Toxicology in Vitro 44 (2017) 142–153

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Comparative assessment of local tolerance of alcohols commonly used in alcohol-based hand rubs for hand hygiene

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ARTICLE INFO

Keywords:
Alcohol-based hand rubs
Hand hygiene
In vitro
Skin irritation
Reconstructed human epidermis
Phototoxicity

ABSTRACT

Hand hygiene plays a key role in nosocomial infection prevention. To achieve users' adherence, products' dermal tolerance is essential. We aimed at making a comparative assessment of skin irritation and phototoxicity of the 3 alcohols commonly used in alcohol-based hand rubs (Ethanol, Propan-2-ol, Propan-1-ol) at 60, 70, 80 or 85% w/w in water or with co-formulates (hydrating, emollient and skin protective agents). *In vitro* validated OECD methods 439 and 432 were used. For irritation, EpiSkin™ Small Model was the chosen Reconstructed Human Epidermis (RhE). For phototoxicity, co-formulates alone or in mixture with and without alcohol were tested using BALB/c 3T3 cell cultures. Whilst Ethanol and Propan-2-ol could not be differentiated and displayed good skin tolerance profiles, Propan-1-ol based products lead to significant viability impairments of RhE at 60, 70 or 80% and at 60% in the presence of co-formulates. However, these results could not be reproduced in another RhE model. Taking also into account bibliographic data on Propan-1-ol, this suggests that our results are probably related to a lack of specificity of the used RhE. Therefore, it can be relevant in case of significant results to use two different RhE models before performing any classification and/or performing any complementary tests.

1. Introduction

In its recommendations for hand hygiene in health care, the World Health Organization (WHO) states that hand hygiene is the first infectious risk prevention measure associated with care. It helps prevent the risk of microbial transmission to patients, health-care workers (HCW) and health-care environment. Non-adherence to hand hygiene is considered the major cause of occurrence of healthcare-associated infections and spread of multi-resistant organisms; it is also recognized as a significant factor in the development of outbreaks (WHO 2009). The 5 indications for hand hygiene identified to effectively interrupt pathogen transmission during care are “Before patient contact”, “Before aseptic task”, “After body fluid exposure risk”, “After patient contact” and “After contact with patient surroundings”. This involves a high number of hand hygiene episodes during some care practices, which can reach up to 30 occurrences per hour (WHO 2009).

Ultimate use of hand hygiene products will depend on user's acceptance and skin tolerability. Currently, products that best suit are Alcohol-based hand rubs (ABHR). Their high content of alcohol, mainly Ethanol, Propan-2-ol or Propan-1-ol, or a combination of two of them, gives them efficiency against a broad spectrum of pathogens (bacteria, yeast, fungi, virus, mycobacterium, *etc.*) in a rather short-time action. They are easy to set up because of their use without water point, as long as hands are visibly clean. However, alcohols are known for their dehydrating effect in relation with their lipid-dissolving effect, and can therefore be responsible of skin dryness, and in some extent, skin irritation. Poor acceptance of alcohol-based hand-rubs was described in some hospitals in relation with the drying effect of alcohols (Larson

Abbreviations: ATCC, American Type Culture Collection; COLIPA, Now Cosmetics Europe – The Personal Care Association; ECVAM, European Centre for the Validation of Alternative Methods; EFSA, European Food Safety Authority; ESAC, ECVAM Scientific Advisory Committee; GHS, Globally Harmonized System; OECD, Organization for Economic Co-operation and Development; SCCS, Scientific Committee on Consumer Safety; UN, United Nations; WHO, World Health Organization

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http://dx.doi.org/10.1016/j.tiv.2017.07.004
Received 5 April 2017; Received in revised form 5 July 2017; Accepted 7 July 2017
Available online 09 July 2017
0887-2333/

et al. 2006).

Throughout Europe and the United States, health professionals are among the occupational groups at greatest risk to present Irritant Contact Dermatitis (ICD) (English 2004, Halioua et al. 2012). It is a significant occupational health problem because ICD will dissuade from complying with hand-hygiene protocols, and consequently increase the possibility of cross-infection as well as HCW self-injuries with possible serious infections (Gould 2003, Larson et al. 2006, WHO 2009). ICD is the clinical response of the skin due to contact with chemical substances that induce a non-immunological cutaneous inflammatory reaction. Even if allergic contact dermatitis does exist as well, ICD is the most common form of occupational skin disease which accounts for nearly 80% of contact dermatitis (Al-Otaibi & Alqahtani 2015). It is caused and modulated by both exogenous and endogenous factors resulting in epidermal barrier damage, damage to keratinocytes and release of pro-inflammatory factors (Mathias & Maibach 1978, Berardesca & Distante 1994). ICD types identified to date are numerous, including acute, chronic and cumulative irritant dermatitis, delayed acute irritant dermatitis, irritant reaction, pustular irritant dermatitis, suberythematous irritation, sensory irritation, friction dermatitis and airborne dermatitis (Chew & Maibach 2003). It can sometimes be a photo-irritation phenomenon, *i.e.* an acute-light induced irritation which occurs on skin exposed to sufficient concentrations of the so-called photo-irritant and doses of activating radiation (Gonzalez & Gonzalez 1996, Deleo 2004). Photo-irritation can occur after the first exposure and clinical signs are the same as the ones of irritation, erythema, edema and desquamation (Gould et al. 1995). It is first observed as an inflammatory reaction, and typically only light-exposed areas are affected (Epstein 1983). Whilst ICD can be prevented by recommending users to avoid skin contact with the products by wearing personal protective equipment (PPE), and exposure to light by wearing clothes or PPE or changing the time of use in the specific case of photo-irritation, this cannot be applied to hand hygiene products.

For all the above mentioned reasons, it is of importance to offer hand hygiene products without any identified irritating potential. For ABHR, the presence of co-formulates, among which humectants and hydrating agents, allow significantly countering the adverse side effects of alcohols. Several studies have been conducted in clinical settings using different commercially available products containing humectants to assess their acceptability. They all converged to the same conclusion that such products are better tolerated by HCW and are associated with a better skin condition (less skin irritation and dryness) when compared with either plain or antimicrobial soap (Boyce & Pittet 2002, Larson et al. 2006).

The prediction of any chemical-induced ICD is a matter of special concern. Whilst it can be partly achieved by a strict selection of ingredients with regard to their toxicological profile, it can also be assessed through experimental test. Since 2003, alternative methods have been developed, especially for cosmetic safety assessment, with the publication of the 7th amendment (EU 2003) of cosmetic directive (now replaced by regulation, EU 2009), introducing a progressive ban of animal testing for cosmetics. Out of validated alternative methods, skin irritation using RhE models is one of them. The EpiSkin™ test method was recognized in 2007 as a stand-alone method scientifically validated to fully replace the Draize skin irritation test according to EU classification (ECVAM Scientific Advisory Committee (ESAC) Statement 2007, Spielmann et al. 2007). This method will only assess acute irritation. ICD types that can also occur with alcohols, notably sensory irritation (subjective symptoms including stinging, burning, tightness, itching or even painful sensations, but without morphological changes) and cumulative irritancy (Phillips et al. 1972) would require separate experiments.

Regarding the phototoxic potential, it can be properly studied *in vitro*, using the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU-PT), OECD guideline No. 432 (OECD. 2004a, EU. B.41. 2008). This is a simple and predictive model for acute phototoxicity, as confirmed by comparative studies of the results of *in vitro* 3T3 NRU-PT *versus in vivo* acute phototoxicity tests in animals and humans (Spielmann et al. 1994, Spielmann et al. 1998, Anon 1998, OECD. 2004a). Despite human skin keratinocytes are the first target cells exposed to sunlight, mouse fibroblasts have been preferred in this *in vitro* test for sensitivity reasons, as shown in the ECVAM/COLIPA pre-validation study screening different photosensitizers, where primary human keratinocytes cultures were less sensitive in relation with their higher potential to absorb UV radiation (Spielmann et al. 1995).

The aim of our research was to make a comparative assessment of local tolerance of alcohols commonly used in hand hygiene alcohol-based products using *in vitro* validated alternative methods. Formulated products, hereafter called hydro-alcoholic solutions (HAS), were also part of the tests battery, in order to take into account the presence of co-formulates in the commercially available products. For the above mentioned reasons, skin irritation and phototoxicity were investigated. Comparative assessment was between the 3 commonly used alcohols in ABHR: Ethanol, Propan-2-ol and Propan-1-ol, and for each alcohol, between 4 different concentrations ranged from 60% w/w to 85% w/w, this range covering the alcohol content encountered in commercialized ABHR.

2. Material and methods

2.1. Chemicals and reagents

2.1.1. Test items

Ethanol (purity 94.377% w/w), Propan-2-ol (purity 99.974% w/w) and Propan-1-ol (purity 99.961% w/w) were purchased from BRABANT Global Solvant (Tressin – France). Propan-1-ol (purity ≥ 99.8%) was also purchased from Merck (Darmstadt - Germany). Co-Formulates arbitrary named Raw-Materials 1, 2, 3 & 4 (RM1, RM2, RM3 and RM4 - hydrating, emollient and skin protective agents) were provided by Laboratoires ANIOS (Lille-Hellemmes, France). For confidentiality reason, RM names and suppliers' identity could not be provided.

Solutions of alcohol (Ethanol, Propan-2-ol and Propan-1-ol) at 60, 70, 80 and 85% w/w in water or in the presence of 2 or 2.5% w/w of co-formulates (mixture of RM1, RM2, RM3 and RM4) and mixture of co-formulates at 2.5 (CoF 2.5) or 10% w/w (CoF_{10}) in Dimethyl sulfoxide (DMSO, purity > 99.9% - Sigma-Aldrich - Saint Quentin Fallavier, France) were provided ready to use by Laboratoires ANIOS. Tested HAS were voluntary different from real HAS marketed products, with co-formulates' concentration determined in preliminary experiments at 2.5% for irritation tests, and 2% for phototoxicity tests, in order to not introduce toxicity level that would have impaired the test conduct or its interpretation.

2.1.2. Other chemicals and reagents

For *in vitro* skin irritation, Dulbecco's phosphate-buffered saline (PBS) without Calcium and Magnesium (GIBCO, Paisley, UK) was used as negative control and rinsing solution. Sodium dodecyl sulfate 5% (SDS, purity ≥ 99% - Biorad, Japan or purity ≥ 98.5% Sigma, USA) was used as positive control. MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide, ≥ 98% - Sigma, Saint Louis, US), Propan-2-ol (purity ≥ 99.5% – Sigma, Steinheim, Germany) and Hydrochloric acid 32% (Merck, Darmstadt, Germany) were used for the determination of the tissues viability.

For *in vitro* phototoxicity test, DMSO (purity ≥ 99.7% - Merck, US) was used for test items dilution and Calcium and Magnesium free HBSS (Hanks Balanced Salt Solution - GIBCO, Paisley, UK) was used for treatment administration in cells cultures (1/200) and cells washings. CPZ (Chlorpromazine hydrochloride, purity ≥ 98%, Sigma, China) was used as positive control. NR (Neutral red: 3-amino-7-dimethylamino-2-methylphenazine hydrochloride, purity ≥ 90% - Sigma Aldrich, Switzerland), absolute ethanol (purity ≥ 99.8% - Merck, Darmstadt, Germany) and acetic acid (purity ≥ 99% - Chem-Lab, Zedelgem,