Molecular iodine is not responsible for cytotoxicity in iodophors

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SUMMARY

Background: Ten percent povidone-iodine (PVP-I) was initially promoted as 'tamed iodine' as the chemical activity of the active biocide, uncomplexed or free molecular iodine (I_2), is reduced 30- to 50-fold compared with Lugol's solution. The idea that I_2 is responsible for topical iodine staining and irritation remains widely held. However, there are no controlled studies that characterize the cytotoxicity and staining of the hydrophobic I_2 species compared with the other hydrophilic iodine species that comprise over 99.9% of the total iodine in topical iodine disinfectants.
Aims: To compare the staining properties of the I_2 species with other topical iodine disinfectants; to evaluate if the concentrations of I_2 in diluted PVP-I used to reduce severe acute respiratory syndrome coronavirus-2 in the nasal cavity are potentially cytotoxic; and to determine if high concentrations of I_2 can be delivered beyond the stratum corneum into the hypodermis, which could provide a mechanistic rationale for I_2 out-gassing.
Methods: Five liquid compositions that contained complexed and uncomplexed (free) I_2 in aqueous and non-aqueous carriers were used to evaluate the interaction of I_2 with mammalian cells in culture as well as human and pig skin.
Findings: Concentrations of I_2 (7800 ppm) that are 1500 times higher than that found in PVP-I can be applied to skin without irritation and staining. I_2 is not cytotoxic at concentrations >100 times higher than that found in PVP-I, and does not contribute materially to staining of skin at concentrations found in Lugol's solution (approximately 170 ppm). I_2 can partition into hypodermis tissue, remain there for hours and out-gas from skin. PVP-I and Lugol's solution are highly effective topical disinfectants, but do not facilitate diffusion of I_2 through the stratum corneum.
Conclusion: The maximum concentration of I_2 found in diluted PVP, approximately 25 ppm, is not cytotoxic or irritating. The potential clinical utility of I_2 has been limited by incorporating this broad-spectrum biocide into acidic aqueous formulations that contain numerous chemical species that contribute toxicity but not biocidal activity. I_2 can be delivered topically into hypodermis tissue without irritation.

Introduction

The global pandemic has induced clinicians to identify agents that can be used in the oral and nasal cavities [1—6].

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Use of an iodine disinfectant is logical as: (i) iodine has established antiviral and antimicrobial activity; (ii) there is no evidence that bacteria can develop resistance to I_2 [7,8] as it reacts with several functional groups, thus providing simultaneous action against multiple molecular targets; (iii) iodine is a naturally occurring biomolecule synthesized in the follicular lumen and other iodine-concentrating tissues; and (d) iodine has a 200-year history of use in and on humans. The nose is the primary nidus of infection for severe acute respiratory syndrome coronavirus-2, and while masks mitigate aerosol spread, they do nothing to reduce viral load in the nasal cavity [9,10]. The 3M Skin and Nasal Antiseptic is 5000 ppm available iodine iodophor that is painted on to the nasal cavity prior to surgery [11], and many clinicians have advocated for use of a diluted iodophor solution with 0.25–1.5% thiosulphate titratable as a prophylactic nasal lavage [12–16] for healthcare personnel. The US Food and Drug Administration has reviewed at least 27 clinical trials that use diluted povidone-iodine (PVP-I) [17] to control coronavirus disease 2019. Oral and nasal application of diluted PVP-I nasal spray and irrigation [18,19] has been adopted, and products with lower total iodine concentrations are also offered for this purpose (ioShield Nasal Barrier Cream, Iotech International, Boca Raton, FL, USA; Halodine Nasal Antiseptic, Miami, FL, USA).

The thiosulphate titratable iodine in PVP-I can vary from the amount stated on the label by 35% as the US Pharmacopeia (USP) definition requires not less than 85% and not more than 120% of the labelled amount of iodine [20]. The term 'iodine' in this sentence means thiosulphate titratable iodine. However, triiodide, hypoiodous acid and I_2 are all reduced by thiosulphate, and the concentration of active biocide in PVP-I is 0.02–0.06% of the label claim. In addition, there is up to 6.6% iodide ion in PVP-I. Consequently, clinicians do not know the concentration of active biocide in the iodophor they use clinically. Nuckolls has highlighted the risks and limitations inherent in the simple dilution of PVP-I to generate a 'low concentration' iodine disinfectant which can have a 10-fold higher concentration of active biocide (i.e. I_2) [21].

Gottardi made the surprising discovery that topically applied I_2 can diffuse into skin and provide prolonged (>12 h duration) epidermal antibacterial activity due to a continuous flux or 'back diffusion' of absorbed I_2 from skin [22]. Dilution of an iodophor such as PVP-I results in an increase of I_2 concentration [23–25] that also increases absorption of I_2 into skin, as the I_2 flux is proportional to exposure time and concentration. The interaction of PVP-I with skin and wounds has been studied extensively, but these studies do not provide insight into the interaction of the I_2 species with skin. Only a limited number of studies have examined the topical properties of pure I_2. Duan *et al.* published acute dermal toxicity results for a low concentration (35 ppm) I_2 composition [26], and Uchiyama *et al.* examined the efficacy of a 400 ppm I_2 composition to reduce meticillin-resistant *Staphylococcus aureus* in the nasal carriage of mice [27]. The present study compares the interaction of I_2 with skin using (i) I_2-glycerine compositions and (ii) several commercially available aqueous iodine compositions.

Methods

Staining of human skin by iodine compositions

The forearm of a volunteer was cleaned with a Dacron wipe (75% alcohol) prior to application of the iodine compositions. Four glass vials (VWR Cat#66022-300) were held simultaneously against a human forearm for 3 min. Each vial contained 2 mL of the following compositions: (i) 10% PVP-I, (ii) 300 ppm I_2 in water, (iii) 7800 ppm I_2-glycerine, (iv) Lugol's solution (3.3% I_2–6.6% NaI), and (e) USP tincture of iodine (47% ethanol; 2% I_2–2.4% NaI). After removal of the vials, the treated area of skin was cleaned twice with an alcohol Dacron wipe to remove residual material, and a stopwatch was started.

Staining of pig skin with 66,000 ppm I_2-glycerine

Thirty microlitres of a 66,000 ppm I_2-glycerine solution was applied to the epidermis of a 1.77-cm^2 piece of pig skin and spread over the surface for 20 s using a sterile disposable plastic loop. The material was retained on the skin surface for 3 min. The stratum corneum was removed, and a SenSafe Iodine Check test strip (Part No. 480018, Industrial Test Systems, Rock Hill, SC, USA) was contacted with the area of hypodermis tissue that had a purplish hue for 1 min to test for the presence of I_2.

Staining of hypodermis pig skin tissue with I_2 compositions

The epidermis and dermal layers of pig skin were separated and discarded. Cubes of hypodermis tissue 5 mm in width and length were sectioned, hydrated for 30 min in water, and patted dry. Five cubes of hypodermis tissue were submerged into 1 mL of each of the following three compositions: PVP-I, Lugol's solution, and glycerine with 15,200 ppm I_2. Each piece of hypodermis tissue was removed after 15 min and rinsed thoroughly with water until a colour change was not detected. The tissue was contacted with a SenSafe Iodine Check test strip for 5 min to check for I_2.

I_2 out-gassing from hypodermis tissue

Ten cubes of hypodermis tissue 5 mm in width and length were hydrated for 30 min, patted dry, submerged in 2 mL of 15,200 ppm I_2-glycerine for 15 min, and then rinsed thoroughly seven times with water. At 15, 30, 45, 60, 75 and 90 min, and every 30 min thereafter until 210 min, a cube was removed and contacted with a SenSafe Iodine Check test strip for 5 min to test for the presence of I_2.

Out-gassing of I_2 from pig skin

Circular pieces (1.77 cm^2) of pig skin were cut, hydrated for 30 min at room temperature (20 °C), and patted dry with a tissue. The pig skin was treated with 10 μL of a 66,000 ppm I_2-glycerine composition, spread over the epidermis for 20 s using a disposable plastic cell spreader. After 10 min, residual material was removed from the epidermis by wiping the top
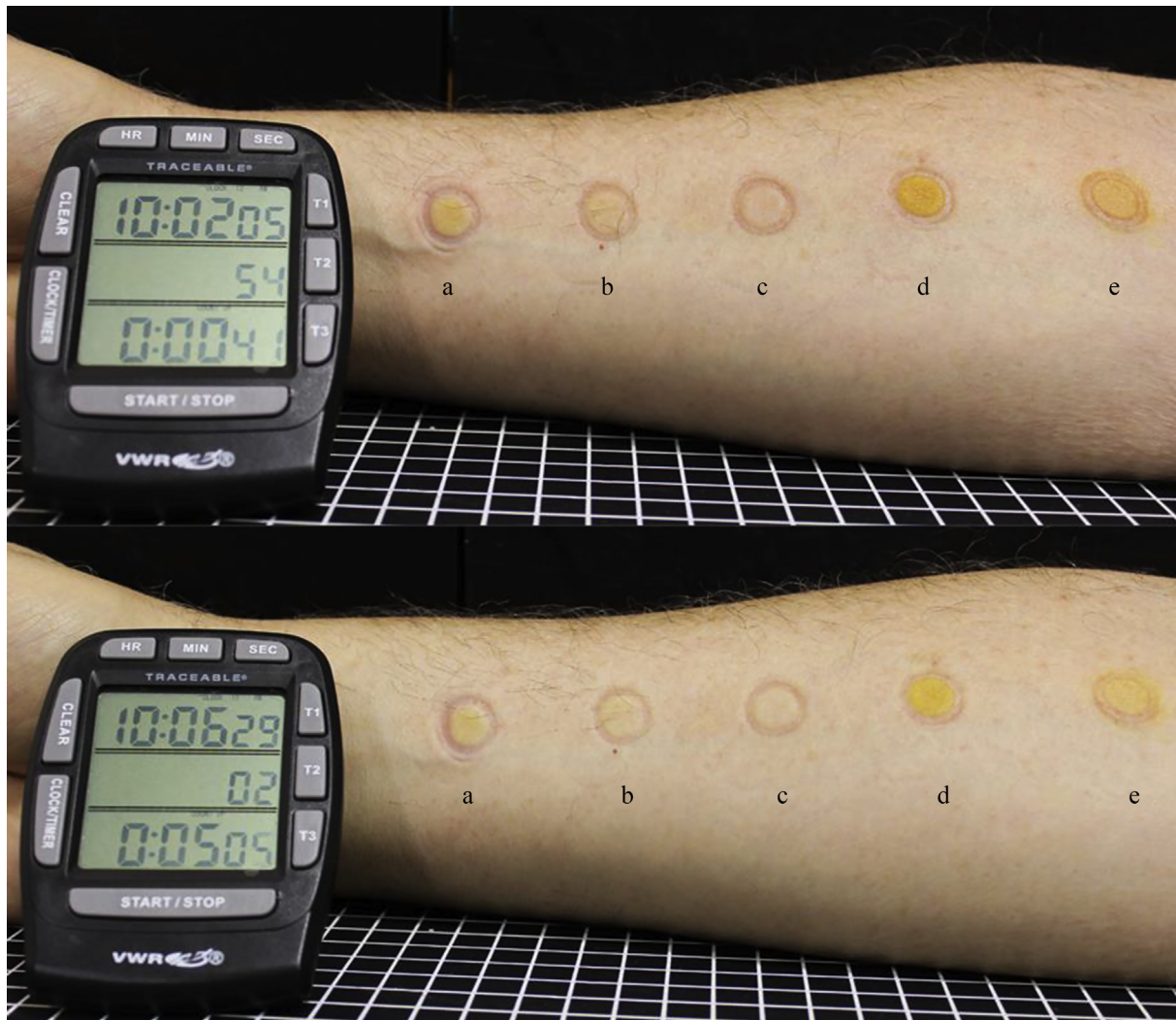
Figure 1. One millilitre of (a) 300 ppm I^2 in water; (b) 10% povidone-iodine; (c) 7800 ppm I^2-glycerine, (d) Lugol's solution and (e) iodine tincture were simultaneously contacted to the forearm of a volunteer for 3 min and residual was removed with a Dacron alcohol wipe. Images were taken immediately after application (top) and after 5 min (bottom).

surface of the pig skin with an alcohol Dacron wipe. Controls were treated with 10 μL of glycerine. Pig skin tissues were loaded individually into a vertical diffusion cell (VDC; Copley Transdermal Tester HDT 1000; Cat. No. 7290, Serial #50290) in an open configuration. Tissues were kept at a constant 34 °C in the VDC to mimic the temperature of human skin. Tissues were placed between two support washers, and the donor chamber was screwed into place. The occlusion disc was used intermittently by resting on top of the donor chamber to avoid loss of I_2 when not in use. I_2 diffusion from skin was measured using 1.0 mM N,N-dimethyl-p-phenylenediamine dihydrochloride (DPD) prepared daily. DPD reagent (1 mL) was loaded on to the pig skin in the donor chamber of the VDC to directly capture I_2 out-gassing from the epidermis. The DPD reagent was removed after a defined contact time that ranged from 30 s to 15 min, transferred to a cuvette, and absorbance was determined at 550 nm (Persee Analytics, Inc., Auburn, CA, USA; Model T6V). Pig skin was rinsed with two 1-mL DI-water rinses between DPD sampling to avoid cross-contamination. The I_2 flux (in μg/cm²-min) was determined by comparing the absorbance of unknowns with a standard curve prepared daily. The between-day mean (*N*=25) and standard deviation of the slope and intercept for the standard curve were 0.05217 A/μg $\pm$ 0.00236 and 0.01163 A $\pm$ 0.00959, respectively.

Cytotoxicity of 24-h exposure to 1500 ppm I_2

Three samples of 1500 ppm I_2-glycerine were evaluated for cytotoxicity using a direct contact test according to ISO 10993-5:2009 [28]. Sterile filter paper with a flat surface with a total surface area of 1.0 cm² was saturated with 0.1 mL of the test article, and placed directly on a cell culture monolayer of mouse fibroblasts (ATCC CRL-2648) in the centre of a 10-cm² well. Triplicate-positive (Encore Latex glove; Ansell Healthcare Products, LLC, Iselin, NJ, USA) and -negative (high-density polyethylene; USP, Lot K0M357) controls were tested in the same manner as the test articles. All wells were incubated for not less than 24 h at 37 $\pm$ 1 °C in a humidified incubator with 5 $\pm$ 1% CO_2. After incubation, the test articles and controls were removed from the wells, and the cell cultures were examined under an inverted microscope with 100X magnification for cytotoxic response.

Figure 2. (a) Iodine staining of pig skin epidermis after application of 30 μL of 66,000 ppm I^2-glycerine to a 1.77-cm^2 circular piece of pig skin. (b) I^2 detected (aqua colour) in purplish hypodermis tissue using a SenSafe Iodine Check test after removal of the epidermal and dermal skin layers.

Out-gassing of I_2 from human skin

The forearm of a volunteer was cleaned with a Dacron wipe (75% alcohol) prior to application of Lugol's solution or PVP-I. A borosilicate glass vial with an inner diameter of 1.1 cm was contacted with an area on the forearm for 3 min, and the treated surface was cleaned with an alcohol Dacron wipe. An identical vial that contained 1 mL of DPD reagent was placed on the treated area for 3 min to capture I_2 diffusing from the skin. I_2 concentrations were determined as described for out-gassing of I_2 from pig skin.

Results

A glycerine composition with 7800 ppm of I_2 did not stain the skin on the forearm of a 70-year-old white male (Figure 1), in contrast to four other topical iodine formulations that contained much lower I_2 concentrations. PVP-I, which contains 2–8 ppm I_2, discoloured skin slightly more than a freshly prepared aqueous 300 ppm I_2 solution. Lugol's solution, which contains 170 ppm free I_2, stained skin more deeply than any of the other materials, although it contains less ($\approx 56\%$) I_2 than the aqueous I_2 solution and 98% less I_2 than the glycerine composition. Lugol's solution
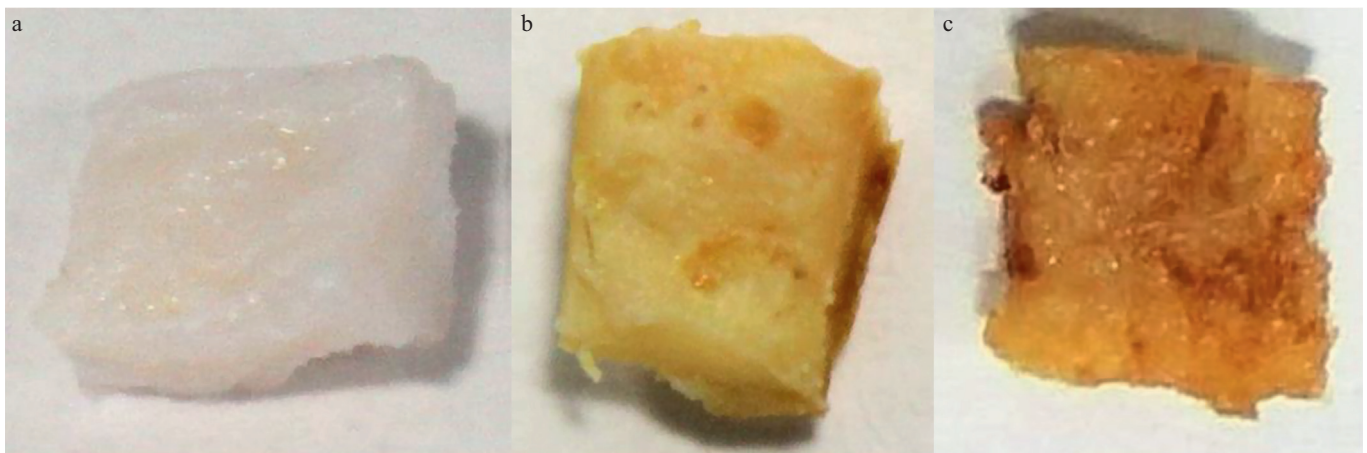
Figure 3. Cubes of hypodermis tissue (5 x 5 mm) were sectioned, hydrated for 30 min and then patted dry. Cubes of hypodermis tissue were submerged in 1 ml of the following three compositions: (a) 10% povidone-iodine (b) Lugol's solution, and (c) glycerine with 15,200 ppm I^2. Each piece of hypodermis tissue was removed after 15 min and rinsed thoroughly with water until a colour change was not detected. The degree of staining was proportional to I^2 exposure.

Table I
Iodine (I_2) flux from hypodermis tissue treated with 15,200 ppm I_2 vs time

Time post exposure (min)	I_2 ppm score[a]	Time to final colour (s)[b]
15	5	25
30	5	20
45	5	18
60	2	120
75	1	180
90	0.5	240
120—210[c]	0	300

[a] SenSafe Iodine Check test strip incorporates a qualitative scale from 0 to 5 ppm I_2.
[b] All observations monitored for 300 s.
[c] The 120-, 150-, 180- and 210-min time points yielded identical results.

and iodine tincture imparted the deepest stains; this implicates triiodide as the staining agent, as both of these products contain high concentrations of uncomplexed triiodide. Staining with I_2-glycerine required 15,000 ppm I_2 (data not shown), and the discolouration from this higher level of I_2 dissipated substantially 10 min post application.

Sodium thiosulphate has been used to remediate skin staining by topical iodine, which suggests that either triiodide, I_2 or both iodine species stain skin. I_2 presents as a yellowish-brown solution in water, in contrast to the violet colour observed in aprotic (non-polar) solvents or in the vapour phase. This study confirmed that pure I_2 can stain skin by applying 30 μL of a 66,000 ppm I_2-glycerine composition on pig skin. A deep reddish-brown stain (Figure 2a) formed on the stratum corneum, and mottled areas were observed in hypodermis tissue that exhibited a purplish colour (Figure 2b). The purplish sections of hypodermis tissue are consistent with the presence of I_2 in a non-polar environment. Contacting a SenSafe Iodine Check test strip with the purplish areas of hypodermis indicated the presence of I_2. The ability of I_2 to partition in hypodermis tissue was further tested by submerging cubes of hypodermis tissue from pig skin in PVP-I, Lugol's solution and 15,200 ppm I_2-glycerine for 15 min. PVP-I did not stain hypodermis tissue, and a 5-min contact with a SenSafe Iodine Check test strip did not detect I_2 (Figure 3a). In contrast, staining was observed in hypodermis tissue treated with Lugol's solution and 15,200 ppm I_2-glycerine (Figure 3b,c); a much darker stain was observed with the higher concentration of I_2 in the glycerine composition. Contacting thoroughly washed hypodermis tissue previously exposed to Lugol's solution or 15,200 ppm I_2-glycerine with a SenSafe Iodine Check test strip for 5 min yielded a concentration of 1 and 5 ppm I_2, respectively; this is consistent with the idea that I_2 diffusion into hypodermis tissue is concentration dependent. After 15 min of exposure to 15,200 ppm I_2-glycerine, I_2 out-gassing was detected from cubes of hypodermis tissue (Table I) for up to 90 min.

I_2 out-gassing from pig skin treated with 66,000 ppm I_2-glycerine and maintained at 34 °C in a vertical diffusion cell was detected for up to 2 h (Figure 4). The I_2 flux decreased exponentially with time, as reported previously by Gottardi [2]. The I_2 flux from human skin measured after a 3-min 10% PVP-I application was 0.49 ± 0.047 μg/cm^2-min (N=5) at 0.5 min post application. Identical measurements made on human skin

with Lugol's solution at 0.5, 30 and 80 min post application were 5.39 ± 1.48, 1.32 ± 0.44 and 0.41 ± 0.24 μg/cm^2-min, respectively. These results are consistent with data reported previously by Gottardi [22], who demonstrated residual antibacterial activity against bacteria in water.

A 1500 ppm I_2-glycerine composition did not exhibit cytotoxicity in a 24-h direct contact test with mouse fibroblasts, which contrasts with cell-based cytotoxicity reports using commercially available topical iodine products [29].

Discussion

In a series of studies funded by the National Aeronautics and Space Administration, Thrall *et al.* demonstrated dramatic differences in the pharmacological and toxicological properties of orally administered iodide vs I_2 in the rat [30—32]; the results support the principal that the functional properties of an iodine composition depend upon the relative concentration of the different iodine species contained therein. Aceves and others have demonstrated dramatic differences in the behaviour of I_2 compared with iodide in different cell lines and tissues [30,31,33,34]. There are more than seven different iodine species in aqueous topical iodine disinfectants [35], in addition to other ingredients (e.g. surfactants, water, pH modifiers, etc.). All of the chemicals in these iodine products can contribute to their functional behaviour [36,37]. The present study observed the interaction of skin with pure I_2, and noted differences between the behaviour of pure I_2 and complex mixtures of iodine species (e.g. PVP-I).

Thiosulphate has been used successfully to treat iodine burns, which suggests that either triiodide, I_2 or both species are implicated as the causal skin staining agent as thiosulphate reduces both iodine species. The differences in human skin staining exhibited by the iodine compositions in this study are inconsistent with the long-held assumption that topical staining is due to I_2 [38]. The complete lack of staining from a 7800-ppm composition of I_2-glycerine contrasts dramatically with the deep persistent stain imparted by both Lugol's solution and iodine tincture. This lack of staining at 7800 ppm I_2 combined with an absence of cytotoxicity with 1500 ppm I_2 in a 24-h direct contact test with fibroblasts suggests that high concentrations of I_2 can be used safely on skin if properly formulated. Skin staining from Lugol's solution and iodine tincture may be due to the binding of triiodide to glycogen in the epidermis [39].

PVP-I is visible on skin but it does not stain skin, as diffusion of triiodide or I_2 into the epidermis is limited due to binding of I_3 by PVP. I_2 stains skin at higher concentrations but the resulting colour differs from that observed with triiodide staining; a difference in colour is also observed in in-vitro experiments that characterize glycogen-iodine complexes [40]. Systemic iodine exposure from topical iodine disinfectants such as PVP-I is well known [41—44]. The total iodine applied topically in a glycerine-I_2 composition would be lower than with a PVP-I composition. For instance, a 1000 ppm I_2-glycerine solution would expose 100 μg of I_2 to a human hand based on 0.1 mL application on to a 400-cm^2 hand, which compares with approximately 10,000 μg of total iodine from PVP-I for an equivalent volume. However, a much higher percentage of the iodine in I_2-glycerine may be absorbed compared with PVP-I. Safran and Braverman evaluated thyroid
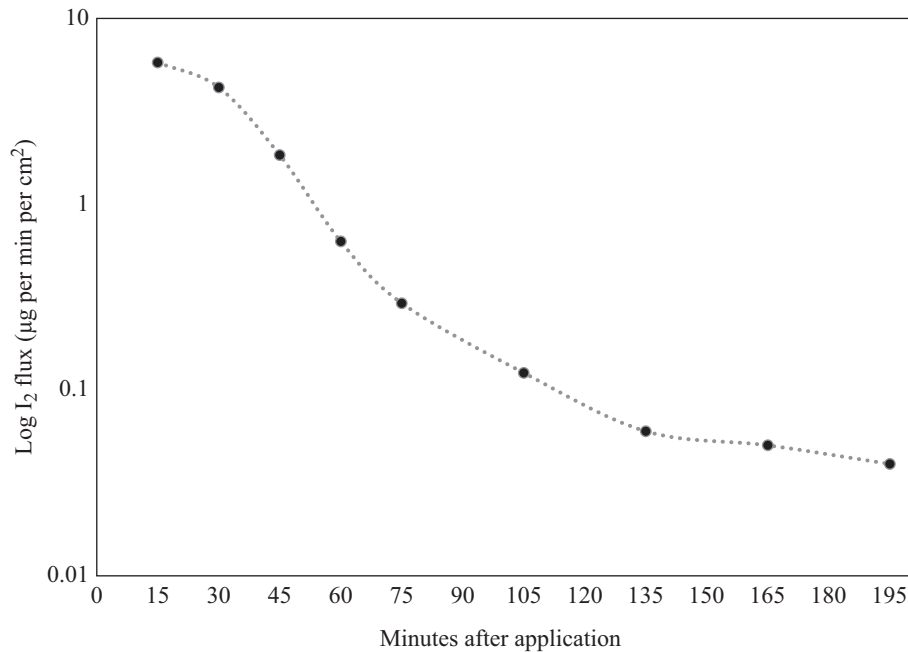
Figure 4. I_2 flux (μg/min-cm^2) at 34°C from a hydrated 1.77-cm^2 piece of pig skin treated with 10 μL of a 66,000 ppm I^2-glycerine composition.

status during daily vaginal douching with PVP-I (0.3% thio-sulphate titratable iodine) in 12 euthyroid volunteers for 14 days, and concluded that 5% of the applied iodine was absorbed [45]. A topical administration of 0.2 mL of an I_2-glycerine composition containing 1000 ppm I_2 used for hand disinfection would administer 200 μg of I_2 on to skin. If one assumes 20% absorption as opposed to the 5% measured by Safran and Braverman, 40 μg would cross the stratum corneum. A maximum of 50% of this I_2 could be available to partition into the thyroid [30–32], which equates to a total thyroid exposure of 20 μg of iodide which is approximately 15% of the recommended daily allowance. Consequently, it would be important to measure the potential for iodine exposure from repeat applications of a high I_2-glycerine composition.

Gottardi first observed out-gassing of I_2 from skin after application of Lugol's solution [2]. In the present study, measurements of I_2 flux from human skin treated with Lugol's solution are consistent with Gottardi's data. The purplish areas of coloured hypodermis in pig skin treated with 66,000 ppm I_2-glycerine suggest the presence of I_2, which is consistent with test results from the DPD test strip. The apparent stability of I_2 absorbed into skin is ostensibly a surprising outcome as I_2 is highly reactive with biological tissue. However, partitioning of I_2 into regions of unsaturated lipid in the hypodermis could provide stability to I_2, which partitions readily into oil and fat. It was observed that I_2 partitioned into hypodermis tissue in proportion to the dose, as tissue treated with 15,200 ppm I_2 contained more I_2 than tissue treated with Lugol's solution (170 ppm). The duration of out-gassing from pig skin treated with 66,000 ppm I_2 was detected for approximately 3.3 h, compared with 4 h for hypodermis tissue treated directly with 15,200 ppm I_2.

Gottardi mentioned that absorption and subsequent post-application diffusion of a topically applied antimicrobial over time is unique in the field of skin disinfection. Some researchers have reported a persistent antimicrobial activity on skin after use of iodine-based handwashes [46–49]. Converting hypodermis tissue into a material that releases I_2 over time confers potential benefits for topical disinfection, and also offers the potential to: (i) treat infectious diseases, (ii) act as an anti-inflammatory as I_2 can interrupt ligand signalling via iodination, and (iii) stimulate wound healing via the sustained delivery of free iodine at concentrations that retain antimicrobial activity without cytotoxicity [50].

It is known that the concentration of I_2 increases as PVP-I is diluted up to approximately 1/100 [23]. Diluted PVP-I has been shown to be less cytotoxic than neat PVP-I; therefore, the literature provides indirect evidence that I_2 is not the cytotoxic species in PVP-I. Additionally, cadexomer iodine, which contains a high concentration of I_2 compared with 10% PVP-I, does not affect fibroblast viability and collage synthesis. In fact, cadexomer iodine stimulated secretion of proinflammatory cytokines (tumour necrosis factor-alpha) in human macrophages [23].

The characterization of PVP-I on product labels is not sufficient to ensure consistent results in cell-based cytotoxicity studies due to variations in pH (can vary between 1.5 and 6.5), iodide concentration, ionic strength, buffering capacity and I_2 concentration [24,25]. Additionally, there is a 15% variance allowed in the level of titratable iodine under the definition in the USP. There is a legitimate concern that formulation variations among different PVP-I compositions (manufacturer-to-manufacturer and lot-to-lot) have influenced cytotoxicity study outcomes unbeknownst to researchers conducting these studies [29,51,52]. Equally problematic are studies that dilute a topical iodine composition and draw incorrect conclusions based on the assumption that I_2 is stable for days after dilution [53]. Some manuscripts have thoughtfully explored the composition of PVP-I [54], but an insufficient understanding of the fundamental chemical properties of PVP-I almost certainly contributes to unfortunate clinical outcomes [55–57].

If one views I_2 as just another antimicrobial chemical, the lack of cytotoxicity observed for the 1500-ppm I_2-glycerine material may appear paradoxical given the elevated antimicrobial activity associated with such a high level of I_2. However, molecular iodine is a naturally occurring biochemical that can function as an antioxidant [58–60], an anti-inflammatory agent [61,62], an antiproliferative agent [35,63–68] and a differentiation agent [69–73]. There are more than 12 different tissues in humans with the ability to concentrate iodine. Based on a recommended daily allowance of 125 μg/day for iodide, the follicular lumen oxidizes 6.25 μg iodide/h into I_2 in a volume of approximately 7.5 mL, which equates to generating 900 ppm I_2/h which is approximately 2 orders of magnitude higher than the I_2 concentration found in PVP-I. Molecular iodine is the only widely used disinfectant molecule that plays an essential role in mammalian biochemistry [74] and, if formulated properly, can be used clinically at highly elevated concentrations.

In conclusion, the highest concentration of I_2 achievable from diluted PVP-I is approximately 25 ppm, which suggests that there is at least a 60-fold margin of safety for I_2 with respect to topical toxicity on human mucous membranes of the nasal cavity, as a 1500-ppm I_2 composition was not found to be cytotoxic. The staining and irritation associated with topical iodine disinfectants is not due to the I_2 species. There are potential clinical benefits that may accrue from the use of high concentrations of topically applied I_2.

Acknowledgements

References

[1] Stathis C, Victoria N, Loomis K, Nguyen SA, Eggers M, Septimus E, et al. Review of the use of nasal and oral antiseptics during a global pandemic. Future Microbiol 2021;16:119–30.

[2] Sarma P, Bhattacharyya A, Prakash A, Kaur H, Prajapat M, Borah M, et al. Yogic Neti-Kriya using povidone iodine: can it have a preventive role against SARS-CoV-2 infection gateway? Indian J Otolaryngol Head Neck Surg 2021. https://doi.org/10.1007/s12070-021-02885-2.

[3] Pelletier JS, Tessema B, Frank S, Westover JB, Brown SM, Capriotti JA. Efficacy of povidone-iodine nasal and oral antiseptic preparations against severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Ear Nose Throat J 2021;100(Suppl.):192S–6S.

[4] O'Brien TP, Pelletier J. Topical ocular povidone-iodine as an adjunctive preventative practice in the era of COVID-19. Asia Pac J Ophthalmol 2021;10:142–5.

[5] Kwok CS, Dashti M, Tafuro J, Nasiri M, Muntean EA, Wong N, et al. Methods to disinfect and decontaminate SARS-CoV-2: a systematic review of in-vitro studies. Ther Adv Infect Dis 2021;8:2049936121998548.

[6] Guenezan J, Garcia M, Strasters D, Jousselin C, Lévêque N, Frasca D, et al. Povidone iodine mouthwash, gargle, and nasal spray to reduce nasopharyngeal viral load in patients with COVID-19: a randomized clinical trial. JAMA Otolaryngol Head Neck Surg 2021;147:400–1.

[7] Lanker Klossner B, Widmer HR, Frey F. Nondevelopment of resistance by bacteria during hospital use of povidone-iodine. Dermatology 1997;195(Suppl. 2):10–3.

[8] Houang ET, Gilmore OJ, Reid C, Shaw EJ. Absence of bacterial resistance to povidone iodine. J Clin Pathol 1976;29:752–5.

[9] Bleier BS, Ramanathan Jr M, Lane AP. COVID-19 vaccines may not prevent nasal SARS-CoV-2 infection and asymptomatic transmission. Otolaryngol Head Neck Surg 2021;164:305–7.

[10] Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon 3rd KH, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. Cell 2020;182:429–46.

[11] The evidence is mounting for 3M™ skin and nasal antiseptic [povidone-iodine solution 5% w/w (0·5% available iodine) USP] preoperative skin preparation: five new studies prove clinical efficacy in reducing the risk of surgical site infections. Int Wound J 2014;11:574–5.

[12] Mady LJ, Kubik MW, Baddour K, Snyderman CH, Rowan NR. Consideration of povidone-iodine as a public health intervention for COVID-19: utilization as 'personal protective equipment' for frontline providers exposed in high-risk head and neck and skull base oncology care. Oral Oncol 2020;105:104724.

[13] Pelletier JS, Tessema B, Frank S, Westover JB, Brown SM, Capriotti JA. Efficacy of povidone-iodine nasal and oral antiseptic preparations against severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Ear Nose Throat J 2021;100(Suppl.):192s–6s.

[14] Chorney SR, Rizzi MD, Dedhia K. Considerations for povidone-iodine antisepsis in pediatric nasal and pharyngeal surgery during the COVID-19 pandemic. Am J Otolaryngol 2020;41:102737.

[15] Frank S, Capriotti J, Brown SM, Tessema B. Povidone-iodine use in sinonasal and oral cavities: a review of safety in the COVID-19 era. Ear Nose Throat J 2020;99:586–93.

[16] Arefin MK. Povidone iodine (PVP-I) oro-nasal spray: an effective shield for COVID-19 protection for health care worker (HCW), for all. Indian J Otolaryngol Head Neck Surg 2021. https://doi.org/10.1007/s12070-021-02525-9.

[17] ClinTrials.gov. National Library of Medicine. Available at: https://clinicaltrials.gov/ct2/results?recrs=&cond=&term=Iodine+and+Covid&cntry=&state=&city=&dist=.

[18] Zarabanda D, Vukkadala N, Phillips KM, Qian ZJ, Mfuh KO, Hatter MJ, et al. The effect of povidone-iodine nasal spray on nasopharyngeal SARS-CoV-2 viral load: a randomized control trial. Laryngoscope 2021. https://doi.org/10.1002/lary.29935.

[19] Wu PH, Cheng PC, Chang CM, Lo WC, Cheng PW. Efficacy of povidone-iodine nasal irrigation solution after sinonasal surgery: a randomized controlled study. Laryngoscope 2021. https://doi.org/10.1002/lary.29818.

[20] US Pharmacopeia. Povidone-iodine USP29-NF24. p. 1780. Available at: http://www.pharmacopeia.cn/v29240/usp29nf24s0_m68160.html.

[21] Nuckolls C. Chemical considerations related to the dilution of commercial 10% povidone-iodine for use in the COVID-19 pandemic. OSF Preprints 2020. https://doi.org/10.31219/osf.io/8kepw.

[22] Gottardi W. The uptake and release of molecular iodine by the skin: chemical and bactericidal evidence of residual effects caused by povidone-iodine preparations. J Hosp Infect 1995;29:9–18.

[23] Berkelman RL, Holland BW, Anderson RL. Increased bactericidal activity of dilute preparations of povidone-iodine solutions. J Clin Microbiol 1982;15:635–9.

[24] Atemnkeng MA, Plaizier-Vercammen J, Schuermans A. Comparison of free and bound iodine and iodide species as a function of the dilution of three commercial povidone-iodine formulations and their microbicidal activity. Int J Pharm 2006;317:161–6.

[25] Atemnkeng MA, Plaizier-Vercammen JA. Comparison of free iodine as a function of the dilution of two commercial povidone-iodine formulations. J Pharm Belg 2006;61:11—3.

[26] Duan Y, Dinehart K, Hickey J, Panicucci R, Kessler J, Gottardi W. Properties of an enzyme-based low-level iodine disinfectant. J Hosp Infect 1999;43:219—29.

[27] Uchiyama S, Dahesh S, Nizet V, Kessler J. Enhanced topical delivery of non-complexed molecular iodine for methicillin-resistant *Staphylococcus aureus* decolonization. Int J Pharm 2019;554:81—6.

[28] International Organization for Standardization. ISO 10993-5:2009 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. Geneva: ISO; 2009. Available at: www.iso.org/standard/36406.html.

[29] Kaspar D, Schwarz W, Claes L, Ignatius A. [Study of the toxicity of povidone-iodine for fibroblast-like cells (BALB-3T3) and primary human chondrocytes]. Arzneimittel-Forschung 2006;56:605—11.

[30] Sherer TT, Thrall KD, Bull RJ. Comparison of toxicity induced by iodine and iodide in male and female rats. J Toxicol Environ Health 1991;32:89—101.

[31] Thrall KD, Bull RJ. Differences in the distribution of iodine and iodide in the Sprague-Dawley rat. Fundam Appl Toxicol 1990;15:75—81.

[32] Thrall KD, Bull RJ, Sauer RL. Distribution of iodine into blood components of the Sprague-Dawley rat differs with the chemical form administered. J Toxicol Environ Health 1992;37:443—9.

[33] Garcia-Solis P, Alfaro Y, Anguiano B, Delgado G, Guzman RC, Nandi S, et al. Inhibition of N-methyl-N-nitrosourea-induced mammary carcinogenesis by molecular iodine (I_2) but not by iodide (I^-) treatment evidence that I_2 prevents cancer promotion. Mol Cell Endocrinol 2005;236:49—57.

[34] Arroyo-Helguera O, Anguiano B, Delgado G, Aceves C. Uptake and antiproliferative effect of molecular iodine in the MCF-7 breast cancer cell line. Endocr Relat Cancer 2006;13:11.

[35] Gottardi W. Iodine and iodine compounds. In: Block SS, editor. Disinfection, sterilization, and preservation. Philadelphia: Lippincott Williams & Wilkins; 1991. p. 152—66.

[36] Napke E, Stevens DG. Excipients and additives: hidden hazards in drug products and in product substitution. Can Med Assoc J 1984;131:1449—52.

[37] Pottel J, Armstrong D, Zou L, Fekete A, Huang XP, Torosyan H, et al. The activities of drug inactive ingredients on biological targets. Science 2020;369:403—13.

[38] World Health Organization. WHO guidelines on hand hygiene in health care: first global patient safety challenge — clean care is safer care. Geneva: WHO; 2009. Available at: https://www.ncbi.nlm.nih.gov/books/NBK144013/.

[39] Montagna W, Chase HB, Hamilton JB. The distribution of glycogen and lipids in human skin. J Invest Dermatol 1951;17:147—57.

[40] Moulay S. Molecular iodine/polymer complexes. J Polymer Eng 2013;33:389—443.

[41] Chabrolle JP, Rossier A. Danger of iodine skin absorption in the neonate. J Pediatr 1978;93:158—9.

[42] Miller KL, Coen PE, White WJ, Hurst WJ, Achey BE, Lang CM. Effectiveness of skin absorption of tincture of I in blocking radioiodine from the human thyroid gland. Health Phys 1989;56:911—4.

[43] Furudate S, Nishimaki T, Muto T. [125]I uptake competing with iodine absorption by the thyroid gland following povidone-iodine skin application. Exp Anim 1997;46:197—202.

[44] Nesvadbova M, Crosera M, Maina G, Larese Filon F. Povidone iodine skin absorption: an ex-vivo study. Toxicol Lett 2015;235:155—60.

[45] Safran M, Braverman LE. Effect of chronic douching with polyvinylpyrrolidone-iodine on iodine absorption and thyroid function. Obstet Gynecol 1982;60:35—40.

[46] Galle PC, Homesley HD, Rhyne AL. Reassessment of the surgical scrub. Surg Gynecol Obstet 1978;147:215—8.

[47] Ayliffe GA, Babb JR, Davies JG, Lilly HA. Hand disinfection: a comparison of various agents in laboratory and ward studies. J Hosp Infect 1988;11:226—43.

[48] Wade JJ, Casewell MW. The evaluation of residual antimicrobial activity on hands and its clinical relevance. J Hosp Infect 1991;18(Suppl. B):23—8.

[49] Paulson DS. Comparative evaluation of five surgical hand scrub preparations. AORN J 1994;60:246—56.

[50] Cooper RA. Iodine revisited. Int Wound J 2007;4:124—37.

[51] Kim S, Ahn Y, Lee Y, Kim H. Toxicity of povidone-iodine to the ocular surface of rabbits. BMC Ophthalmol 2020;20:359.

[52] Li SH, Wang Y, Gao HB, Zhao K, Hou YC, Sun W. Experimental study on the toxicity of povidone-iodine solution in brain tissues of rabbits. Int J Clin Exp Med 2015;8:14863—70.

[53] Robison LM, Sylvester PW, Birkenfeld P, Lang JP, Bull RJ. Comparison of the effects of iodine and iodide on thyroid function in humans. J Toxicol Environ Health A 1998;55:93—106.

[54] Zamora JL. Chemical and microbiologic characteristics and toxicity of povidone-iodine solutions. Am J Surg 1986;151:400—6.

[55] Glick PL, Guglielmo BJ, Tranbaugh RF, Turley K. Iodine toxicity in a patient treated by continuous povidone-iodine mediastinal irrigation. Ann Thorac Surg 1985;39:478—80.

[56] Kurt TL, Morgan ML, Hnilica V, Bost R, Petty CS. Fatal iatrogenic iodine toxicity in a nine-week old infant. J Toxicol Clin Toxicol 1996;34:231—4.

[57] D'Auria J, Lipson S, Garfield JM. Fatal iodine toxicity following surgical debridement of a hip wound: case report. J Trauma 1990;30:353—5.

[58] Venturi S, Donati FM, Venturi A, Venturi M, Grossi L, Guidi A. Role of iodine in evolution and carcinogenesis of thyroid, breast and stomach. Adv Clin Path 2000;4:11—7.

[59] Kupper FC, Carpenter LJ, McFiggans GB, Palmer CJ, Waite TJ, Boneberg EM, et al. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. Proc Natl Acad Sci USA 2008;105:6954—8.

[60] Berking S, Czech N, Gerharz M, Herrmann K, Hoffmann U, Raifer H, et al. A newly discovered oxidant defence system and its involvement in the development of *Aurelia aurita* (Scyphozoa, Cnidaria): reactive oxygen species and elemental iodine control medusa formation. Int J Dev Biol 2005;49:969—76.

[61] Beukelman CJ, van den Berg AJ, Hoekstra MJ, Uhl R, Reimer K, Mueller S. Anti-inflammatory properties of a liposomal hydrogel with povidone-iodine (Repithel) for wound healing in vitro. Burns 2008;34:845—55.

[62] Moore KTA, Harding KG. Iodine released from the wound dressing Iodosorb modulates the secretion of cytokines by human macrophages responding to bacterial lipopolysaccharide. Int J Biochem Cell Biol 1997;29:163—71.

[63] Dugrillon A, Gartner R. Delta-iodolactones decrease epidermal growth factor-induced proliferation and inositol-1,4,5-trisphosphate generation in porcine thyroid follicles — a possible mechanism of growth inhibition by iodide. Eur J Endocrinol 1995;132:735—43.

[64] Gartner R, Rank P, Ander B. The role of iodine and delta-iodolactone in growth and apoptosis of malignant thyroid epithelial cells and breast cancer cells. Hormones 2010;9:60—6.

[65] Rosner H, Torremante P, Moller W, Gartner R. Antiproliferative/cytotoxic activity of molecular iodine and iodolactones in various human carcinoma cell lines. No interfering with EGF-signaling, but evidence for apoptosis. Exp Clin Endocrinol Diabetes 2010;118:410—9.

[66] Shrivastava A, Tiwari M, Sinha RA, Kumar A, Balapure AK, Bajpai VK, et al. Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving the mitochondria-mediated pathway. J Biol Chem 2006;281:19762—71.

[67] Aceves C, Garcia-Solis P, Arroyo-Helguera O, Vega-Riveroll L, Delgado G, Anguiano B. Antineoplastic effect of iodine in mammary

cancer: participation of 6-iodolactone (6-IL) and peroxisome proliferator-activated receptors (PPAR). Mol Cancer 2009;8:33.

[68] Rösner H, Möller W, Groebner S, Torremante P. Antiproliferative/cytotoxic effects of molecular iodine, povidone-iodine and Lugol's solution in different human carcinoma cell lines. Oncol Lett 2016;12:2159–62.

[69] Moreno-Vega A, Vega-Riveroll L, Ayala T, Peralta G, Torres-Martel JM, Rojas J, et al. Adjuvant effect of molecular iodine in conventional chemotherapy for breast cancer: randomized pilot study. Nutrients 2019;11:1623.

[70] Anguiano B, Garcia-Solis P, Delgado G, Aceves Velasco C. Uptake and gene expression with antitumoral doses of iodine in thyroid and mammary gland: evidence that chronic administration has no harmful effects. Thyroid 2007;17:851–9.

[71] Mendieta I, Nunez-Anita RE, Nava-Villalba M, Zambrano-Estrada X, Delgado-Gonzalez E, Anguiano B, et al. Molecular iodine exerts antineoplastic effects by diminishing proliferation and invasive potential and activating the immune response in mammary cancer xenografts. BMC Cancer 2019;19:261.

[72] Youson JH, Sower SA. Theory on the evolutionary history of lamprey metamorphosis: role of reproductive and thyroid axes. Comp Biochem Physiol 2001;129:337–45.

[73] Heyland A, Moroz LL. Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. J Exp Biol 2005;208:4355–61.

[74] Kessler J, Obinger C, Eales G. Factors influencing the study of peroxidase-generated iodine species and implications for thyroglobulin synthesis. Thyroid 2008;18:769–74.