Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 46 (2008) 1940–1948
www.elsevier.com/locate/foodchemtox

Chronic ethanol ingestion alters xenobiotic absorption through the skin: Potential role of oxidative stress

R.M. Brand [a,*], J.L. Jendrzejewski [b]

[a] Departments of Dermatology and Medicine, University of Pittsburgh Medical Center, 190 Lothrop Street, 145 Lothrop Hall, Pittsburgh, PA 15213, United States
[b] Department of Internal Medicine, Evanston Northwestern Healthcare, Evanston IL, United States

Received 7 November 2007; accepted 19 January 2008

Abstract

Alcohol ingestion is correlated with several skin disorders and it has been proposed that changes in skin properties may be an early indicator of alcohol misuse. Topically applied ethanol is an effective transdermal penetration enhancer; however, little is known about the effects of chronic ethanol ingestion on skin. Rats were pair fed a diet containing 36% ethanol for twelve weeks. The animals were then switched to a non-ethanol diet and were monitored for up to four weeks. Non-invasive measurements for changes in dermal blood flow using laser Doppler velocimetry (LDV), damage to skin barrier via transepidermal water loss (TEWL) and changes in skin moisture content were obtained for the experimental duration. At 0, 1 day or 1, 2, 3, 4 weeks after alcohol removal rats were euthanized and their skin was analyzed for alcohol and aldehyde dehydrogenase, and lipid peroxidation. Transdermal penetration of the herbicide paraquat, industrial solvent dimethyl formamide (DMF), insect repellant *N*,*N*-diethyl-*m*-toluamide (DEET) and herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was also determined. Transdermal absorption, LDV, TEWL, skin alcohol and aldehyde dehydrogenase, as well as lipid peroxidation significantly increased after continuous ethanol exposure ($p < 0.05$). These factors remain elevated for up to four weeks after termination of ethanol consumption, showing that skin changes induced by alcohol are not immediately reversible and reflect fundamental changes in the skin itself. This work provides a starting point for examining the link between ethanol ingestion and skin disorders associated with alcohol use.

Keywords: Ethanol ingestion; Skin; Skin barrier; LDV; TEWL; Penetration; Oxidative stress

1. Introduction

The potential benefits of alcohol use have recently been highlighted (Standridge et al., 2004). To recommend regular alcohol consumption in moderation, it is essential for physicians to weigh benefits and risks of its use. Well-known complications such as liver disease and chronic pancreatitis are relatively rare in moderate/low alcohol use. Alcohol abuse has been associated with development of several skin disorders including psoriasis, rosacea, discoid eczema, palmer erythema and spider nevi (Higgins and du Vivier, 1992). Chronic alcohol abuse is also a predisposing factor for necrotizing wound infections and delayed wound healing (Dhaene et al., 1986; Faunce et al., 2003; Lawlor et al., 1992; Radek et al., 2005). Melanoma and basal cell carcinoma onset have also been correlated with alcohol ingestion (Freedman et al., 2003a,b; Fung et al., 2002; Le Marchand et al., 2006). Our knowledge regarding the mechanisms, by which alcohol consumption triggers these effects, however, is limited, but the pathogenesis of ethanol effects on the skin is consistent with increases in generation of reactive oxygen species (ROS). Allergic and inflammatory skin diseases are mediated by oxidative stress (Drewa et al., 2002; Leveque et al., 2004; Yildirim et al., 2003), and it has been hypothesized

* Corresponding author. Tel.: +1 412 648 3399; fax: +1 412 648 9731.
E-mail address: brandr@dom.pitt.edu (R.M. Brand).

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.01.034

that the pathogenesis of melanoma is driven by an altered redox state that is generated during malignant melanoma tumorigenesis (Meyskens et al., 2004).

Chemical exposures in the home, workplace or outdoors are unavoidable with some of these compounds posing health risks. The skin is the major site of absorption for many chemicals; therefore, a compromised dermal barrier could increase the level of toxins entering the body. Both chronic and acute ethanol ingestion enhance transdermal penetration of xenobiotics (Brand et al., 2004,2006; Squier et al., 2003). The question therefore arises if the same mechanism is responsible for increased xenobiotic absorption with both exposure patterns. Previously, we have demonstrated that vasodilation triggered by a single episode of ethanol ingestion is not responsible for the observed increase in transdermal absorption (Brand et al., 2007a). Furthermore, increases in transdermal absorption could also be due to ROS generated damage to the lipid bilayers of the stratum corneum that are principally responsible for skin barrier function. Acute alcohol consumption causes changes in skin lipids, leading to increases in both lipid peroxidation and transepidermal water loss (TEWL) (Brand et al., 2007a). The mechanisms by which chronic ethanol ingestion impact skin barrier function are not clear. We hypothesized that chronic alcohol consumption will cause alterations in the skin that are similar to those that occur in alcoholic liver disease. These changes include increases in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as well as free radical generation (Skrzydlewska et al., 2002), leading to barrier damaging lipid peroxidation. To test the hypotheses, rats were pair fed a diet containing 36% ethanol for twelve weeks. During this time, changes in dermal blood flow using laser Doppler velocimetry (LDV), damage to the skin barrier via TEWL and alterations in skin moisture content were non-invasively measured. After 12 weeks, the animals were switched to a non-ethanol diet and were monitored for up to four weeks. At 0, 1 day, or 1, 2, 3, 4 weeks rats were euthanized and their skin was analyzed for alcohol and aldehyde dehydrogenase, and lipid peroxidation. Transdermal penetration of the herbicide paraquat, the industrial solvent dimethylformamide (DMF), the commonly used insect repellant *N*,*N*-diethyl-*m*-toluamide (DEET) and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was also determined at each time point.

2. Methods

2.1. Animal model

All animals were maintained in an AAALAC approved facility and were treated according to the NIH Guide for the Care and Use of Laboratory Animals. Male Wistar rats were fed the Lieber DeCarli liquid diet, where 36% of the calories are due to ethanol for 12 weeks. The diet contains 35% fat and 18% of total protein, 36% ethanol and the remainder as carbohydrate (Lieber and DeCarli, 1989). Rats were pair fed to controls receiving a diet where ethanol was replaced isocalorically with carbohydrate ($n = 9$). After 12 weeks the liquid diet was replaced with standard chow and animals were euthanized at either just before the transition or 1 day, 1, 2, 3, 4 weeks after chow feeding.

2.2. Non-invasive measurements

Dermal blood flow, TEWL and skin moisture were non-invasively measured prior to ethanol feeding and 1 day, 1, 2, 3, 4, 6, 8, 10 and 12 weeks after starting alcohol and 1 day, 1, 2, 3, or 4 weeks after returning to a chow diet. The back of each rat was carefully shaved using dog grooming clippers (Oster, blade #40) taking care not to abrade the skin, prior to measurement. Animals were held in a comfortable position without the use of anesthesia since there was concern that it could interfere with the LDV blood flow measurements (Fizanne et al., 2003; McKaigney et al., 1986). The laser of the Moorlab server (Moor Instruments, Wilmington Delaware) was placed on the shaved region of the back and blood flow was measured for 60 s. Average readings were taken from stable portions of the curve and the procedure was repeated. TEWL was measured using a closed chamber evaporimeter (VapoMeter SWL2g; Delfin Technologies Ltd, Kuopio Finland) that monitors the increases in relative humidity inside the chamber and reports them as g/m^2-h (Fluhr et al., 2006). Skin moisture content was determined in the dermis using a Moisture MeterD (Delfin Technologies Ltd, Kuopio Finland). A S15 probe with an effective measurement depth of 1.5 mm was used to measure dermal moisture by exposing the skin to a 300 MHz low power electromagnetic wave. The measured dielectric constant is proportional to tissue moisture levels (Nuutinen et al., 2004). Stratum corneum moisture was measured using a Scalar Moisture Checker (SCALAR AMERICA, Sacramento CA) which monitors skin conductance levels to calculate the absolute water content (Asano-Kato et al., 2001).

2.3. Diffusion studies

An *in vitro* flow-through diffusion cell system with chambers containing a surface area of 0.8 cm^2 was used for transdermal penetration experiments (PermeGear, Riegelsville, PA). The donor solutions were 100 μL of either (1) 2,4-D amine (Agriliance, St. Paul, MN), diluted to 1.2% (1.2 mg) and spiked with 50,000 dpm [^{14}C] 2,4-D (specific activity 92.6 μCi/mg, Sigma St. Louis, MO), (2) Paraquat, (Gramoxone Extra, ZenecaAg/Syngenta, Wilmington DE), diluted in to 0.93% (0.93 mg) in water mixed with 50,000 dpm [^{14}C] paraquat – methyl ^{14}C dichloride (specific activity 125.7 μCi/mg, Sigma St. Louis, MO), (3) DEET (Sigma St. Louis, MO) diluted to 19% (19 mg) in acetone (since it is insoluble in water) spiked with 50,000 dpm [^{14}C] DEET (specific activity 209.1 μCi/mg, Sigma St. Louis, MO) or (4) DMF (Sigma St. Louis,

MO) diluted to 3% (3 mg) in water spiked with 50,000 dpm [^{14}C] dimethyl formamide (DMF, specific activity 752.4 μCi/mg Sigma St. Louis, MO). Each concentration was chosen because it reflects common usage of the product. The dermal side was in contact with the continuously perfused receiver fluid comprised of Hanks balanced saline solution (HBSS) and the system was maintained at 32 °C. Fractions were collected in 120-minute increments and were assayed for radioactivity using liquid scintillation counting (Packard, Tricarb Model 1600CA).

2.4. Enzyme assays

A 15 mg piece of skin was snap frozen and pulverized using a BioPulverizer (BioSpec Products, Bartlesville, OK). The powder was placed into 1 ml of saline and centrifuged with the effluent being used for all assays. Alcohol dehydrogenase was assayed using a spectrophotometric method (Boleda et al., 1989). Briefly, a cuvette was filled with 1.4 ml of 0.05 M sodium pyrophosphate buffer, 1.4 ml of 0.025 M NAD^+ and 0.1 ml ethanol. The reaction was initiated by the addition of 0.1 ml of skin homogenate to the cuvette. The increase in absorbance at 340 nm and 25 C was monitored for 5 min. Aldehyde dehydrogenase was assayed following the method of Bostian and Betts (Bostian and Betts, 1978). The cuvette contained 2.2 ml distilled water, 0.3 ml of 1.0 M Tris–HCL buffer, 0.1 ml of 0.005 M acetaldehyde, 0.1 ml of 3.0 M KCl, 0.1 ml of 0.33 M α-2-mercaptoethanol and 0.1 ml of 0.02 M NAD^+. The reaction was started by the addition of the 0.1 ml of skin homogenate as a source of aldehyde dehydrogenase.

2.5. Lipid peroxidation

Lipid peroxidation triggers the breakdown of polyunsaturated fatty acids, leading to the formation of malondialdehyde, which then reacts with thiobarbituric acid (TBAR) to give a red species absorbing at 535 nm. One ml of the sample was combined with 2.0 ml of TCA–TBA–HCl reagent (15%w/v trichloroacetic acid; 0.375%w/v thiobarbituric acid and 0.25 N hydrochloric acid), heated for 15 min and then centrifuged for 10 min at 1000*g*. The absorbance was determined at 535 nm against a blank containing all reagents minus the sample. The malondialdehyde concentration of the sample was calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Buege and Aust, 1978).

2.6. Data analysis

The data were normalized to account for any variability in feeding regime that may have occurred. The measured value from each ethanol-fed animal was divided by the corresponding result from its pair fed control ($n = 9$) for each assay and non-invasive measurement. The enhancement ratio (ER) was calculated for each pair of animals and the mean ± s.e.m for each ER were determined. The enhancement ratio was further analyzed to determine if it was statistically different from 1.0 (which would indicate no enhancement) using a two-tailed, one sample *t*-test with significance set at $p < 0.05$ (GraphPad Prism, GraphPad, San Diego, CA) (Brand et al., 2006).

Transdermal absorption data were analyzed by computing cumulative percent penetrated vs. time. The enhancement ratio (ER) was determined by dividing the amount through skin from ETOH-treated rats at 24 h by the amount through control skin at 24 h, for each pair tested ($n = 9$). This direct comparison between control and ethanol-fed rat was the basis for the statistical analysis described above.

3. Results

3.1. Chronic ethanol consumption triggers enzymatic changes in the skin

The effect of the Lieber–DeCarli diet on skin enzymes was examined by assaying rat skin for ADH and ALDH levels. Fig. 1 demonstrates that chronic ethanol consumption upregulated these skin enzymes associated with alcohol metabolism with enhancement ratios of 1.6 ($p < 0.01$) for both enzymes when compared with non-alcohol ingesting animals. This enhancement continued for at least two weeks after cessation of ethanol ingestion even though skin ethanol levels returned to baseline 24 h after alcohol removal (data not shown).

3.2. Alcohol ingestion causes reactive oxygen species formation in the skin

The hypothesis that alcohol may trigger changes in skin barrier function by inducing oxidative stress was tested by examining the ability of ethanol and its metabolites to cause oxidative damage to skin lipids, as indicated by lipid peroxidation, using the TBAR assay (Buege and Aust, 1978). Lipid peroxidation was enhanced 2.3 fold after 12 weeks of ethanol ingestion when compared to pair fed controls ($p < 0.01$). While the level of lipid peroxidation dropped rapidly upon termination of alcohol exposure (ER = 1.3, $p < 0.05$), it does remain significantly elevated for an additional two weeks (Fig. 2).

3.3. Chronic ethanol consumption induces physical changes in the skin

The functional integrity of the skin barrier can be measured non-invasively by monitoring the permeability of water through the stratum corneum quantitated as TEWL (Baker and Kligman, 1967). Alcohol is a potent vasodilator known to act on the skin as characterized by ethanol-induced flushing. LDV is a technique that non-invasively measures dermal blood flow using light transmitted from a helium–neon laser light source and is therefore a sensitive

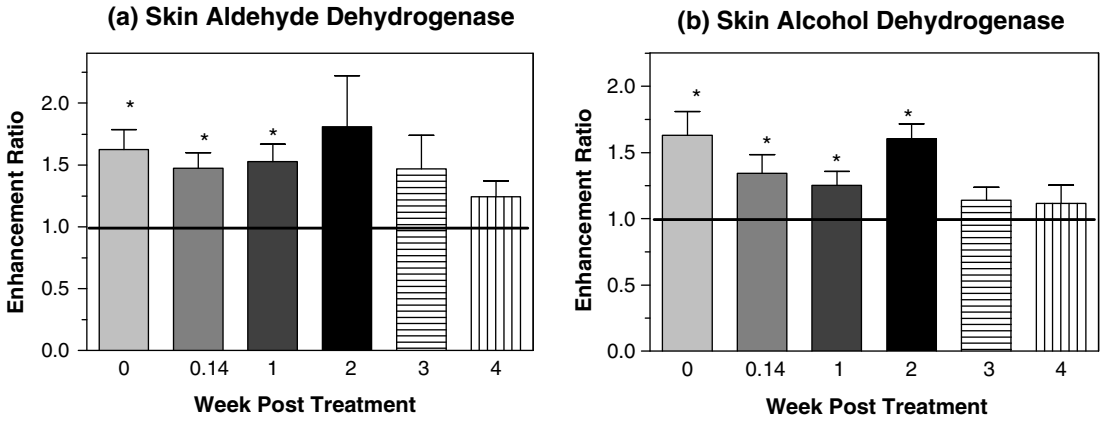

Fig. 1. Effect of ethanol consumption on (a) alcohol dehydrogenase and (b) aldehyde dehydrogenase as a function the duration on chow diet after 12 weeks of ethanol consumption. Enhancement Ratio = value ETOH animal/value control animal. Data are presented as mean ± s.e.m ($n = 9$). * = $p < 0.05$.

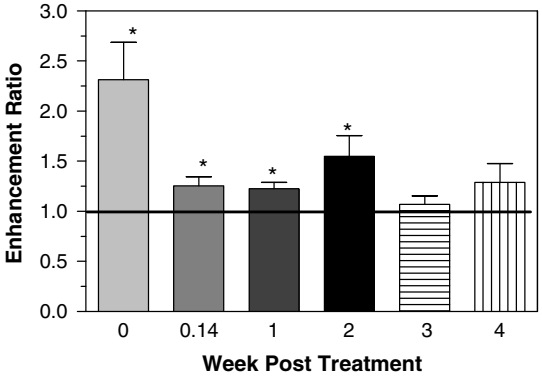

Fig. 2. Lipid peroxidation as determined by malondialdehyde levels as a function the duration on chow diet after 12 weeks of ethanol consumption. Enhancement Ratio = value ETOH animal/value control animal. Data are presented as mean ± s.e.m ($n = 9$). * = $p<0.05$.

indicator of changes in local skin blood flow (Guy et al., 1985; Holloway and Watkins, 1977).

Dermal blood flow, TEWL and skin moisture levels were measured throughout the course of the experiment. Animals were pair fed the Lieber–DeCarli diet for 12 weeks and then were switched to a chow diet for either 1 day, or 1, 2, 3, or 4 weeks. Fig. 3 presents the enhancement ratio for each of these parameters. The data as evidenced by an ER > 1.0 demonstrate that chronic ethanol ingestion triggered increased skin blood flow (Fig. 3a, LDV) within one day of beginning ingestion. This effect continued for four weeks after removal of alcohol from the diet. Additionally, the TEWL measurements demonstrate the skin barrier was altered by chronic ethanol consumption, but this effect was more gradual in onset than the changes in dermal blood flow (Fig. 3b TEWL ER > 1.0). TEWL remained elevated for at least three weeks after alcohol cessation. Dermal moisture levels also increased with alcohol consumption, but this may be a reflection of increased blood flow as the patterns are similar (Fig. 3c). Furthermore, stratum corneum moisture levels were reduced by drinking alcohol (Fig. 3d).

3.4. Alcohol ingestion increases transdermal absorption of xenobiotics

Fig. 4 demonstrates the impact of alcohol consumption on the absorption of the xenobiotics tested. Both the cumulative penetration curve through skin from animals euthanized while still on an ethanol diet (day 0) and the enhancement ratio (ER) as a function of time after cessation of ethanol ingestion are presented. The ER is defined as the cumulative amount absorbed at 24 h through skin from ethanol treated animals/absorption through skin from pair fed control animals. Ethanol increased the transdermal penetration for each of the four compounds tested with Fig. 4a and b showing paraquat, Fig. 4c and d demonstrating DMF, Fig. 4e and f giving DEET results and Fig. 4g and h showing 2,4-D. Paraquat had the greatest enhancement ratio at day 0 (3.2) followed by 2,4-D (2.3), DMF(1.9) and DEET (1.4). The transdermal absorption of DMF remained significantly elevated ($p < 0.05$) for at least four weeks after cessation of ethanol treatment. Penetration of the other xenobiotics also remained elevated through skin from ethanol-fed animals for the duration of the experiments but the enhancement was not statistically significant.

4. Discussion

These studies characterized increases in transdermal penetration and the mechanisms by which these changes occurred in rats given ethanol chronically. The data demonstrate greater levels of skin enzymes associated with ethanol metabolism and higher generation of reactive oxygen species that parallel those found in the liver after alcohol consumption. Furthermore, ethanol ingestion induces changes in the skin leading to increased internalization of hazardous chemicals. The prototype xenobiotics used reflect exposures in an industrial, agricultural and home environment. There is a link between toxic chemical exposures and multiple diseases, some of which are also

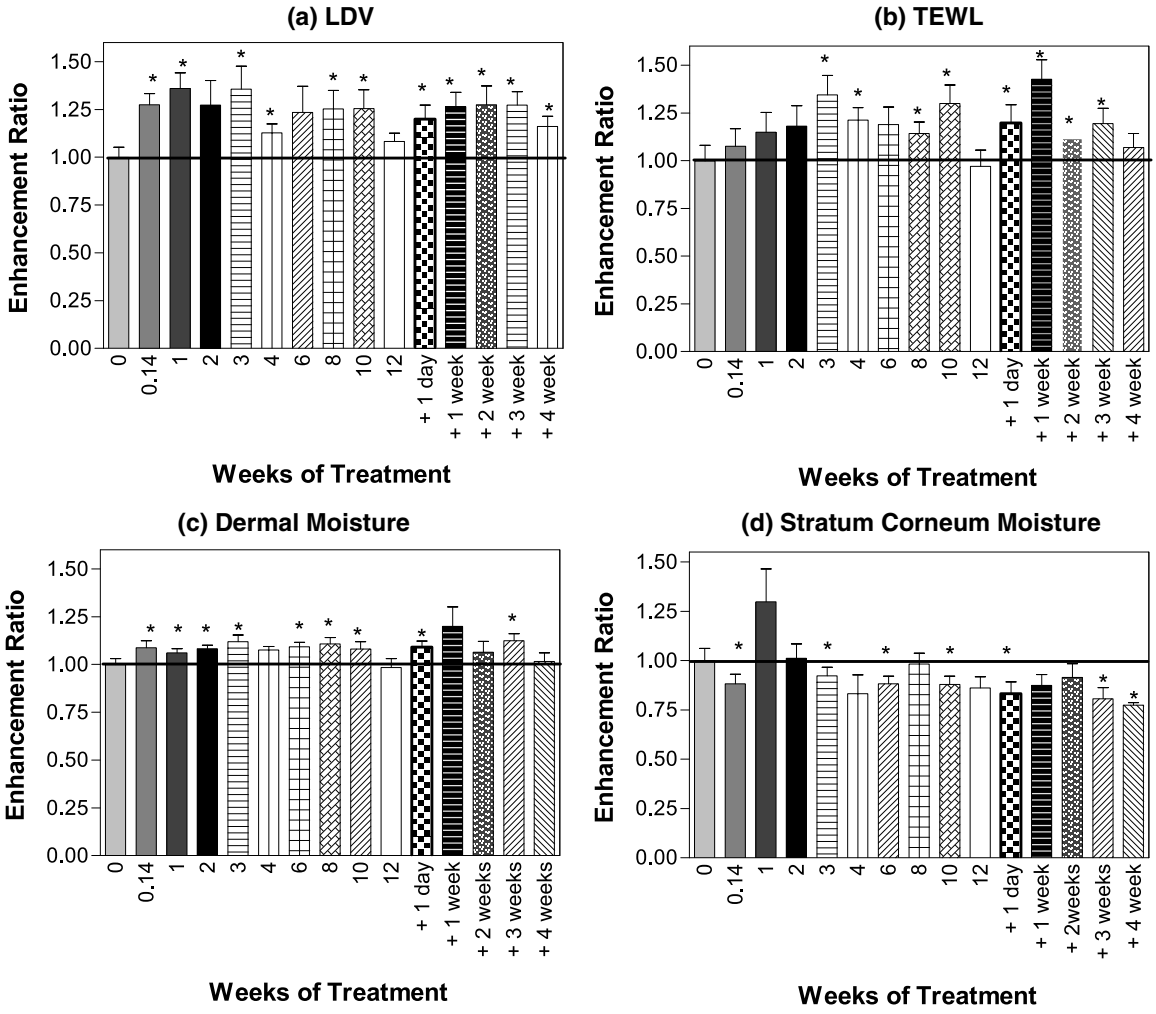

Fig. 3. Impact of ethanol consumption on (a) LDV, (b) TEWL, (c) dermal moisture and (d) SC moisture measurements as a function the duration on chow diet after 12 weeks of ethanol consumption. Enhancement Ratio = value ETOH animal/value control animal. Data are presented as mean ± s.e.m ($n = 9$). * = $p < 0.05$.

associated with alcohol consumption. It is possible that the interaction between these chemicals have a greater impact in disease development than previously anticipated. Directly comparing the results of the experiments presented in this work with those from an earlier study examining changes after acute alcohol consumption (Brand et al., 2007a) supports the idea that multiple mechanisms may be responsible for ethanol-induced changes in the skin.

The Lieber–DeCarli ethanol-fed rat model leads to clinically relevant blood alcohol levels above 100 mg/dL, 20 mM or 0.1% ethanol (Lieber and DeCarli, 1989). It is an accepted model for human alcohol consumption as it causes the usual ethanol-induced hepatic changes, including hepatomegaly, elevated triglycerides and ultrastructural changes (Casey et al., 1987; Lieber, 1993). Rat skin is also an appropriate model for human dermal absorption. While the exact quantity of xenobiotic penetration will vary between species, delivery trends tend to be similar, with rat skin averaging 3.1 greater permeability (range 1.7–5.8) than human skin (Barber et al., 1992; Priborsky and Muhlbachova, 1990).

Alcohol ingestion induces multiple enzymes in the liver to assist in its metabolism, with the main pathways involving the enzymes ADH and ALDH or cytochrome P450-2E1. Much of the toxicity of ethanol is associated with the redox changes and oxidative stress generated by these enzymes during the course of ethanol metabolism (Lieber, 2004). The impact of alcohol consumption on these enzymes in the skin however is unknown. The skin is a major source of extrahepatic alcohol dehydrogenase giving it the highest capacity for ethanol oxidation outside the liver. While its activity per mg of tissue is quite low (2.8 mU/g tissue), in rats, the size of the skin makes it a major factor with a total level of 88.0 ± 15 mU/organ which is 1/40th of the livers level and 23% of the total extrahepatic rate. Rat skin contains both the isoenzymes ADH-1 and ADH-2, but not ADH-3 (Boleda et al., 1989). Chronic ethanol consumption induced skin ADH

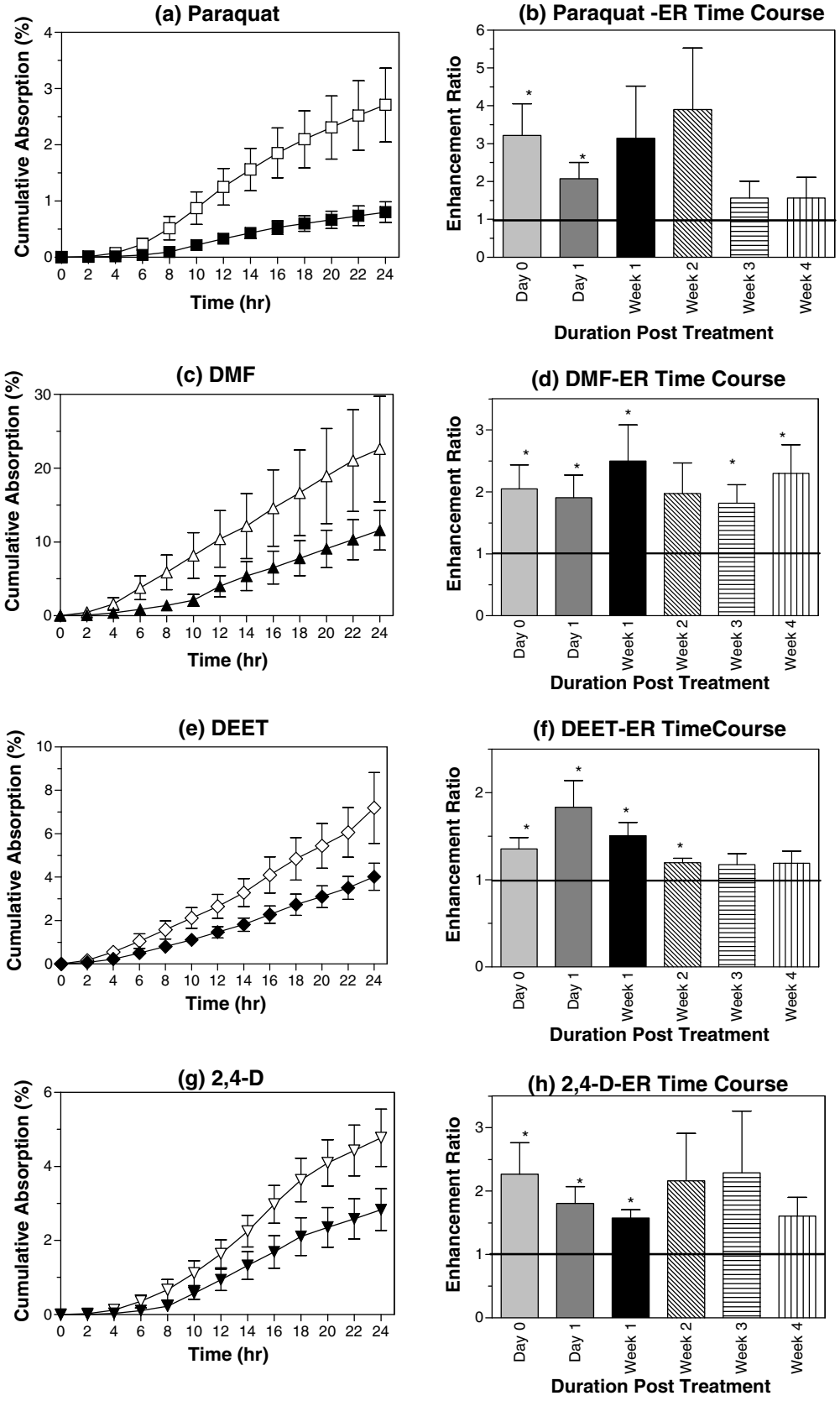

Fig. 4. Transdermal absorption of (a) paraquat, (c) DMF, (e) DEET, (g) 2,4-D taken from animals after 12 weeks of ethanol ingestion (day 0). Open symbols are ethanol-fed; closed symbols were fed a control diet. Enhancement ratios (ER) for transdermal penetration studies as a function the duration on chow diet after 12 weeks of ethanol consumption (b) paraquat, (d) DMF, (f)DEET, (h) 2,4-D. Enhancement Ratio = % penetration ETOH animal skin/% penetration control animal skin after 24 h. Data are presented as mean ± s.e.m ($n = 9$) $^{*} = p < 0.05$.

levels by 60% compared to pair fed controls and this enhancement remained for at least two weeks after alcohol cessation. Aldehyde dehydrogenase levels also increased by 60% after chronic ethanol consumption then gradually decreased to near control levels at four weeks of abstinence.

Lipid peroxidation results from the generation of reactive oxygen species and consists of the oxidative degradation of glycerophospholipids, sphingolipids, unsaturated fatty acids and cholesterol (Lasch et al., 1997). Ethanol ingestion triggers an increase in lipid peroxidation, as indicated by the formation of maldionaldehyde in the liver, heart, brain and testes (Kasdallah-Grissa et al., 2006). It also induces lipid peroxidation in skin of alcohol consuming animals. Chronic ethanol consumption for 12 weeks increased malondialdehyde levels by 230%. Skin lipid peroxidation dropped rapidly upon alcohol termination but remained elevated by 30–50% for an additional two weeks. These withdrawal levels are closer to those found after acute alcohol treatment. Lipid peroxidation levels significantly increased two hours after a single ethanol ingestion of either 6 or 10 g/kg ethanol by 63% and 40%, respectively (Brand et al., 2007a).

Disruptions in the lipid bilayers of the stratum corneum can compromise the functional integrity of the skin barrier and these changes can be monitored non-invasively through transepidermal water loss (Baker and Kligman, 1967; Grubauer et al., 1989). Water loss through the skin generated by transepidermal diffusion (independent of sweating) is a steady passive process limited by the stratum corneum. There is a correlation between increasing TEWL levels and percutaneous penetration (Levin and Maibach, 2005). SC lipid peroxidation levels correlate well with changes in TEWL levels after barrier disruption (Weber et al., 2003). A single dose of 10 g/kg alcohol increases TEWL by 12% in Wistar rats (Brand et al., 2007a) and by 30% in the flexor forearm and 50% in the forehead of humans (Jacobi et al., 2005). TEWL is not very sensitive to immediate changes in blood flow (Baker and Kligman, 1967), so ethanol-induced vasodilation would not responsible for the TEWL changes observed in this study. Rodrigues et al. (2004) found that a 90% decrease in LDV levels triggered just a 3% increase in TEWL levels, while restoring the blood flow back to original levels caused only a 7% reduction in TEWL. In the chronically fed animals, TEWL increased gradually, reached a maximum 35% increase after three weeks of ethanol ingestion, and remained elevated for three weeks after alcohol cessation.

In a previous study, we demonstrated both a trend toward increasing TEWL levels after ethanol ingestion and increases in lipid peroxidation, indicating that even a single incident of alcohol consumption causes changes in skin lipids (Brand et al., 2007a). Topically, ethanol is an effective penetration enhancer used in commercially available transdermal patches and topical preparations (Cornwell and Barry, 1995; Walters, 1989). It acts directly on the skin by increasing lipid fluidity near the polar interface by as much as 70% and altering the polar head group region of the lipid bilayers (Marjukka Suhonen et al., 1999). This increases the effective interfacial area of the bilayer leading to greater diffusivity and partitioning especially for hydrophilic permeants (Kim et al., 1992; Krill et al., 1992). Thus it is possible that both acute and chronic ethanol ingestion increase transdermal penetration by altering the lipid bilayer within the stratum corneum, even at the low doses associated with drinking.

Ethanol consumption at levels sufficient to damage the liver can interfere with skin barrier function by altering the fatty acids released from the liver. Epidermal lipid generation requires linoleate and arachidonate, but since they cannot be made in the skin, the liver provides them (Wertz and Downing, 1989). In the absence of sufficient levels of linoleate, oleate substitution occurs. Alcohol consumption decreases the level of linoleate while increasing the release of oleate and palimate into the blood stream (Dan and Laposata, 1997) allowing the development of normal intercellular lamellae, but an incomplete dermal barrier (Wertz and Downing, 1989,1990). Furthermore, the extended duration of increased xenobiotics penetration would also be consistent with alcohol-induced changes in fatty acid composition. This would support the hypothesis that ethanol-induced changes in skin barrier are directly due to liver damage and is consistent with the data presented in this study. However, previous work in our laboratory has found changes in xenobiotic permeability after a single dose of ethanol consumption (Brand et al., 2006,2007b) and acute ethanol ingestion would not be sufficient to trigger the liver changes.

The increases in blood flow, TEWL, lipid peroxidation and alcohol and acetaldehyde dehydrogenase all correlated with greater transdermal absorption of the topically applied xenobiotics triggered by ethanol ingestion. The enhancement magnitude for percutaneous penetration is dependent on the xenobiotic tested. These results are similar to earlier studies that demonstrate that both chronic and acute ethanol consumption increase the transdermal penetration of multiple xenobiotics. Rat skin is more permeable to tritiated water and the tobacco carcinogen nitrosonornicotine (Squier et al., 2003) as well as to small hydrophilic and moderately hydrophobic herbicides after chronic alcohol ingestion (Brand et al., 2004). Greater transdermal absorption is also found for the herbicide paraquat, the industrial solvent DMF, the commonly used insect repellant DEET and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) after rats are gavaged with a single dose of ethanol (Brand et al., 2006,2007b). Changes in blood flow, however were found to be independent of transdermal absorption in our system since injection of the vasoconstrictor epinephrine and vasodilator prilocaine did not alter the penetration of xenobiotics (Brand et al., 2007a) so it is unlikely to be responsible for penetration enhancement after chronic treatment.

The impact of withdrawal on the skin was examined by monitoring animals for up to four weeks after alcohol ces-

sation. Previously, we demonstrated that the transdermal penetration of the herbicine 2,4-D partially recovers 1–2 weeks after removal of alcohol from the diet (Brand et al., 2004). The data presented in this study are consistent with the earlier report, but have examined the effect of withdrawal on the skin in more detail. The stratum corneum is completely replaced every 3–4 weeks in normal skin so we hypothesized that the effects of alcohol would be reversed within four weeks of termination. The results, however, do not support this hypothesis and indicate that the residual effects of alcohol are interfering with the recovery of the skin barrier. The precise mechanism by which this occurs however, was not examined in this work and it is not possible to determine with certainty the mechanisms involved.

The data are consistent with alcohol-induced oxidative stress causing lipid peroxidation within the skin leading to a disruption of the dermal barrier. The induction of ADH and ALDH in skin are potential pathways by which alcohol generates these reactive oxygen species, within the skin. Additionally, there may be other by-products generated from ethanol during metabolism that can interfere with skin barrier function. The ability of ingested ethanol to act as a skin solvent, interfering with the lipids themselves was not examined, but would be a possible mechanism to explain the increases in transdermal penetration in both chronic and acute models. Changes in lipid composition of the skin, resulting in impaired barrier function after chronic ethanol ingestion, were not directly tested in this work, but increases in TEWL and decreases in stratum cornuem moisture are consistent with these changes. This mechanism, however would likely only be a factor in chronic alcohol and not acute intake. Dermal blood flow is increased with alcohol ingestion and this may contribute to the increased penetration, however earlier studies in the acute feeding model demonstrated that greater blood flow was not responsible for higher transdermal absorption. These observations make it less likely that ethanol-triggered vasodilation is the primary mechanism responsible for increased xenobiotic absorption after chronic ingestion.

Ethanol induces changes in the skin that mimic those seen in the liver including increases in enzymes related to alcohol metabolism, oxidative stress and blood flow and these changes remain for at least four weeks after ethanol withdrawal. It is not possible to determine whether skin alterations from chronic ethanol consumption are due solely to metabolic changes within the skin or if ethanol effects on the liver are indirectly responsible for the skin modifications. Further studies are necessary to better explore the liver–skin axis to determine the interrelationship between alcohol consumption and skin disorders.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors would like to acknowledge the assistance of Jennifer Koestier, Nirmita Kumar and Harrison Brand. This work was supported by NIH Grant Number 1 R21 AA015199-01.

References

Asano-Kato, N., Fukagawa, K., Tsubota, K., Urayama, K., Takahashi, S., Fujishima, H., 2001. Quantitative evaluation of atopic blepharitis by scoring of eyelid conditions and measuring the water content of the skin and evaporation from the eyelid surface. Cornea 20, 255–259.

Baker, H., Kligman, A.M., 1967. Measurement of transepidermal water loss by electrical hygrometry. Instrumentation and responses to physical and chemical insults. Arch. Dermatol. 96, 441–452.

Barber, E.D., Teetsel, N.M., Kolberg, K.F., Guest, D., 1992. A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundam. Appl. Toxicol. 19, 493–497.

Boleda, M.D., Julia, P., Moreno, A., Pares, X., 1989. Role of extrahepatic alcohol dehydrogenase in rat ethanol metabolism. Arch. Biochem. Biophys. 274, 74–81.

Bostian, K.A., Betts, G.F., 1978. Kinetics and reaction mechanism of potassium-activated aldehyde dehydrogenase from *Saccharomyces cerevisiae*. Biochem. J. 173, 787–798.

Brand, R.M., Charron, A.R., Dutton, L., Gavlik, T.L., Mueller, C., Hamel, F.G., Chakkalakal, D., Donohue Jr., T.M., 2004. Effects of chronic alcohol consumption on dermal penetration of pesticides in rats. J. Toxicol. Environ. Health A 67, 153–161.

Brand, R.M., Jendrzejewski, J.L., Charron, A.R., 2007a. Potential mechanisms by which a single drink of alcohol can increase transdermal absorption of topically applied chemicals. Toxicology 235, 141–149.

Brand, R.M., Jendrzejewski, J.L., Henery, E.M., Charron, A.R., 2006. A single oral dose of ethanol can alter transdermal absorption of topically applied chemicals in rats. Toxicol. Sci. 92, 349–355.

Brand, R.M., McMahon, L., Jendrzejewski, J.L., Charron, A.R., 2007b. Transdermal absorption of the herbicide 2,4-dichlorophenoxyacetic acid is enhanced by both ethanol consumption and sunscreen application. Food Chem. Toxicol. 45, 93–97.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Casey, C.A., Kragskow, S.L., Sorrell, M.F., Tuma, D.J., 1987. Chronic ethanol administration impairs the binding and endocytosis of asialoorosomucoid in isolated hepatocytes. J. Biol. Chem. 262, 2704–2710.

Cornwell, P.A., Barry, B.W., 1995. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int. J. Pharmaceut. 117, 101–112.

Dan, L., Laposata, M., 1997. Ethyl palmitate and ethyl oleate are the predominant fatty acid ethyl esters in the blood after ethanol ingestion and their synthesis is differentially influenced by the extracellular concentrations of their corresponding fatty acids. Alcohol Clin. Exp. Res. 21, 286–292.

Dhaene, M., Thys, J.P., Askenasi, R., Toussaint, C., 1986. Pneumococcal cellulitis. Am. J. Emerg. Med. 4, 225–226.

Drewa, G., Krzyzynska-Malinowska, E., Wozniak, A., Protas-Drozd, F., Mila-Kierzenkowska, C., Rozwodowska, M., Kowaliszyn, B., Czajkowski, R., 2002. Activity of superoxide dismutase and catalase and the level of lipid peroxidation products reactive with TBA in patients with psoriasis. Med. Sci. Monit. 8, BR338–BR343.

Faunce, D.E., Garner, J.L., Llanas, J.N., Patel, P.J., Gregory, M.S., Duffner, L.A., Gamelli, R.L., Kovacs, E.J., 2003. Effect of acute ethanol exposure on the dermal inflammatory response after burn injury. Alcohol Clin. Exp. Res. 27, 1199–1206.

Fizanne, L., Fromy, B., Preckel, M.P., Sigaudo-Roussel, D., Saumet, J.L., 2003. Effect of isoflurane on skin-pressure-induced vasodilation. J. Vasc. Res. 40, 416–422.

Fluhr, J.W., Feingold, K.R., Elias, P.M., 2006. Transepidermal water loss reflects permeability barrier status: validation in human and rodent in vivo and ex vivo models. Exp. Dermatol. 15, 483–492.

Freedman, D.M., Sigurdson, A., Doody, M.M., Mabuchi, K., Linet, M.S., 2003a. Risk of basal cell carcinoma in relation to alcohol intake and smoking. Cancer Epidemiol. Biomarkers Prev. 12, 1540–1543.

Freedman, D.M., Sigurdson, A., Doody, M.M., Rao, R.S., Linet, M.S., 2003b. Risk of melanoma in relation to smoking, alcohol intake, and other factors in a large occupational cohort. Cancer Cause. Control 14, 847–857.

Fung, T.T., Hunter, D.J., Spiegelman, D., Colditz, G.A., Rimm, E.B., Willett, W.C., 2002. Intake of alcohol and alcoholic beverages and the risk of basal cell carcinoma of the skin. Cancer Epidemiol. Biomarkers Prev. 11, 1119–1122.

Grubauer, G., Feingold, K.R., Harris, R.M., Elias, P.M., 1989. Lipid content and lipid type as determinants of the epidermal permeability barrier. J. Lipid Res. 30, 89–96.

Guy, R.H., Tur, E., Maibach, H.I., 1985. Optical techniques for monitoring cutaneous microcirculation recent applications. Int. J. Dermatol. 24, 88–94.

Higgins, E.M., du Vivier, A.W., 1992. Alcohol and the skin. Alcohol Alcoholism 27, 595–602.

Holloway Jr., G.A., Watkins, D.W., 1977. Laser Doppler measurement of cutaneous blood flow. J. Invest. Dermatol. 69, 306–309.

Jacobi, U., Bartoll, J., Sterry, W., Lademann, J., 2005. Orally administered ethanol: transepidermal pathways and effects on the human skin barrier. Arch. Dermatol. Res. 296, 332–338.

Kasdallah-Grissa, A., Mornagui, B., Aouani, E., Hammami, M., Gharbi, N., Kamoun, A., El-Fazaa, S., 2006. Protective effect of resveratrol on ethanol-induced lipid peroxidation in rats. Alcohol Alcoholism 41, 236–239.

Kim, Y.-H., Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., 1992. Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeant in hairless mouse skin: Mechanisms of action. Int. J. Pharmaceut. 80, 17–31.

Krill, S.L., Knutson, K., Higuchi, W.I., 1992. Ethanol effects on the stratum corneum lipid phase behavior. Biochim. Biophys. Acta 1112, 273–280.

Lasch, J., Schonfelder, U., Walke, M., Zellmer, S., Beckert, D., 1997. Oxidative damage of human skin lipids. Dependence of lipid peroxidation on sterol concentration. Biochim. Biophys. Acta 1349, 171–181.

Lawlor, M.T., Crowe, H.M., Quintiliani, R., 1992. Cellulitis due to Streptococcus pneumoniae: case report and review. Clin. Infect Dis. 14, 247–250.

Le Marchand, L., Saltzman, B.S., Hankin, J.H., Wilkens, L.R., Franke, A.A., Morris, S.J., Kolonel, L.N., 2006. Sun exposure, diet, and melanoma in Hawaii Caucasians. Am. J. Epidemiol. 164, 232–245.

Leveque, N., Robin, S., Muret, P., Mac-Mary, S., Makki, S., Berthelot, A., Kantelip, J.P., Humbert, P., 2004. In vivo assessment of iron and ascorbic acid in psoriatic dermis. Acta Derm. Venereol. 84, 2–5.

Levin, J., Maibach, H., 2005. The correlation between transepidermal water loss and percutaneous absorption: an overview. J. Control Release 103, 291–299.

Lieber, C.S., 1993. Biochemical factors in alcoholic liver disease. Semin. Liver Dis. 13, 136–153.

Lieber, C.S., 2004. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 34, 9–19.

Lieber, C.S., DeCarli, L.M., 1989. Liquid diet technique of ethanol administration: 1989 update. Alcohol Alcoholism 24, 197–211.

Marjukka Suhonen, T., Bouwstra, J.A., Urtti, A., 1999. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J. Control Release 59, 149–161.

McKaigney, J.P., Carmichael, F.J., Saldivia, V., Israel, Y., Orrego, H., 1986. Role of ethanol metabolism in the ethanol-induced increase in splanchnic circulation. Am. J. Physiol. 250, G518–G523.

Meyskens Jr., F.L., Farmer, P.J., Anton-Culver, H., 2004. Etiologic pathogenesis of melanoma: a unifying hypothesis for the missing attributable risk. Clin. Cancer Res. 10, 2581–2583.

Nuutinen, J., Ikaheimo, R., Lahtinen, T., 2004. Validation of a new dielectric device to assess changes of tissue water in skin and subcutaneous fat. Physiol. Meas. 25, 447–454.

Priborsky, J., Muhlbachova, E., 1990. Evaluation of in vitro percutaneous absorption across human skin and in animal models. J. Pharm. Pharmacol. 42, 468–472.

Radek, K.A., Matthies, A.M., Burns, A.L., Heinrich, S.A., Kovacs, E.J., Dipietro, L.A., 2005. Acute ethanol exposure impairs angiogenesis and the proliferative phase of wound healing. Am. J. Physiol. Heart Circ. Physiol. 289, H1084–H1090.

Rodrigues, L.M., Pinto, P.C., Magro, J.M., Fernandes, M., Alves, J., 2004. Exploring the influence of skin perfusion on transepidermal water loss. Skin Res. Technol. 10, 257–262.

Skrzydlewska, E., Ostrowska, J., Stankiewicz, A., Farbiszewski, R., 2002. Green tea as a potent antioxidant in alcohol intoxication. Addict. Biol. 7, 307–314.

Squier, C.A., Kremer, M.J., Wertz, P.W., 2003. Effect of ethanol on lipid metabolism and epithelial permeability barrier of skin and oral mucosa in the rat. J. Oral Pathol. Med. 32, 595–599.

Standridge, J.B., Zylstra, R.G., Adams, S.M., 2004. Alcohol consumption: an overview of benefits and risks. South. Med. J. 97, 664–672.

Walters, K.A., 1989. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Marcel Dekker, New York, pp. 197–247.

Weber, S.U., Thiele, J.J., Han, N., Luu, C., Valacchi, G., Weber, S., Packer, L., 2003. Topical alpha-tocotrienol supplementation inhibits lipid peroxidation but fails to mitigate increased transepidermal water loss after benzoyl peroxide treatment of human skin. Free Radic. Biol. Med. 34, 170–176.

Wertz, P.W., Downing, D.T., 1989. Stratum corneum: biological and biochemical considerations. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Marcel Dekker, Inc, New York, pp. 1–22.

Wertz, P.W., Downing, D.T., 1990. Metabolism of linoleic acid in porcine epidermis. J. Lipid Res. 31, 1839–1844.

Yildirim, M., Inaloz, H.S., Baysal, V., Delibas, N., 2003. The role of oxidants and antioxidants in psoriasis. J. Eur. Acad. Dermatol. Venereol. 17, 34–36.