

Molecular Iodine Misconceptions: A Novel Formulation Approach to Topical Iodine

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Perspective

My team of chemists have formulated molecular iodine (I₂) so it can be studied independent of other iodine species. The initial impetus for this effort was to allow for a higher concentration of biocidal I₂ that would not evaporate into the atmosphere when applied to skin. The COVID pandemic increased the use of diluted PVP-I for nasal and oral prophylaxis which induced us to perform basic toxicological evaluations of pure I₂. We did not believe that elevated concentrations of I₂ would stain and irritate skin as literature suggests. Since dilution of Povidone Iodine (PVP-I) for nasal prophylaxis results in higher concentrations of I₂ than neat 10% PVP-I, we wanted to demonstrate that this practice was likely to be safe. It was further reasoned that these formulations would allow an evaluation of I₂ outgassing from skin as originally demonstrated by the leading expert of iodine disinfection in the 20th century, Waldemar Gottardi [1]. These questions were addressed and a novel approach for topical iodine disinfection is presented using high concentrations of I₂ that can destroy pathogens for extended periods of time without staining or damaging skin [2].

The data we generated challenges two long-held beliefs that have inhibited the use of pure I₂ in clinical and OTC settings. The scientific and medical communities believe that I₂ is responsible for staining and cytotoxicity associated with aqueous iodine products such as PVP-I and Lugol's Solution [3]. There is no controlled data in the literature that supports this assumption, and our data also calls this assumption into question. We demonstrated that I₂ does not stain or irritate skin; this observation indicates that ingredients other than I₂ in topical iodine disinfectants are responsible for these negative characteristics. Additionally, it is reasonable to ask,

why wouldn't pure I₂ compositions be a preferred topical anti-microbial as compared to PVP-I and Lugol's Solution?

Lack of staining – we demonstrated, in a straight-forward manner, how an emollient formulation containing pure I₂ does not stain skin. The pure I₂ emollient, with a concentration of 7500 ppm (>100 fold higher than I₂ ppm found in PVP-I), was compared against 4 other topical formulations [2]. The pure I₂ emollient was the only test area without any staining on the author's left forearm (**Figure 1**). This experiment negates the argument that I₂ is responsible for staining from iodophors such as PVP-I or from Lugol's Solution.

Biocompatibility with Ex vivo Human Skin

Biocompatibility of I₂ (500, 2500, and 10000 ppm) with human skin was evaluated by performing an MTT assay. Adipose tissue was removed from freshly acquired human skin tissue. Intact human skin tissue explants (12 mm) were produced via biopsy punch and trimmed to an even thickness. Punched explants were transferred to the insert of a 6-well transwell cell culture plate with two explants per well. One mL of Roswell Park Memorial Institute 1640 medium (ATCC MTT Reagent containing 2% penicillin/streptomycin) was placed in each well below the transwell insert. Test agent (10 µL) were placed on top of explants and spread across the surface of the explant with sterile inoculation loops to ensure even coverage without allowing any formulation to go over the sides of the explant. Phosphate buffered saline (PBS), Tween 20, Triton X 100, SDS, and glycerin were run as controls. Cell viability was calculated by dividing the mean absorbance measured for test articles by the mean value for PBS. Reported. Triplicate (n=3 for MTT) samples were prepared per test item and timepoint and shown below in **Table 1**. At 3 and 7 hours



Figure 1. One milliliter of (a) 300 ppm I₂ in water; (b) 10% povidone-iodine; (c) 7800 ppm I₂-glycerin, (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). The glycerin composition with 7800 ppm of I₂ did not stain the skin on the forearm of this 70-year-old white male and author of this manuscript, Jack Kessler Ph.D. in contrast to the four other topical iodine formulations that contained much lower I₂ concentrations.

Table 1. Cell Viability of Ex vivo Human Skin Cells (MTT assay – all values normalized to PBS).

Test Agent	Cell Viability Compared to PBS (mean ± SEM)	
	3h	7h
Glycerin	140.3 ± 13.4	59.1 ± 2.84
10% Tween 20	110.3 ± 6.0	46.7 ± 5.5
1% Triton X 100	83.7 ± 11.1	22.3 ± 0.4
5% SDS	59.9 ± 12.7	21.24 ± 3.8
500 ppm	114.5 ± 8.9	58.0 ± 4.5
2500 ppm	158.0 ± 5.15	54.0 ± 5.4
10,000 ppm	127.4 ± 18.2	67.3 ± 11.3

there was no difference in skin cell viability between glycerin (carrier control) and any of the I₂ treatments. At 7 hours the 10,000 ppm I₂ control exhibited a higher mean cell viability than the glycerin control while Tween-20 (mild irritant), Triton-X100 (moderate irritant) and SDS (strong irritant) all reduced cell viability.

Antiproliferative activity of I₂ – Iodinated lipids were identified as mediators of the Wolff-Chaikoff effect over 25 years ago [4,5] and speculation on their effect on cancer cells followed [5-7]. Interaction of molecular iodine with lipid membranes leads to the formation of 5-hydroxy-6-iodo-8,11,14-eicosatrienoicdeltalactone, an iodinated arachidonic acid which can be abbreviated as 6-IL. Experimental demonstration of cancer cell apoptosis [8-11] was demonstrated and Carmen Aceves has systematically explored the role of non-thyroidal iodine in tumor cell lines [12-20] and she is currently conducting studies to determine the effect of molecular iodine as adjuvant for conventional chemotherapy patients [21]. Thomasz and co-workers have recently demonstrated that 6-IL induces apoptosis in a xenograft colon cancer (HT29 cells) in a dose dependent manner [22,23]. IL-6 reduced tumor mass and microvessel density and downregulated VEGF and VEGF-R2. Aceves, Thomasz and others have proposed several possible pathways to explain the effect of IL-6 on angiogenesis including inhibition of Cox2 [24] and PPAR γ agonism [6,8-10,20,25,26]. It is clear that more research is needed to elucidate a potential antiproliferative role for 6-IL/I₂. However, it is also reasonable to speculate that IL- δ could be used as a chemotherapeutic agent for the treatment of cancer tissues, alone or combined with other therapies.

I₂ Cytotoxicity

The metabolic activity of human skin treated with I₂ concentrations up to 15,000 ppm was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). *Ex vivo* human skin tissue (HST) procured post-surgically under an IRB exempt protocol was used to evaluate tissue compatibility with I₂. Adipose tissue was removed from freshly acquired HST and tissue explants were produced via biopsy punch. Explants were transferred to 6-well transwell cell cultures plates with two explants per well. RPMI 1640 (1 mL) containing 2% penicillin/streptomycin was placed in each well below the transwell insert. Ten microliters of test agent (carrier control and I₂-carrier at concentrations between 500 and 10,000 ppm) were placed on top of explants in quadruplicate (n=3) per test item. The percent viability of the human skin cells was quantified 3 & 7 hours post application and there was no difference in cell viability between the glycerin control and any of the I₂ treated tissues. We demonstrated the absence of cytotoxicity with a test using an elevated concentration of I₂ (1500 ppm) in a 24-hour direct contact test with mouse fibroblasts [2]. If pure I₂ is not responsible for cytotoxicity in topical iodine disinfectants, which ingredients are? Leaving the observations in this study aside, the argument that I₂ is responsible for cytotoxicity in PVP-I can be seen as

questionable when we consider that that a 1/100 dilution of PVP-I, contains higher amounts of I₂ but exhibits less toxicity [27-29].

The active biocide in topical iodine disinfectants is the I₂ molecule [27,30-32]. Once absorbed into the skin, I₂ immediately begins outgassing [1]. While outgassing from skin, the I₂ molecule can inactivate pathogens [1,33]. Observations indicate that I₂ partitions into the hydrophobic regions of the dermis/hypodermis. The I₂ slowly diffuses from the hydrophobic tissue for 6 or more hours until I₂ can no longer be measured [2].

A careful reading of the medical and chemical literature related to “iodine” illuminates several current dynamics: (a) the medical community understandably associates the word “iodine” with thyroid hormones; (b) does not understand aqueous “iodine” chemistry which determines the properties of the topical iodine products currently in use [33,34]; and (c) there is an abundance of independent experimental data based on distinct experimental approaches that demonstrate an anti-inflammatory [35-39] and antiproliferative activity for molecular iodine and IL-6.

The data presented here may suggest a new model for surgical lavage [40-44], skin antisepsis and provides a practical I₂ dosage form to explore clinical utility as an anti-inflammatory agent [35-37,39,45-49]. This formulation approach provides the possibility of converting skin into an antimicrobial material for hours while providing emollience.

Is pure I₂ a natural antimicrobial with broader clinical utility?

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