drugs into lipid bilayer membranes plays an important role in potency, as such partitioning affects drug concentrations in the vicinity of GRs.

Partition coefficients of drugs between lipid bilayer vesicles (liposomes) and water provide fundamental information related to drug interactions with biomembranes. In particular, most drugs usually partition into the cell membrane via passive diffusion; such information about drug partitioning has enhanced our understanding of the pharmacodynamics and pharmacokinetics of drugs. Quantitative structure–activity relationship studies of drugs have suggested that the partition coefficients obtained for liposome/water systems are more useful than those obtained for n-octanol/water systems.

We have reported that second-derivative spectrophotometry is an important and useful analytical technique that can be applied to determine the partition coefficients ($K_p$) of certain psychotropic phenothiazine20 and benzodiazepine21 drugs between phosphatidylcholine (PC) small unilamellar vesicles (SUVs) and water, without necessitating the troublesome separation procedures required to eliminate the SUVs, which cause strong light-scattering. Thus, the derivative method has been used in several studies to determine the partition coefficients of drugs between lipid vesicles and water.10–14

In this study, we used second-derivative spectrophotometry to determine the $K_p$ values between PC SUVs and water of the following six anti-inflammatory steroid drugs: dexamethasone (DMS), betamethasone (BMS), triamcinolone acetonide (TCLA), flucinolone acetonide (FCLA), betamethasone 17,21-dipropionate (BMSDP), and clobetasol propionate (CBSP). In addition to the elimination of background signal effects, an important feature of the derivative method for the determination of the $K_p$ values is that small changes in a spectrum are enhanced.15–17 The effects of cholesterol, a major lipid constituent of biomembranes, on the partitioning of these drugs to the PC-cholesterol bilayer of SUVs were also investigated. Furthermore, the relevance of $K_p$ values for the reported therapeutic potency of these six steroid drugs as dermatological treatments is discussed.

**Experimental**

**Calculation of Molar Partition Coefficients** The molar partition coefficient ($K_p$) of a steroid drug between SUVs and water is defined as,5,18

\[
K_p = \frac{[\text{mol of drug in lipid}]}{[\text{mol of lipid}]} \cdot \frac{[\text{mol of drug in water}]}{[\text{mol of water}]}
\]

This is further described as,5,18

\[
K_p = \frac{([S_L]/[S_W])/[L]}{([S_L]/[S_W])/[W]}
\]

(1)

where $[S_L]$ and $[S_W]$ represent the concentrations of a steroid drug in SUVs and water, respectively, and $[L]$ and $[W]$ are the molar concentrations of lipids (PC + cholesterol) in SUV and water (55.3 mol% at 37°C), respectively.

If the background signal effect based on SUVs is eliminated in the second derivative spectra, the derivative intensity difference ($AD$) of a steroid drug before and after the addition of SUVs at a specific wavelength is proportional to the concentration of the steroid drug in the SUVs. As described...
where $\Delta D_{\text{max}}$ is the $\Delta D$ value when all of the steroid drug species in the sample solution are assumed to partition into the SUVs. The values of $K_p$ and $\Delta D_{\text{max}}$ can be calculated from the experimental values of [L] and $\Delta D$ by applying a nonlinear least-squares calculation to Eq. 2. Here, the calculations were performed with a personal computer.$^5$

### Results and Discussion

**Absorption and Second Derivative Spectra** The absorption spectra of 40 $\mu$M DMS and FCLA in sample solutions containing various amounts of PC SUV at 37 °C are shown in Fig. 2. Both drugs show small spectral changes according to the increases in the PC concentration. Also, no isobestic point was observed due to the incomplete baseline compensation resulting from the intense light-scattering of the PC SUVs. It is usually difficult to cancel the effects of strong background signals to obtain a flat and zero-level baseline. Thus, further spectral data for calculating the $K_p$ values could not be obtained from these absorption spectra.

The second derivative spectra calculated from the absorption spectra in Fig. 2 are illustrated in Figs. 3a and b, respectively. In these spectra, derivative isosbestic points can be clearly seen at 265 and 283 nm in the case of DMS, and at 274 nm for FCLA, thus confirming that the influence of the residual background signal of the PC SUVs was entirely eliminated in the second derivative spectra, and that both drugs were present in two states,$^{23}$ i.e., in the bulk water and in the PC bilayer of the SUVs. Moreover, the spectral intensity changes of both drugs were clearly enhanced in the second derivative spectra, which enabled us to obtain the exact
\( \Delta D \) values. Similar results were obtained for BMS, TCLA, BMSDP, and CBSP, and for all of the drugs in the PC-cholesterol SUV experiments.

**Calculated \( K_p \) Values** The \( \Delta D \) values used to calculate the \( K_p \) and \( \Delta D_{max} \) values were obtained from the derivative values at wavelengths of 273 nm for DMS, BMS, TCLA, BMSDP, and CBSP, and at a wavelength of 267 nm for FCLA. Using the obtained \( \Delta D \) values, the \( K_p \) and \( \Delta D_{max} \) values were calculated by a nonlinear least-squares calculation applied to Eq. 2, and the results are summarized in Table 1. The relative standard deviation (R.S.D.) of each \( K_p \) value in Table 1 remained below 10%, thus confirming the precision achieved by the second derivative method.

It has been known that the \( K_p \) values of certain amphiphilic drugs show concentration dependence due to the molecular association or formation of their micelles.8,24 To see whether the \( K_p \) values of these steroid drugs are affected by their concentrations, the \( K_p \) values of DMS and BMS were measured at several concentrations. The results in Table 2 show that the \( K_p \) values of each drug were similar for its concentrations employed. The results revealed that neither of these drugs associated nor formed micelles in the bulk water, which in turn confirmed the validity of applying partition theory to account for the interactions between these anti-inflammatory steroid drugs and the PC bilayer.

**Lipophilicity–Structure Relationship Based on \( K_p \) Values** The differences between the \( K_p \) values shown in Table 1 indicate the dependence of lipophilicity on molecular structure (see Fig. 1). Thus, using each \( K_p \) value as an index for the lipophilicity of the corresponding steroid drug, the effects of chemical structure on lipophilicity can be discussed.

BMS is a stereoisomer of DMS that differs from DMS in terms of the configuration of the C16-methyl group, i.e., BMS has a \( \beta \)-configuration and DMS an \( \alpha \)-configuration. Since there is only a small difference between the \( K_p \) values of DMS and BMS, it can be concluded that the configuration of the C16-methyl group does not affect the partitioning of these steroid drugs into the SUV bilayer.

TCLA and DMS differ structurally in that TCLA has an acetonide group between C16 and C17, whereas DMS has methyl (C16) and hydroxyl (C17) groups, respectively. The \( K_p \) value of TCLA was found to be approximately 1.3 times higher than that of DMS, showing that the replacement of the methyl and hydroxyl groups by an acetonide group slightly increases the lipophilicity of DMS. Moreover, the \( K_p \) value of FCLA (which has a fluorine atom at C6 with an \( \alpha \)-configuration, whereas TCLA has a hydrogen atom) increased to approximately 1.6 times that of TCLA, revealing that the substitution of the H atom with an F atom considerably increases the affinity for PC bilayers.

Table 1 also shows remarkably large \( K_p \) values for BMSDP and CBSP. The structure of BMSDP is such that the two hydroxyl groups at the C17 and C21 positions of BMS are esterified by propionic acids. It was confirmed that the esterification of the hydroxyl group with propionic acid greatly strengthened the hydrophobicity of the drug, and resulted in a large increase in the \( K_p \) value, i.e., the \( K_p \) value of BMSDP increased to approximately 10.5 times that of BMS.

In CBSP, the propionate group at the C21 position of BMSDP is substituted by a chlorine atom; there was a further increase (1.2 times) in the \( K_p \) value of CBSP with respect to that of BMSDP, which renders it the largest among \( K_p \) values for these six anti-inflammatory steroid drugs.

**Effects of Cholesterol Content on \( K_p \) Values** Cholesterol is a prominent nonpolar lipid constituent of many biological membranes and it influences interactions between various drugs and biomembranes. Therefore, the effects of cholesterol on the partitioning of these steroid drugs to PC-cholesterol bilayers of SUVs were examined. The \( K_p \) values for PC-cholesterol SUVs were determined with a range of cholesterol contents of up to 30 mol%, and are plotted as the ratios to the corresponding \( K_p \) values obtained for PC SUVs against the cholesterol content in Fig. 4. The results show that all of the \( K_p \) values decreased considerably with increases in cholesterol content, e.g., at a 30 mol% cholesterol content, the \( K_p \) values decreased to approximately 35—50% of their values at a 0 mol% cholesterol content. It is likely that the decrease in membrane fluidity due to cholesterol25,26 prevents the interaction between anti-inflammatory steroid drugs and lipid bilayer membranes.

In Fig. 5, the fractions of DMS and CBSP partitioned to

![Image](image-url)
the PC-cholesterol (0—30 mol% cholesterol) SUVs are shown as a plot of the ΔD/ΔD_{max} values versus lipid concentration. Solid lines represent the theoretical curves calculated from Eq. 2 using the obtained K_{p} and ΔD_{max} values. The experimental values at each cholesterol content show a good fit with the calculated curves, indicating the validity of the obtained K_{p} values. Similar results were obtained for BMS, TCLA, FCLA, and BMSDP.

Comparison of K_{p} Values with the Dermatological Potency of Steroid Drugs As noted above, the affinity of drugs for biomembranes influences their clinical potency, since potency is related to both the absorption of the drug and its concentration in the membranes of relevant tissues or cells. Therefore, the obtained K_{p} values were expected to be reflected in the clinical potency of these steroid drugs. Here, we attempted to confirm the relevance of the K_{p} values to the clinical potency of dermatological treatment, since via the percutaneous absorption, the affinity of drugs for PC bilayers is more directly reflected in the clinical potency of those drugs than via oral or injective administration.

Steroid drugs used in dermatological treatments have been classified into several categories according to their therapeutic potency. In a recent report, “guideline for therapy for atopic dermatitis 2004,” several steroid drugs used in topical preparations are classified into 5 categories. In Table 3, the logarithm of the K_{p} values (log K_{p}) of these steroid drugs and the logarithm of their reported partition coefficients (log P) determined for n-octanol/water systems are listed together with this dermatological therapeutic potency (Potency) described in the guideline. Herein, BMS is not referred to in the guideline. However, BMS is widely known to have potency equivalent to that of DMS and therefore, it could be reasonably classified as having medium-level potency (Table 3).

The order of the log K_{p} values in Table 3 indeed coincides with that of the dermatological therapeutic potency of these steroid drugs. The results clearly indicate that the potency of steroid drugs as dermatological treatments significantly depends on their K_{p} values.

On the other hand, the order of the log P values of the steroid drugs listed in Table 3 does not show complete agreement with that of the dermatological therapeutic potency of these drugs, i.e., the log P value of CBSP (Strongest), the most therapeutically effective of the drugs listed, is smaller than that of BMSDP (Very strong). Thus, it was confirmed that the K_{p} value of a drug measured in a liposome/water system is a more superior index for evaluating the lipophilicity of a drug than is the log P value measured in a n-octanol/water system.

In conclusion, it should be emphasized that the K_{p} values of steroid drugs for the PC liposome/water system were easily and accurately determined using the second-derivative spectrophotometric method. K_{p} values can therefore serve as indices when assessing lipophilicity—structure relationships, and they are of significant relevance with respect to the therapeutic potency of steroid drugs used as treatments for various dermatological conditions.

Acknowledgements The authors would like to thank Ms. Mayo Fukunaga for her assistance with the experiments.

References


Table 3. The log K_{p} and log P Values, and Dermatological Therapeutic Potency for the Six Steroid Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>log K_{p}</th>
<th>log P</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBSP</td>
<td>5.41</td>
<td>3.83</td>
<td>Strongest</td>
</tr>
<tr>
<td>BMSDP</td>
<td>5.33</td>
<td>4.07</td>
<td>Very strong</td>
</tr>
<tr>
<td>FCLA</td>
<td>4.69</td>
<td>2.48</td>
<td>Strong</td>
</tr>
<tr>
<td>TCLA</td>
<td>4.48</td>
<td>2.30</td>
<td>Medium</td>
</tr>
<tr>
<td>DMS</td>
<td>4.36</td>
<td>1.83</td>
<td>Medium</td>
</tr>
<tr>
<td>BMS^*</td>
<td>4.31</td>
<td>1.94</td>
<td>Medium</td>
</tr>
</tbody>
</table>

\( a \) Refer to ref. 30. \( b \) Refer to ref. 29. \( c \) Refer to ref. 31.

Fig. 5. Fractions (ΔD/ΔD_{max}) of DMS (Closed) and CBSP (Open) in PC-Cholesterol SUV Membranes at Various Cholesterol Contents as a Function of Lipid Concentration.

The solid lines show the theoretical curves calculated from Eq. 2 using the experimental values of K_{p} and ΔD_{max}. The symbols are the experimental values. Cholesterol (mol%): (circle) 0, (square) 10, (triangle) 20, (diamond) 30.