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The uptake and release of molecular iodine by the skin: chemical and bactericidal evidence of residual effects caused by povidone-iodine preparations

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Summary: The residual effect of iodine-based disinfectants is caused by a dynamic back-diffusion which is the reverse of the absorption occurring during application. A very sensitive photometric method was used to measure the iodine flux of the back-diffusion after treating the skin with povidone-iodine preparations and Lugol's solution. After removal of the preparation the intensity of the iodine flux decreases with time and correlates with the amount of iodine being resorbed. The latter depends on the concentration of free molecular iodine of the preparation, the contact time and the thickness of the horny layer of the treated skin. With Lugol's solution (approximately 170 ppm free molecular iodine) iodine flux could be observed 12–24 h after an application of 20–60 s, while with low-level povidone-iodine preparations (0·5–20 ppm free iodine) the measurable flux lasted only 0·5–1 h after an application time of 3 min.

The bactericidal activity of the back-diffusing iodine from skin previously treated with a commercial povidone-iodine preparation was assayed by using an inoculation with *Micrococcus luteus*. This showed a logarithmic reduction of 0·4 in bacterial concentration. Since the skin was washed with soap for 1 min after application this bactericidal action reflected the residual effect of the back-diffusing iodine.

Introduction

Skin disinfectants may exert a persistent bactericidal activity after application, a phenomenon which may occur even after the removal of the preparation by washing away with water. This 'persistent action' or 'residual effect' is usually regarded as an advantage. It is due to retention of the bactericidal agents on the skin or to alterations in the skin surface that provokes its antibacterial properties. The origin of this phenomenon may relate to the following three mechanisms:

(1) Films of substances attached to the skin by non-covalent bonds
Disinfectant films (e.g. chlorohexidine, surface-active substances like quaternary ammonium or amphoteric compounds) are bound to the skin mainly

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by weak adsorption (Van der Waal) forces, but also, in the case of quaternary ammonium compounds, by real ionic bonds.

(2) Chlorine, covalently bound onto the skin
So-called chlorine covers are formed at the uppermost layer of the horny skin when it comes in contact with solutions of active chlorine compounds. They are built up mainly by N–Cl units which are formed by halogenation of NH_2 groups of the protein matrix of the skin.[1]

(3) Molecular iodine dissolved in the skin
In contrast to chlorine-based disinfectants which contain oxidizing and therefore bactericidal –O–Cl or >N–Cl compounds, the bactericidal action of iodine-based disinfectants is caused by free molecular iodine (I_2).[2] Because of its excellent penetration and its poor reactivity with protein constituents (it reacts under the conditions prevailing at disinfection only with S–H functions[3]) iodine easily enters the skin where it forms a solid solution. In the case of Lugol's solution (strong iodine solution corresponding to USP XXI) this produces a dark brown staining which cannot be removed by washing with soap and water or by moistening with reducing material. It decreases only very slowly and in the case of strong iodine loads can be observed even after 12 h.[4] The decrease in the absorbed iodine has two causes: one part diffuses into deeper skin layers where it is reduced to iodide and produces an increase of serum-iodide,[5] while the other part diffuses back out of the skin. An iodine atmosphere thus develops at the skin surface which can be detected by the iodometric glove method developed for quantifying chlorine covers.[4] However, this method is not sensitive enough to measure the back-diffusing iodine of povidone-iodine preparations which have substantially lower free iodine concentrations (2–10 ppm) than Lugol's solution (approximately 170 ppm). Nevertheless, since povidone-iodine preparations can also cause an increase of serum-iodide,[6] iodine from these compounds must be absorbed by the skin.

The purpose of this investigation was to measure back-diffusing iodine after povidone-iodine application and to determine whether it is strong enough to exert persistent bactericidal activity.[7–10] In order to measure only the real residual effects of povidone-iodine caused by back-diffusion, the skin was thoroughly washed after the application in order to remove any remaining traces of the povidone-iodine preparation which would simulate a pseudo-residual effect.

Methods

Chemical assessment of residual effects caused by iodine
As with chlorine, measurement of the residual effect of iodine is based on the determination of oxidation capacity. However, with chlorine the covalently bound halogen (Cl^{+1}) is located on the skin surface while the

iodine is contained inside the skin. Thus, while the complete oxidation capacity of residual chlorine can be easily measured by application of an appropriate reagent solution to the skin, with iodine only the amount diffusing back in a given time can be measured.

Measuring principle. The iodine emerging from a defined section of the skin (area F), is absorbed during a given measuring time (t_m) by an aqueous solution (volume V) of diethyl-p-phenylenediamine[11] (DPD). Iodine combines with DPD forming a red dye ($\lambda_{max} = 553$ nm) proportional to the absorbed iodine quantity [$cI_2 = A_{553} \times \varepsilon^{-1} \times d^{-1}$ ($\varepsilon_{553} = 20500\ mol^{-1}\ cm^{-1}$, d = optical path length)]. This procedure estimates mass-flux Φ with the dimension mass/area × time (dim [Φ] = m l^{-2} t^{-1}). Using the DPD reagent the iodine-flux at the time t is given by

$$\Phi_{I2(t)} = \frac{A_{553} \times V \times 2{\cdot}538 \times 10^5}{\varepsilon_{553} \times F \times t_m} \left[\mu g\ cm^{-2}\ min^{-1}\right] \quad (1)$$

with the terms volume (V), area (F) and measuring time (t_m) expressed as ml, cm^2 and min. The measuring time is given by $t_m = t_2 - t_1$, while the time over which the measured flux ($= \Phi_{I2(t)}$) is averaged comes to $t = (t_1 + t_2) \div 2$, where t_1 = start of measured time and t_2 = end of measured time.

Application. The mouth of a small Erlenmeyer flask (volume 20–25 ml, opening diameter of approximately 2·5 cm) containing approximately 2–3 ml of the iodine preparation to be investigated was applied to the skin of the upper or forearm. With this procedure a reproducible loading of the skin with a safe dose of iodine was achieved. After the chosen application time of 0·5–8 min the flask was removed and the treated skin area thoroughly washed with lukewarm water for 30 s and dried.

Assessment of the iodine flux. One millilitre of the DPD reagent (1 Multicol-reagent tablet A, Benckiser Company, A-5400 Hallein, in 15 ml water) was transferred to a 1-cm cuvette (breadth 0·5 cm) for measuring the absorption at 553 nm; this was used as a blank. The solution was then transferred to a small Erlenmeyer flask with a slightly narrower mouth (area F) than the one used for the application. The mouth of the flask was placed concentrically onto the skin area that had been treated with the iodine preparation and left there with occasional shaking during the measuring time t_m. Then the absorption of the solution at 553 nm was again measured and the flux calculated with Equation 1.

Three-minutes value. Exactly 2 min after the end of the iodine application (which corresponded to the beginning of the washing step) the back-diffusing iodine was absorbed for 2 min by the DPD reagent. Thus the

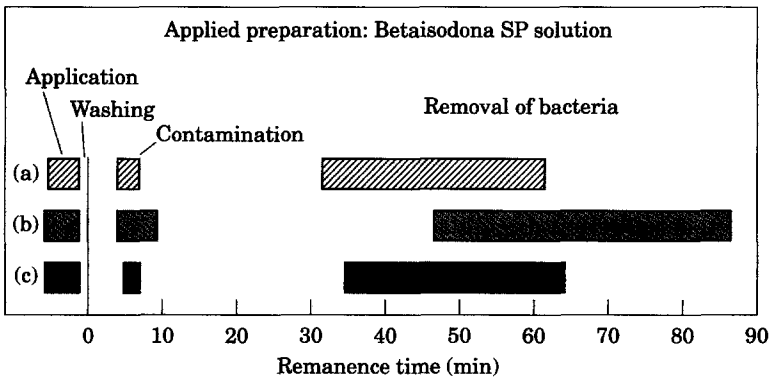

Figure 1. Execution and results of the bacteriological experiments. Results were as follows: (a), RF = 0·40 ± 0·31, $n = 12$; (b), RF = 0·40 ± 0·46, $n = 16$; (c), RF = 0·41 ± 0·25, $n = 16$. Mean RF = 0·40 ± 0·35.

iodine flux was measured 3 min after the end of application, i.e. at $t_{rem} =$ 3 min (see below).

Remanence time. This is the time between the removal of the iodine preparation by washing ($= t_0$) and the time the flux was measured: $t_{rem} = (t_1 + t_2 - 2t_0) \div 2$

Detection limit. Using the results of measurements on skin not treated with iodine the detection limit was calculated with $A_{det.lim} = A_{553(blind)} + 3\ S_{(blind)}$ and Equation (1) to be 0·002–0·003 $\mu g I_2\ cm^{-2}\ min^{-1}$ (measuring time 4 min, 1 ml DPD reagent, $F = 2{\cdot}55\ cm^2$.

Bacteriological assessment of residual effects caused by the back-diffusing iodine

Micrococcus luteus was used as the test organism since it has previously been used for evaluating residual effects of disinfectants.[12] Test areas were marked on both forearms; one of them was treated with the povidone-iodine preparation before inoculation, and the other was the control. The temporal course of the different steps of the experiments is described in Figure 1.

Application. Both forearms were washed for 1 min with soap, flushed with water for 15 s and dried. One forearm was then treated for 5 min with a total of 50 ml of povidone-iodine (Betaisodona SP solution) so that every minute a fresh 10 ml portion of preparation was added. Then the treated forearm was thoroughly washed with soap for 1 min, rinsed with water and dried.

Contamination. Sixteen circular skin areas (diameter 2·5 cm) were marked

symmetrically on both forearms with a water-resistant ink. Each was inoculated with 25 µl of a 1:100 dilution of a 24–48 h CSL-culture of *M. luteus* (ATCC 9341).

Removal of bacteria. Twenty-five to 45 min after inoculation the mouths of sterile 25 ml flasks (Erlenmeyer type, with narrow neck, cross-section 2·5 cm) filled with 2 ml of the collecting solution (CSL-bouillon with 3% Tween 80) were placed over the marked skin areas. After gentle shaking of the flask for 1 min a colony count was performed on the collecting solution.

Evaluation. The logarithms of the cfu values for test and control areas were used to derive a quantitative measure for the disinfecting power of the back-diffusing iodine:

$$RF_{rem} = \log \text{ cfu}_{\text{not disinfected}} - \log \text{ cfu}_{\text{disinfected}}$$

Results

Chemical investigations

Temporal course of the iodine flux. Figure 2 shows the decrease of iodine flux with time after applying: (1) povidone-iodine solutions with different concentrations of free molecular iodine but the same contact time (3 min); and (2) Lugol's solution with different contact times (10–60 s). The decrease in iodine flux with time approximates to parallel lines when plotted on a double logarithmic scale. Thus only one measured value is needed to estimate the intensity of the iodine flux for a given time. The area under the curves (calculated by numeric integration) gives the total amount of iodine diffusing out during this period, which is proportional to the total iodine absorbed by the skin.

Influence of contact time. Figure 2 also shows that iodine flux is dependent on the contact time (experiments with Lugol's solution) and the concentration of free molecular iodine (experiments with povidone-iodine). The influence of contact time on iodine flux was evaluated by applying a commercial preparation (Betaisodona SP solution, with 10·2 ppm free iodine) for 0·5–8 min and measuring the three-minutes-value. As shown in Figure 3 the iodine flux increases strongly with the contact time.

Influence of free iodine concentration. In Figure 4 the three-minutes values of povidone-iodine preparations adjusted in the range of $[I_2] = 0{\cdot}5$–48 ppm free molecular iodine and Lugol's solution ($[I_2]$ approximately 170 ppm) are plotted against their concentration of free iodine. Because the povidone-iodine preparations were applied for 3 min and Lugol's solution for only

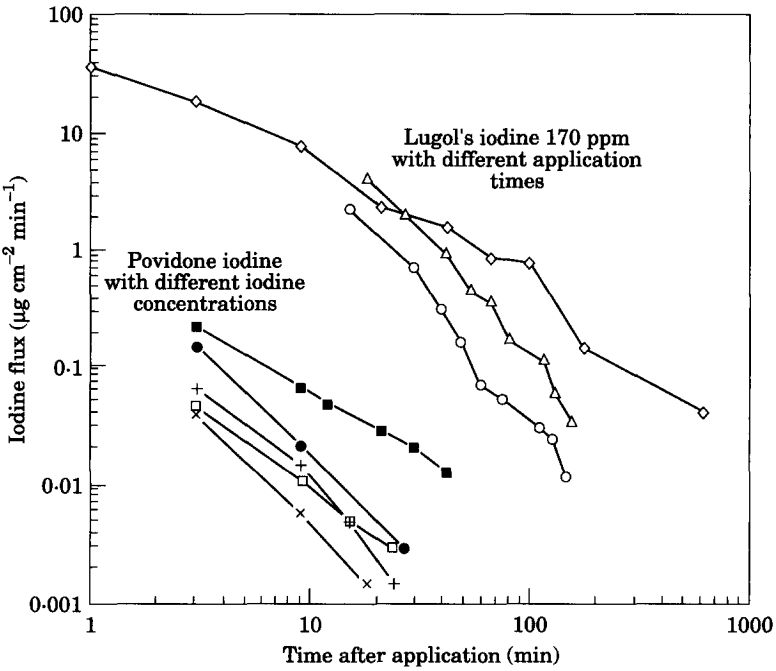

Figure 2. Temporal course of the iodine flux after application of povidone-iodine and Lugol's solution. (-■-), 20 ppm; (●), 10 ppm; (+), 7 ppm; (-□-), 2 ppm; (X), 0·5 ppm; (-◇-), 1 min; (-△-), 40 s; (-○-), 20 s.

1 min these different preparations cannot be compared directly. Nevertheless, as expected the iodine flux increases with the concentration of free iodine.

Bacteriological investigations

As can be seen in Figure 1 the residual (i.e. back-diffusing) iodine of the applied povidone-iodine preparation induced a bactericidal effect represented by a mean logarithmic reduction value of $RF_{rem} = 0{\cdot}4$. Though not very high, the equal results of three experiments are reliable, showing a significance at the 99, 99·9 and in one case even the 99·99% level by the Student *t*-test.[13]

Discussion

Chemical and physical aspects

Using a new, sensitive photometric method, it was possible to measure the back-diffusion of molecular iodine absorbed by the skin, even for povidone-iodine preparations where the absorbed quantities are so low that staining of the skin is not observed.

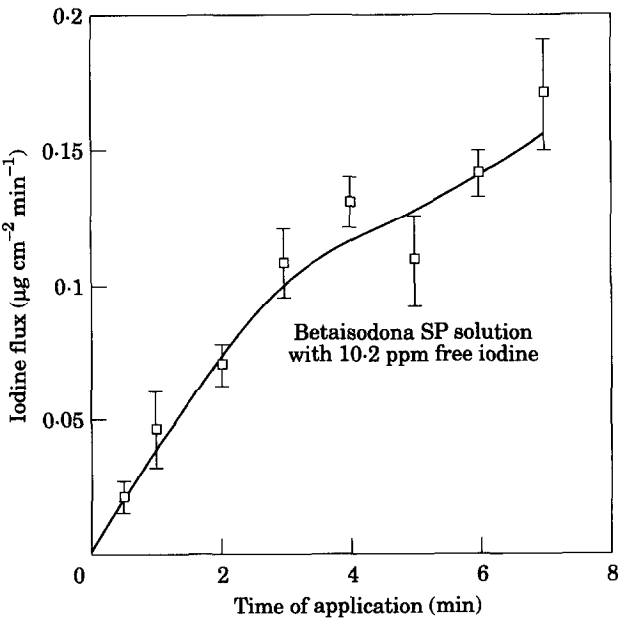

Figure 3. Influence of the time of application on the intensity of the back-diffusion. (□), MW ± SD; (−), three-minutes value.

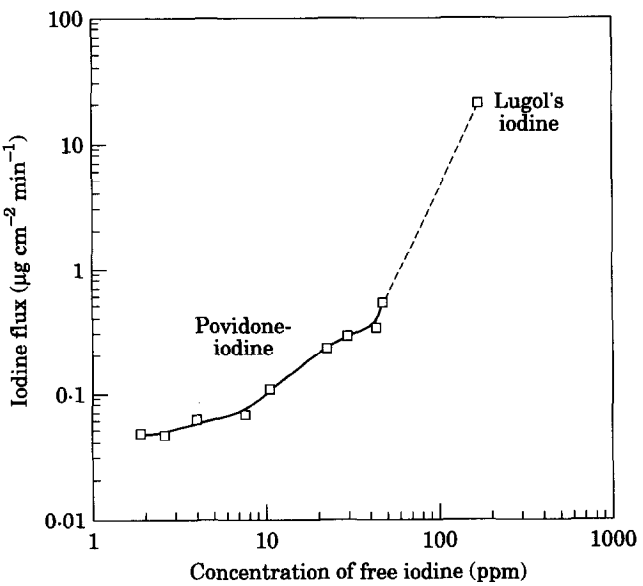

Figure 4. Influence of the concentration of free iodine of the applied preparation on the intensity of the back-diffusion. (−□−), three-minutes value.

With the three-minutes value an important parameter was established, which enables a prediction of the persistence of the back-diffusion of iodine: from the regressions of the curves of the povidone-iodine solutions in Figure 2, $\log \Phi = B + S \times \log t$ ($S = -1{\cdot}7 \pm 0{\cdot}5$), it follows $S = \text{tg}\ \alpha = (\log \Phi_2 - \log \Phi_1)/(\log t_2 - \log t_1)$, which, rearranged to $\Phi_2 = \Phi_1 \times (t_2/t_1)^S$ allows one to estimate the iodine flux at a time t_2 from the known one at time t_1.

With Lugol's solution the detectable persistence of iodine lasts up to 24 h, but with povidone-iodine preparations with their substantially lower concentration of free molecular iodine it was only 30–60 min. However, the application time was 3 min in these experiments compared with 5 min for surgical scrub. For povidone-iodine preparations a mean iodine flux for the first 3 h can be assumed to range from $0{\cdot}1$–$1{\cdot}0\ \mu g I_2\ cm^{-2}\ min^{-1}$ at t = 1 min to $0{\cdot}01$–$0{\cdot}1\ ng I_2\ cm^{-2}\ min^{-1}$ at t = 3 h. Though the iodine flux decreases rapidly, the back-diffusion proceeds very much more slowly than the uptake. Applying the physical laws of diffusion one can draw the conclusion that the intensity of the flux of the back-diffusing iodine is related to the total amount of incorporated iodine. The easily measurable three-minutes value, therefore, also allows estimations of iodine resorption which may have implications for toxicity.

Bacteriological aspects

It was found that the back-diffusing iodine evokes a residual bactericidal action on the skin. The resorption of iodine, one of the few constraints of iodine-based preparations, turns out to be an advantage. On the one hand the back-diffusing iodine exhibits a persistent activity against bacteria spread on the skin after removal of the preparation (see Figure 1), and on the other, the good penetrating qualities also suggest an activity against resident bacteria. Hartmann[12] found that the reduction of the resident flora was significantly higher using a povidone-iodine preparation than it was with isopropanol. This is in contrast to the usual findings for the activity of alcohols.

A unique feature of skin disinfection is that the active agent (i.e. the free molecular iodine) both enters the skin and comes out of it. Because of these residual properties, povidone-iodine preparations are useful for topical application before surgical procedures. According to Gloebel,[11] the increase of the iodide level in body fluids after repeated application of povidone preparations is fully reversible and does not affect the concentration of T_3 or T_4.

General aspects of iodine resorption and health risks

The extent of total iodine resorption induced by the application of a preparation containing free molecular iodine is affected by the following parameters, the first three of which being proportional to the iodine uptake: (1) the concentration of free molecular iodine; (2) the contact time; (3) the dimension of the treated area; and (4) the nature of the treated skin.

Excessive iodine resorption should be avoided. The application of iodine-containing preparations to large areas, as in surgical procedures, should be done with low concentration preparations such as povidone-iodine (2–10 ppm free molecular iodine). If only small areas are to be disinfected, more concentrated preparations such as Lugol's solution or iodine tincture can be used. Because of their high free iodine content these reagents can be recommended for injuries contaminated by HIV or by hepatitis B virus.

Residual effects

Washing the treated area with soap removes all residual films of surface active agents like chlorhexidine or quaternary ammonium compounds, leaving no persistent bactericidal activity. However, washing with soap has virtually no influence on 'chlorine covers' and iodine absorbed in the skin. Chlorine covers can be removed by wetting the skin surface with a solution of a reducing substance (e.g. sodium thiosulphate), but this procedure has no effect on the residual activity of iodine which depletes only by back-diffusion over time.

Wade and Casewell[9] compared three aqueous preparations, non-medicated bar soap, 4% chlorhexidine digluconate skin cleanser (Hibiscrub) and 7·5% povidone-iodine surgical scrub (Betadine), and two alcoholic preparations, 60% isopropanol and 0·5% chlorhexidine digluconate in 70% isopropanol (Hibiscol), and showed a striking residual action of Hibiscol. However, the authors rinsed the treated skin with water after application of the aqueous preparations while the alcoholic ones were only dried. Thus, with Hibiscol a film of the active agent remained on the skin which was not the case with Betadine and Hibiscrub.

To prevent such misleading conclusions the term 'residual activity' should be standardized in such a way that its evaluation has to include a defined procedure for removal of the applied preparation, at least by rinsing with water.

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