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Case Report

Determination of Chemical Agent Optical Clearing Potential Using In Vitro Human Skin

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Background and Objectives: Optical clearing is a method that temporarily reduces the optical scattering of biological tissues.

Study Design/Materials and Methods: To determine the optical clearing potential (OCP) of various chemical agents, we examined the change in reduced scattering coefficients of in vitro human skin after application directly to the dermal side.

Results/Conclusions: We found that the mean agent OCP did not correlate with refractive index or osmolality. Lipophilic hyperosmotic agents had a mean OCP less than unity, indicating increased optical scattering. Lasers Surg. Med. 36:72–75, 2005. © 2005 Wiley-Liss, Inc.

Key words: hyperosmotic agents; glycerol; scattering; dermatology; port wine stains

INTRODUCTION

In biomedical applications, light is used for diagnostic imaging and therapeutics. The penetration depth of light in biological tissue is restricted by absorption and scattering events. At visible and near infrared wavelengths, optical scattering dominates over absorption and is much more significant in reducing light penetration into biological tissues.

Optical clearing is a method for inducing a transient reduction in optical scattering [1]. Studies have demonstrated the increased penetration depth and contrast in optical imaging [1–8]. Using a novel chemical agent (CA), Khan et al. [9] have demonstrated for the first time in vivo optical clearing of human skin. Various mechanisms for optical clearing have been proposed, including refractive index matching [1,5], dehydration [5,10,11], and collagen dissociation [12].

Hyperosmotic CAs with refractive indices similar to that of collagen have been shown to increase tissue transparency. From results obtained with three CAs, Vargas et al. [13] suggested that increased light transmittance is proportional to CA osmolality. In this study, we quantitatively characterized a select group of hyperosmotic CAs. By measuring the optical clearing potential (OCP, defined below), we set out to determine whether there is a correlation between changes in optical scattering and osmolality and refractive index.

MATERIALS AND METHODS

Chemical Agents

We studied three different groups of CAs: hydroxy-terminated, organic solvents, and organic acids as summarized in Table 1. Refractive index was measured with a refractometer (Cole-Parmer, Vernon Hills, IL). Osmolality was measured with a freezing point osmometer (Advanced Instruments, Norwood, MA). Isotonic saline was used as a negative control (i.e., no optical clearing expected). Glycerol (13 M)was used as a positive control (i.e., optical clearing expected).

Skin Samples

Cryopreserved, dermatomed human skin (Community Tissue Services, Dayton, OH) was thawed to room temperature (~25°C). A single edged razor blade was used to cut the skin into ~2.5 cm × 2.5 cm samples. Sample thickness varied between 0.4 and 1 mm, as determined by placing the sample between two glass slides of known thickness and measuring the thickness of the sample/glass combination with a micrometer (Mitutoyo, City of Industry, CA). To minimize error due to sample compression, we recorded the sample thickness once the fine adjustment screw on the micrometer clicked once. Measurements on each sample were initiated within 2 hours after thawing.

Franz Diffusion Chamber

After removal from between the glass slides, each skin sample was mounted in a Franz diffusion cell [14]. The lower reservoir was filled with isotonic saline. Each sample

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TABLE 1. Characteristics of the Different Chemical Agents Investigated in This Study

Chemical agent	Chemical type	Refractive index	Osmolality (mOsm/kg)
Saline	Negative control	1.33	280
Glycerol	Hydroxy-terminated, positive control	1.47	14,550
50% TMP	Hydroxy-terminated	1.43	6830
100% TMP	Hydroxy-terminated	1.47	13,660
1,3-butanediol	Hydroxy-terminated	1.44	22,050
1,4-butanediol	Hydroxy-terminated	1.44	26,900
Ethylene glycol	Hydroxy-terminated	1.43	22,640
MPDiol glycol	Hydroxy-terminated	1.44	23,460
DMSO	Organic solvent	1.47	12,900
Linoleic acid	Organic acid	1.47	N/A
Oleic acid	Organic acid	1.46	N/A
P-0062	Hydroxy-terminated prepolymer	1.48	1643

was positioned above the lower reservoir, with the epidermal side in contact with the saline. The upper reservoir was placed on top and clamped in place. Any air bubbles in the lower reservoir were then removed and additional saline supplied to ensure physical contact with the epidermal side. The exposed dermis in the upper reservoir was 3 cm^2. The CA was applied to the upper reservoir (i.e., the dermal side of the sample) for 20 minutes. Thereafter, the sample was removed from the chamber, cleaned with tissue paper, and the thickness assessed again as described above.

Reflectance/Transmittance Measurements

To measure diffuse reflectance and total light transmittance of the skin samples, we used an integrating sphere based method [15]. The sample/glass combination was irradiated with collimated 633 nm HeNe laser light. The sample was placed either at the exit or entrance port of an integrating sphere (Labsphere, North Sutton, NH) for reflectance or transmittance measurements, respectively. Diffuse reflectance was measured by positioning the sample so that specularly reflected light propagated through the entrance port and hence did not contribute to the measurement. Total transmittance was measured by covering the exit port with a Spectralon coated port plug. Light was detected with a silicon photodiode (Labsphere). Data were acquired with a PC based data acquisition system (National Instruments, Austin, TX). System calibration was performed with calibration standards (Labsphere) and neutral density filters (Newport Corp., Irvine, CA) for reflectance and transmittance measurements, respectively. To minimize complications due to potentially heterogeneous optical clearing, three measurements of reflectance or transmittance were acquired from different locations near the center of the sample and averaged.

Determination of Optical Clearing Potential

Measurements of diffuse reflectance, total transmittance, and sample thickness were used as input to an inverse adding doubling method [16] to estimate the reduced scattering coefficient $\mu_s' \cdot \mu_s'$ values were estimated from measurements taken before and after CA application. OCP was defined as

$$\mathrm{OCP} = \frac{\mu_s'(before)}{\mu_s'(after)} \tag{1}$$

where $\mu_s'(before)$ and $\mu_s'(after)$ represent values determined after and before CA application, respectively. An OCP greater (less) than unity indicates a reduction (increase) in μ_s'.

With the inverse adding double software, one input parameter is tissue refractive index, which is expected to change during optical clearing. We assumed a constant value of 1.4. From a preliminary error analysis, the maximum error in μ_s' was ~5% when tissue refractive index varied between 1.38 and 1.45.

RESULTS

Of the CAs under study, the hydroxy-terminated CAs demonstrated the highest OCP (Fig. 1, group A). The OCP of saline, the negative control under investigation, was approximately unity. The positive control glycerol had a mean OCP of 3. The organic solvent DMSO had a mean OCP of 1.5. Neither of the two organic acids under investigation demonstrated any potential for optical clearing (Fig. 1, group C); in fact, both had OCP values less than unity, suggesting increased skin turbidity. P-0062, a hydroxy-terminated prepolymer, possessed an OCP similar to that of the other hydroxy-terminated CAs.

Refractive index and osmolality of the CAs do not follow similar trends (Table 1, Fig. 2). Refractive indices of the hyperosmotic CAs varied between 1.43 and 1.48. Osmolality values varied over the wide range of 1600 and 27,000 mOsm/kg. The osmometer was incapable of freezing the two organic acids because of the use of ethanol as a solvent in these measurements, and thus the corresponding osmolality values could not be measured.

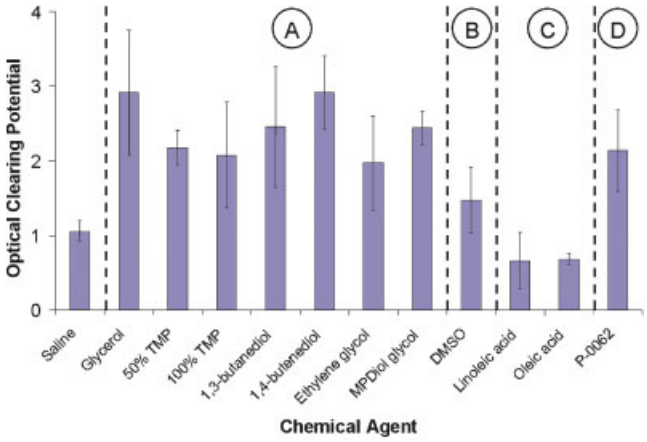

Fig. 1. OCP of various CAs under study. **A**: Hydrophilic hydroxy-terminated CA; (**B**) hydrophilic organic solvent; (**C**) lipophilic organic acids; and (**D**) hydrophilic hydroxy-terminated prepolymer. [Figure can be viewed in color online via www.interscience.wiley.com.]

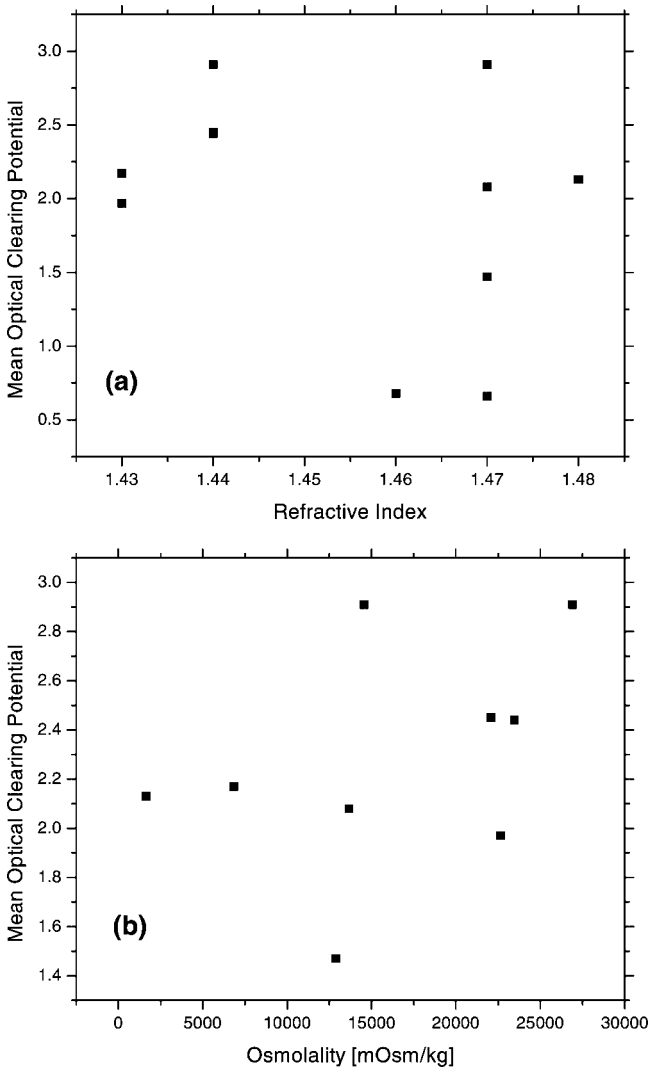

Fig. 2. Mean OCP as a function of CA (**a**) refractive index and (**b**) osmolality. Mean OCP has a very weak correlation with either CA characteristic. Note that saline was excluded from both graphs, and linoleic acid and oleic acid from (b).

DISCUSSION

The experiments presented herein suggest that, within the studied parameter space, refractive index matching and osmolality are not the only factors governing the efficacy of a CA for optical clearing (Fig. 2). Measured OCP values after 20 minute application time did not correlate with either CA refractive index or osmolality.

In clinical applications, the CAs would probably be applied to the epidermal side and allowed to diffuse into the epidermis and dermis. The stratum corneum barrier of the epidermis restricts severely transdermal delivery of the hydrophilic CAs used in this study. Once in the epidermis and dermis, the CA can readily diffuse. Our goal in this study was to introduce the concept of OCP to determine whether there is a correlation between changes in optical scattering and osmolality and refractive index. Since a reduction in optical scattering is desired in the epidermis and dermis, we applied the CAs to the dermal side to obviate problems caused by the stratum corneum.

OCP is affected by the hydrophilic/lipophilic nature of the CA (Fig. 1). All CAs investigated in this study were hydrophilic, with the exception of lipophilic oleic and linoleic organic acids, which also possessed the lowest OCP values (less than unity, indicating an increase in scattering). Since the CAs were applied to the hydrophilic dermal side of each sample, our results suggest that only hydrophilic CAs diffused into the skin. One chemical of interest is P-0062, which is a hydrophilic hydroxy-terminated prepolymer used as an ingredient in a chemical formulation to reduce optical scattering in in vivo human skin [9]. The relatively high mean OCP value ($\sim$2) of P-0062 explains the successful preliminary in vivo results obtained with this CA.

In summary, we present a controlled technique for screening CAs, using the concept of OCP. With this technique, we identified a macroscopic reason for the success of in vivo optical clearing [9].

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