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對皮膚經皮水分散失之影響

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計畫主持人：張火炎
共同主持人：蔡瑞真，許漢銘，石東生

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Topical exposure to carbon disulfide induces epidermal permeability alterations in physiological and pathological changes

Tzu-Chieh Chou a,b, Jui-Chen Tsai c, Hamm-Min Sheu d, Chauying-J. Jen e, Tung-Sheng Shih f, Ho-Yuan Chang a,∗

a Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
b Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
c Institute of Clinical Pharmacy, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
d Department of Dermatology, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
e Department of Physiology, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
f Institute of Occupational Safety and Health, Council of Labor Affairs, No. 99 Lane 407, Heng-Ke Road, Shijr City, Taipei, Taiwan

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Abstract
Carbon disulfide (CS2) has been suggested its possible skin toxicity. Neither a dose-response relationship nor any mechanism of CS2-exposure regarding epidermal permeability alterations has been postulated. The objectives of this study were to evaluate the dose-dependent association and the pathological changes with CS2 topically applied to mouse epidermis. Four concentrations of CS2 (0% (controls), 10%, 15%, and 20% in ethanol) were topically applied to a 1.8 cm2 area of the lateral abdomen of female nude mice for 10 min. Time-series transepidermal water loss (TEWL) profile, morphological examinations by both light microscopy (hematoxylin/eosin stain and Nile Red stain) and electronic microscopy, and lipid analysis by high performance thin-layer chromatography (HPTLC) were used to evaluate the epidermal impairment. We found no recovery occurred within 72 h exposure to 20% CS2 in contrast to substantial recovery found in 10% and 15% CS2-exposure. Clear dose-dependent fashions were shown in TEWL elevations, recovery retardation, and lipid extraction across the ethanol (control), 10%, 15%, and 20% CS2 exposures. Two mechanistic pathways were raised to account for CS2-induced epidermal alterations: intercellular

Abbreviations: CS2, carbon disulfide; TEWL, transepidermal water loss; HPTLC, high performance thin-layer chromatography; FFA, free fatty acid; Chol, cholesterol; Cer, ceramide

∗ Corresponding author. Tel. +886 6 235 3535x5597; fax +886 6 274 3748.
E-mail address: h7154@mail.ncku.edu.tw (H.-Y. Chang).

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lipid depletion and keratinocyte damage. A study with different test animal species is warranted owing to the discrepancies in epidermis between nude mice and other species. © 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Carbon disulfide; Epidermal permeability barrier function; Barrier recovery; Morphological examination; Transepidermal water loss; Stratum corneum lipids

1. Introduction

Numerous environmental stresses and industrial toxicants such as UV radiation and organic solvents have documented to pose skin toxicity (Tsai et al., 2001; Kanikkannan et al., 2002; Heck et al., 2004; Monteiro-Riviere et al., 2004). Carbon disulfide (CS₂) has widely been used as a solvent in industries and has raised great public attention owing to its easy re-contamination in the general environment from the primary pollution sources. In western countries, CS₂ has been one major concern for the American population because it has been identified in more than 200 of the 1430 current or former USEPA National Priorities List (NPL) hazardous waste sites since 1996 (ATSDR, 1996a). In Asia, CS₂ has been in the list of the detectable chemicals in the effluent from wastewater treatment plants in Tokyo, Japan (Hwang et al., 1995). CS₂ has exerted great toxicity in neurological, cardiovascular, reproductive, and hepatic systems (Beauchamp et al., 1983; Graham et al., 1995; Luo et al., 2003). Despite speculation for more than three decades that CS₂ might be a strong skin toxicant because of its high lipophilicity and reactivity (Pirila et al., 1971), there is little data about its dermatological toxicity. Our recent study revealed that skin contact with CS₂ among occupationally exposed workers could result in hand dermatitis, and that the toxicity could be aggravated by simultaneous co-exposure to sulfuric acid. An alarmingly high prevalence (61.5%) of irritant hand dermatitis has been observed in those exposed to CS₂ in the rayon manufacturing process (Chou et al., 2004a). Another study carried out in the occupational settings also demonstrated the chronic and repeated exposure to rayon manufacturing chemicals containing saturated CS₂ aqueous solution can pose significant elevation of basal transepidermal water loss (TEWL) (Chou et al., 2004b). There is a need to explore the underlying mechanisms behind skin impairment after topical application of CS₂, and to establish the dose-response relationship between CS₂ and skin damage.

TEWL has been extensively used to evaluate the skin barrier impairment resulting from contact with solvents, tape-stripping, and pathological conditions (Grubauer et al., 1989a; Aalto-Korte and Turpeinen, 1993; Grösser et al., 1997). It is generally believed that the change of basal TEWL is a sensitive indicator for evaluating skin disease (Seidenari and Giusti, 1995), and that the dynamics of barrier recovery after impairment can provide an in-depth understanding of the functional difference of skin barriers that depart from basal conditions (Denda et al., 2003). Morphological examination by light and electron microscopy can obtain the information of pathological and ultra-structure changes on skin after contact with irritants (Fartasch, 1997; Ahn et al., 2001). Barrier disruption and recovery, however, are reportedly associated with the removal and synthesis of lipids enriched within stratum corneum interstices (Elias and Feingold, 1992). Nude mice model has been extensively documented in percutaneous absorption studies (van den Akker et al., 2000; Sheu et al., 2002; Venter et al., 2001; Tsai et al., 2002). In the present study, nude mice were topically exposed at various levels of CS₂ to determine the dose-response relationship, to measure TEWL immediately after topical exposure to evaluate the change of barrier function (Aalto-Korte and Turpeinen, 1993), and to measure the time-series changes of TEWL within 72 h to determine barrier recovery rates (Taljebini et al., 1986). The exposed skin was further examined by light and electron microscopy and CS₂-extracted lipids were further analyzed to investigate the possible components responsible for skin impairment and recovery.

2. Methods

2.1. Experiments in topical exposure to CS₂ and TEWL determination

CS₂ and ethanol (HPLC grade) were obtained from Tedla Company Inc. (Fairfield, OH, USA). Female
nude mice (BALB/c-nu, 8–12 w/o; Taiwan National Animal Laboratory Center, Taipei) were topically exposed to 1 mL of four levels of CS₂/ethanol solutions (0/100, 10/90, 15/85, and 20/80, % in v/v) on a 1.8 cm² area of the lateral abdomen epidermis for 10 min (n = 3 for each experiment). The exposure concentrations were designed based on findings in previous pioneer experiments: a plateau of TEWL over 50 gm⁻² h⁻¹ consistently occurred when the topical CS₂-exposure levels were over 20% (data not shown). Throughout the experiment, the mice were maintained in an experimental chamber with a temperature and humidity of 24.9 ± 1.5 °C and 48.8 ± 4.1%. A 45.5 mg ml⁻¹ dose of chloral hydrate (TCI-EP, Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) was intraperitoneally injected as an anesthetic 5–10 min before the experiment. TEWL measurements (Tewameter TM 210; Courage + Khazaka Electronic GmbH, Koln, Germany) were not considered valid until the standard deviation of the last five measurements was less than 0.5 gm⁻² h⁻¹. A 72 h time-series profile of TEWL from the end of the exposure was established for each experiment. Barrier recovery (BR%) was determined at 4, 8, 12, 24, 48, and 72 h using the following equation (Zhai et al., 1998):

\[
BR(\%) = \left[ 1 - \frac{\text{TEWL}_{ti} - \text{TEWL}_{b}}{\text{TEWL}_{t0} - \text{TEWL}_{b}} \right] \times 100%
\]

where TEWL_{ti}, TEWL at indicated time (ti); TEWL_{b}, TEWL baseline measured before the exposure; TEWL_{t0}, TEWL immediately after exposure.

2.2. Microscopic examination

For light microscopic observations, skin specimens were taken from ethanol and CS₂-exposed skin, and also from the unexposed epidermis of the controls. The specimens were fixed in 10% formalin, routinely processed, and stained with hematoxylin and eosin (HE stain). For fluorescence localization of stratum corneum neutral lipids, a modified Nile Red staining of unfixed, frozen tissue section was achieved according to the method of Sheu et al. (2003). In brief, a stock solution of Nile Red (1 mg ml⁻¹) in acetone was prepared and stored at −20 °C without any exposure to light. Nile Red stain (20 µl stock solution plus 1 ml of 75% glycerol) was freshly prepared just before use. After adequate centrifugation, one drop of the staining solution was applied to the frozen sectioned sample for 10 min at room temperature in darkness. Four percent potassium hydroxide (KOH) was then added, and the sections were observed by fluorescence microscope (BH2; Olympus, Tokyo, Japan) with the excitation and emission wavelengths of 455 and 530 nm, respectively. Neutral lipids were visualized as yellow-gold fluorescent structures.

2.3. Ruthenium tetroxide staining for stratum corneum intercellular lipid bilayers and electron microscopy examination

Stratum corneum intercellular lamellae structures from 20% exposed skin were examined by electron microscopy following by ruthenium tetroxide fixation (Sheu et al., 1999). In brief, skin samples of nude mice were minced at ≤1 mm³ and kept still for fixation at least 16 h at 4 °C in a solution containing 2% glutaraldehyde and 2% paraformaldehyde, with 0.06% calcium chloride in 0.1 M sodium cacodylate buffer at pH 7.3. Post-fixation of samples was carried in 0.2% cyanide in 0.1 M sodium cacodylate buffer, pH 6.8, in darkness for 1 h at room temperature. Samples were embedded in epoxy resin following by dehydration in a graded series of ethanol and propylene oxide. A Hitachi 7000 electron microscope was used to examine stratum corneum intercellular lipid bilayers while thin sections were post-stained with uranyl acetate and lead citrate.

2.4. Lipid analysis

Because the epidermal permeability barrier is driven by intercellular lipid bilayers in the stratum corneum and among them, free fatty acid (FFA), cholesterol (Chol), and ceramides (Cer) are the predominant components (Schurer and Elias, 1991). Therefore, this study focused only on these three lipids. At the end of the topical exposure to four various concentrations of CS₂, the extracting solutions were used to identify FFA (test by palmitic acid), Chol, Cer (test by ceramides III and IV) from stratum corneum by high performance thin-layer chromatography (HPTLC). Lipids-extracted solutions obtained from topical exposure to four various concentrations of CS₂ were blown to dry by nitrogen (purity >99.9%) and re-dissolved in 1.0 mL chloroform/methanol (2:1) and were then spotted on a TLC plate (No. 1.05641; Merck, Darmstadt, Ger-
many) by a sample spray-on system (Linomat IV; CAMAG, Muttenz, Switzerland) and were further separated by two solvent mixtures developed by Weerheim and Ponec (2001) with minor modifications. The first solvent mixture was chloroform/acetone/methanol (76/8/16) developed to the 3.5 cm level, and the second solvent mixture was n-hexane/chloroform/hexyl-acetate/acetone/methanol (6/80/0.1/10/4) developed to the 9.5 cm level. After drying, the chromatogram was sprayed with 10% CuSO4 solution and charred in a 180 °C oven for 10 min. Bands were identified by comparison with standard lipids. The standards reagents regarding palmitic acid (99% in purity), cholesterol (>99% in purity), ceramide III (99% in purity), ceramide IV (99% in purity) and other separation solvents (HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The sum of three types of extracted lipids was expressed as total extracted lipids. A complete extraction lipid spectrum of stratum corneum was obtained from an unexposed healthy mouse skin for the quantitative determination of extraction percentage of each test lipids. The extraction percentage of each test lipid from CS2-exposed skin relative to that from unexposed skin was calculated by comparing their densitometric areas with CAMAG TLC software using an absorbance reflection mode at 560 nm. The animal study protocol was pre-approved by the Animal Research Committee in National Cheng Kung University Medical Center.

2.5. Statistical analyses

A paired t test was used to compare the TEWL measurements before and after exposure. One-way analysis of variance (ANOVA) was used to test the TEWL differences between the four CS2-exposed groups, and the Tukey honest significant difference (HSD) test was used in post hoc comparisons. Pearson correlation test was used to evaluate whether there is a trend across various CS2-exposures. Statistica software release 6 (StatSoft, Tulsa, OK, USA) was used to perform statistical analyses. Statistical significance was set at P < 0.05.

3. Results

3.1. Effects of CS2-exposure on TEWL

The TEWL differences between post- and pre-exposure were 3.5 ± 1.1, 9.8 ± 0.9, 14.5 ± 1.6, and 38.3 ± 4.0 g m⁻² h⁻¹ (mean ± S.E.M.) for ethanol, 10%, 15%, and 20% CS2-exposed, respectively (Table 1). Although there were no significant pre-exposure differences in TEWL between four groups (P = 0.10), all post-exposure TEWL measurements showed significant elevation from their pre-exposure ones (P < 0.05), suggesting that both ethanol and CS2 are epidermal permeability barrier disruptors. In addition, clear linear trends of post-exposure TEWL and TEWL difference were associated with increases in CS2-exposure levels (P < 0.001), revealing that CS2-induced barrier disruption is dose-dependent.

Sequential TEWL profiles within 72 h after the various exposures are shown in Fig. 1a. There were no significant differences between baseline and post-exposure TEWL measurements for the 10% and 15% CS2-exposed groups, except immediately after the exposure (t₀). Almost all TEWL measurements in

### Table 1
Comparison of transepidermal water loss (TEWL) measurements during various pre- and immediately after CS2-exposures

<table>
<thead>
<tr>
<th></th>
<th>Ethanol</th>
<th>10% CS2</th>
<th>15% CS2</th>
<th>20% CS2</th>
<th>ANOVA testb</th>
<th>Linear trend testc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exposure</td>
<td>7.3 (0.7)</td>
<td>9.2 (0.8)</td>
<td>9.7 (0.7)</td>
<td>10.4 (0.9)</td>
<td>0.10</td>
<td>–</td>
</tr>
<tr>
<td>Immediately after exposure</td>
<td>10.8 (1.2)</td>
<td>19.0 (1.4)</td>
<td>24.3 (1.4)</td>
<td>48.7 (3.8)</td>
<td>P &lt; 0.001 ***</td>
<td>P &lt; 0.001 ***</td>
</tr>
<tr>
<td>Difference between pre- and immediately after exposure</td>
<td>3.5 (1.1) *</td>
<td>9.8 (0.9) **</td>
<td>14.5 (1.6) **</td>
<td>38.3 (4.0) **</td>
<td>P &lt; 0.001 ***</td>
<td>P &lt; 0.001 ***</td>
</tr>
</tbody>
</table>

a TEWL measurement comparisons tested by one-way ANOVA. Tukey honest significant difference (HSD) used in post hoc comparisons.
b Tested by Pearson correlation. (–) indicates test not performed due to insignificance in ANOVA test.
c Differences between pre-exposure and immediately after exposure were tested by paired t test.

* P < 0.05
** P < 0.01
*** P < 0.001.
The unexposed and ethanol-exposed skins (Fig. 2a and b) demonstrated similar morphological changes in the epidermis under HE stain, indicating that the damage to epidermal cells was negligible. In the 10% and 15% CS₂-exposed groups, mild discrete cell death was shown in the viable epidermis (Fig. 2c and d). More frequent discrete cell deaths were found in 20% CS₂-exposure skin (Fig. 2e), indicating 20% CS₂-exposure could pose more toxic effect on viable epidermis.

Fluorescence microscopic examination with Nile Red staining for neutral lipids from unexposed skin was evenly distributed in stratum corneum layers (Fig. 3a). Lipid droplets were observed in the surface of stratum corneum after chemicals exposure. Ethanol exposure showed no significant effect on neutral lipid lamellae in the stratum corneum layers (Fig. 3b). CS₂, on the other hand, extracted the neutral lipid contents from the outer toward the inner stratum corneum layers. Almost all neutral lipids were depleted by 15% and 20% CS₂ solutions, but an observable amount of lipids remained in the deepest layers of the stratum corneum after 10% and higher-CS₂-exposure (Fig. 3c and e). The result demonstrated that the skin barrier function impairment after various CS₂-exposures might result from viable epidermis cell damage and stratum corneum lipids extraction.

3.3. Ruthenium tetroxide staining for stratum corneum intercellular lipid bilayers and electron microscopy examination

A marked decrease in the number of intercellular lipid lamellae was found at 20% CS₂-exposed skin (Fig. 4a). Moreover, intercellular lipid lamellae were almost depleted and only cornified envelopes remained in the stratum corneum (Fig. 4b), which was consistent with the observation in Nile Red stained skin after 20% CS₂-exposure (Fig. 3e). The diffuse destruction of lipid lamellae was noticed in lower stratum corneum (Fig. 4a), revealing that the perturbation at

the recovery period for the 20% CS₂-exposed group, however, showed significant elevation from baseline TEWL. Recovery rates increased with the increasing time after the cessation of topical CS₂-exposure for the 10% and 15% CS₂-exposed groups, but there was no clear pattern of recovery in the 20% CS₂-exposed group (Fig. 1b). While none of the CS₂-exposed groups reached 50% recovery in the first 8 h, the 10% and 15% CS₂-exposed groups eventually reached up to more than 100% and about 80% after 72 h, respectively. Recovery rates for 20% exposed group were always below 50% throughout the 72 h experimental period, indicating that mouse skin topically exposed with 20% CS₂ for 10 min is irreversibly damaged.

3.2. Light microscopic observation

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Fig. 2. Histological findings of skin after the various exposures of ethanol-based CS₂ concentration solutions. No noted differences were observed in epidermis after ethanol exposure (b) compared with unexposed mouse skin (a). Mild discrete cell death in the epidermis (indicated by arrows) could be found in 10% and 15% CS₂-exposed skin (c and d). The epidermis of 20% CS₂-exposure, however, showed more frequent cell death (e). Scale bars: 20μm.
deeper stratum corneum in the skin after 20% CS2-exposure occurred. Abundant and elongated membrane structures were discovered whereas the lipid bilayers did not commence to form within the interstices at the stratum granulosum and stratum corneum (SG–SC) interface (Fig. 4b). Massive lamella bodies were detected in SG–SC interface but lipid lamellae in some lamella bodies were deprived, possibly owing to the achieve-
Fig. 5. The lipid analysis extracted by four various CS₂-exposure (for each experiment, n = 3). Comparing with ethanol exposure group, all CS₂-exposure groups revealed significant differences except the 10% CS₂-exposure in FFA, Cer, and total extracted lipids. A linear trend between the concentrations of CS₂-exposure and lipid content was shown for all lipid analyses (correlation coefficients were 0.87, 0.82, 0.82, and 0.86, P < 0.01 for Chol, FFA, Cer, and total extracted lipids, respectively). Notes used in the figure: Chol, cholesterol; FFA, free fatty acid; Cer, ceramide, mean ± S.E.M., *P < 0.05, **P < 0.01, ***P < 0.001.

management of the exocytosis in the stratum corneum (Fig. 4c).

Fig. 4. Ruthenium tetroxide staining of lipid lamellae and epidermis of mice skin after the 20% CS₂-exposures. (a) A marked decrease in the number of intercellular lipid lamellae (indicated by arrows), the diffuse destruction of lipid lamellae of lower stratum corneum (indicated by arrowheads), and abundant and elongated membrane structures were observed whereas the lipid bilayers did not commence to form within the interstitics at the stratum granulosum and stratum corneum (SG–SC) interface (indicated by asterisk). (b) Intercellular lipid lamellae were almost depleted and only cornified envelops remained in the stratum corneum (indicated by open arrows). (c) Quite a few lamella bodies were found in SG–SC interface, lipid lamellae in some of them, however, were not shown (indicated by open arrowheads), possibly owing to achievement of the exocytosis in the stratum corneum. (d) Cell death was shown in the deeper layers of viable epidermis. (a) ×60,000, (b) ×100,000, (c) ×40,000 and (d) ×6000.

3.4. Effects of CS₂-exposure on the lipids of the stratum corneum

In the HPTLC lipid-component-analysis for the CS₂-extracted solutions, Chol and Cer were the major lipids depleted, and FFA was the least depleted by topical CS₂-exposure. In general, the degree of lipids extraction increased significantly (P < 0.05-0.001) following by the increases in CS₂-content, and significant linear trends between CS₂-exposed concentrations and lipid-extraction percentages were shown (correlation coefficients were 0.87, 0.82, 0.82, and 0.86, respectively, P < 0.01 for Chol, FFA, Cer, and total extracted lipids, Fig. 5).

4. Discussion

CS₂, used worldwide in a great number of industries, is strongly toxic to the neurological, cardiovascular, reproductive, and hepatic systems (Stetkiewicz and Wronska-Nofer, 1998; Luo et al., 2003). Our previous studies have demonstrated CS₂ could be a potent skin toxicant by the findings of an alarmingly high prevalence (61.5%) of irritant hand dermatitis and significant elevation of basal TEWL observed in those who chronically and repeatedly exposed to CS₂ in the rayon man-
We found topical CS2-exposure revealed a clear dose-response pattern of increasing TEWL and lipid extraction. Cell death in viable epidermis were observed for 20% CS2-exposed group, indicating that higher concentrations of CS2 also could induce epidermal cell damage after exposure. This result was consistent with the remarkably low barrier recovery rate after higher CS2-exposure (20% CS2) of mouse skin (Fig. 1b).

Lipophilic or non-polar chemicals such as halogenated hydrocarbons, aromatic hydrocarbons, and CS2 have been extensively used either as raw materials or solvents during manufacturing processes in general industrial environments (ATSDR, 1995, 1996b). Concerns regarding their percutaneous absorption potential and lipids extraction capability on skin has been continuously raised (Grandjean, 1990). Petroleum ether, for example, a non-polar solvent, causes only insignificant TEWL elevation after 10 min topical exposure although it can remove abundant lipids from the stratum corneum (Grubauer et al., 1989b). Jet fuel (multicomponent mixtures of aromatic and aliphatic hydrocarbons), on the other hand, causes a significant increase in TEWL as well as mild edema and inflammation on skin after 5h topical exposure (Monteiro-Riviere et al., 2001). In this study, CS2, another non-polar chemical, reveals a more skin-irritating potential because it caused both significant TEWL elevation and pathologic impairment for skin topically exposed to 20% CS2 for only 10 min exposure. Moreover, severe skin barrier damage caused by a high concentration CS2 could not only be attributed to stratum corneum lipid extraction, damage to keratinocytes in viable epidermis might also contribute to barrier disruption.

Quite a few studies have demonstrated the stratum corneum lipid lamellae could be impaired resulting from chemicals exposure. Pure acetone treatment can deprive the intercellular lipids from the stratum corneum almost completely (Tsai et al., 2001) and can also disturb the arrangement of lipid lamellae with desmosomes and lamella bodies unchanged in hairless mice skin (Fartasch, 1997). Moreover, numerous se-cretions of lamella bodies with almost depleted lipid lamellae were observed after 30 min of acetone treatment in hairless mice skin, indicating the elevated lipids secretion from lamellar bodies could occur while skin was exposed to lipid-perturbation solvents (Menon et al., 1992). Jet fuel exposure, another example, has been found to cause skin barrier perturbation on hairless rat skin in two aspects: TEWL elevation and lipid extraction (Kanikkannan et al., 2001, 2002; Singh and Singh, 2001). Monteiro-Riviere and colleagues further demonstrated the JP-8 + 100, a commercialized jet fuel, is capable to extract some lipids from stratum corneum of procine skin and to induce separation or expansion within intercellular lipid lamellae and desmosomes. Furthermore, the vacuoles appear in stratum corneum near the SG–SC interface and packed filaments in stratum granulosum become loose in the procine skin after 4-day application of jet fuel (Monteiro-Riviere et al., 2004). In this study, almost complete lipid extraction in intercellular interstices was observed for 20% CS2-exposure mice. Moreover, the number of lamella bodies increased greatly and lipid lamellae were disappeared in some lamella bodies, similar to that for acetone-treated hairless mice, as aforementioned (Fig. 4c). Diffuse destruction of lipid lamellae was also found in this study, similar to that for jet fuel-exposed pig skin (Monteiro-Riviere et al., 2004). The above findings suggest the mechanistic process involved in the skin barrier perturbation for CS2-exposure is the depletion of lipids in stratum corneum.

Pure ethanol (0% CS2) exposure was used as a control solvent in this study, as in quite a few previous studies. Zhao and Singh conducted a study to investigate the percutaneous absorption enhancement of propranolol hydrochloride (PHLC) on porcine epidermis and pure ethanol was also used as a solvent. They found TEWL elevation for the pure ethanol treatment was 22.1 g m⁻² h⁻¹, significantly less than that for limonene/ethanol solution (34.3 g m⁻² h⁻¹) (Zhao and Singh, 1999). Goffin et al. performed another study to measure the effect of various solvents, including hexane, ethanol, methanol, hexane–ethanol, chloroform, chloroform–methanol and hexane–methanol, upon human stratum corneum. They found ethanol was the second least aggressive among all test solvents (Goffin et al., 1997). On the other hand, Nielsen exposed human skins to four different detergents in in vitro experiments and he found ethanol could enhance percutaneous penetration without compromising the overall integrity of the skin barrier (Nielsen, 2000). Taken together, topical exposure to pure ethanol could cause mild but significant barrier
disruption in abovementioned studies. In our current study, we found the topical exposure to pure ethanol also caused a significant elevation of TEWL in control-group mice, consistent with the previous studies. The TEWL increase resulting from ethanol exposure, however, was less than one-third that in the 10% CS2-exposed group and less than 10% that in the 20% CS2-exposed group (Table 1), indicating that ethanol was a mild skin barrier disruptor and had negligible influence on the conclusion about the ability of CS2 to cause epidermal barrier damage.

Determination of recovery rate was another index of skin barrier function, which reflected the responses of various components of the repair process to a particular phase of the recovery response (Taljebini et al., 1996). Several steps are involved in the barrier repair process: (1) the immediate secretion of pre-formed lamellar bodies; (2) the increase of epidermal lipids synthesis; (3) the formation and further secretion of new lamellar bodies; and (4) the extracellular remodeling of secreted lamellar-body-derived lipids (Proksch et al., 1993). These steps were related to stratum corneum lipids synthesis; the remodeling occurred at the interface between SG–SC in viable epidermis (Mauro et al., 1998). Our morphological examination results showed only mild damages for viable epidermis cells for 10% and 15% CS2-exposure, and more than 50% recovery both in 10% and 15% CS2-exposure groups after 12 h of exposure. In contrast, 20% CS2-exposure caused obvious cell deaths in viable epidermis, which might account for the disproportionately low recovery rate for the 20% CS2-exposed group.

The nude mouse was used as the test animal in this study and it is a well-known athymic mutant with immunological deficiency. Several lines of evidences showing the epidermal structure of nude mouse is different from that of hairless mice and human (Brysk et al., 1986). Extrapolation of the conclusion in this study to other species should be with precaution. A further study with different test animal species is warranted.

In summary, this study has demonstrated the mouse skin topically exposed to CS2 could result in dose-dependent TEWL elevation, recovery retardation, and lipid extraction. Two mechanistic pathways were provided to account for CS2-induced epidermal alterations: intercellular lipid depletion and keratinocyte damage.

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References


