New Aspects of the Tolerance of the Antiseptic Povidone-Iodine in Different ex vivo Models

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Key Words
Povidone-iodine  Irritating potency  Tissue toxicity  Eye resorption  Nasal tolerance  Cartilage tolerance  Methicillin-resistant Staphylococcus aureus

Abstract
Investigating new possibilities for the application of 1% (v/v) iodophors, povidone-iodine (PVP-I) was better tolerated in the HET-CAM or explant test than 1% (w/v) silver nitrate or tetracycline. After application to the eye, at least 2.6% of used iodine were adsorbed. Therefore PVP-I is more effective than silver nitrate or erythromycin, meaning a possible alternative for the prevention of ophthalmia neonatorum. PVP-I is more active against methicillin-resistant Staphylococcus aureus (MRSA) in a human ex vivo skin model, which results in a complete eradication of S. aureus in the nasal cavity of volunteers after 2 daily applications and will be better tolerated by human nasal ciliary epithelium than chlorhexidine. Having the same clinical tolerance as mupirocin, PVP-I is a useful alternative for the antiseptic therapy of germ carriers of MRSA. The synthesis of proteoglycans in articular cartilage of bovine sesamoid bones was increased after application of 5% (v/v) PVP-I without any increase in catabolism revealing possibilities for the use as irrigation solution in the joint.

Objective
In the German-speaking countries, the following fields of application have established themselves for povidone-iodine (PVP-I): hand disinfection in cases of intolerance of alcohols, antisepsis of the skin, antisepsis of the oral cavity with the special indications of prevention of mucositis and application prior to tooth extraction in the sulcus gingivae to prevent bacteremia, antisepsis of the vagina and orificium urethrae, wound antisepsis except burns after transplantation, antiseptic irrigation of body cavities except peritoneum and intraoperative application to the bowel before surgical anastomosis [1]. Given the wide therapeutic scope of the iodophors, their clinical efficacy, their high therapeutic range (table 1), their good tolerance for wounds [2] and their inhibition of bacterial mediators of inflammation as well as of mediators of the host cells [3], we carried out supplementary studies on the field of application of the eye and examined the suitability of iodophors for the biotopes nasal cavity and cartilage tissue.
Studies to Define the Ways of Using PVP-I as Prophylaxis against Ophthalmia neonatorum

HET-CAM (Irritating Potency)

Method. The study was carried out in the classical manner [4] by way of an objective quantification of the response. For this purpose, the reaction at the chorioallantoic membrane was recorded with an S-VHS-C video camera (Panasonic NV-S 70 E) and the image evaluated by morphometry using the image analyzer Kontron IBAS 2000. In effecting a comparison with the stereomicroscopic assessment [4], an area of <128% (median) could be ascertained as tolerable range for the application of antiseptics to the eye.

Results and Discussion. With an exposure time of 30 s, 1% PVP-I meets the requirements for tolerance of an eye antiseptic with a median of 128%, whereas 1% AgNO₃ induces complete lysis and hence fails to meet the requirements of tolerance.

Table 1. Calculated therapeutic range as quotient of oral LD₅₀ for rats (mmol/kg) and minimal microbicidal concentration at 5 min exposure

<table>
<thead>
<tr>
<th>Agent</th>
<th>Quotient</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td></td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Octenidine</td>
<td></td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>PVP-I</td>
<td></td>
<td>500</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Table 2. Growth rate (%) in explant test with prepared peritoneal tissue

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>Exposure min</th>
<th>Growth rate, % (control = 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP-I</td>
<td>10%</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 mg</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Iodine resorption after conjunctival and periorbital application of PVP-I on one eye

<table>
<thead>
<tr>
<th>PVP-I concentration, %</th>
<th>Renal iodine excretion 24 h after surgery, μg I⁻/g creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>calculated increase</td>
</tr>
<tr>
<td>10</td>
<td>5,000</td>
</tr>
<tr>
<td>1.5</td>
<td>750</td>
</tr>
</tbody>
</table>

Results and Discussion. Tetracycline proved to be highly toxic to tissue, as compared to PVP (table 2), although the latter was undiluted (i.e. 10% PVP-I).

Explant Test (Tissue Tolerability)

Method. 1 mm × 1 mm pieces of peritoneum of neonatal rats (inbreeding Lew 1 A) were exposed to the agents, thereafter washed with Ringer's solution and cultivated in 24-well culture plates for 10 days [2].

Result and Discussion. Tetracycline proved to be highly toxic to tissue, as compared to PVP (table 2), although the latter was undiluted (i.e. 10% PVP-I).

Resorption by Eye Antisepsis

Method. Prior to the extraction of the lens and subsequent implantation of a posterior chamber lens, antisepsis of the conjunctiva and the periorbital skin was carried out, each by way of a swab freshly impregnated with PVP-I solution (calculated average application 0.4–0.8 ml). For the antisepsis of the conjunctiva, the PVP-I solution was applied on an Ethikeil swab, to wipe the conjunctiva for about 10 s. Prior to surgery and after 24 h, the iodide in the urine was determined by the modified Wawschinek method [5]. Creatinine was analyzed by the Jaffé method using system packs from Boehringer Mannheim and a Hitachi 717 device.

Results and Discussion. The elevated excretion of iodine is clearly below the theoretic maximum of full resorption (table 3). In no case was any iodine contamination (for Germany, quotient >300) ascertained; in fact, the values were within the desirable range of the WHO classification [6].

2.5% PVP-I on the eye causes severe burning so that we recommend 1.25% PVP-I on the grounds of our findings on tolerance and efficacy. With this concentration, Neisseria gonorrhoeae is reduced >5 log within 15 s, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa within 30 s and adenoviruses type 2 are inactivated within 5 min by >4 log [7]. Chlamydia trachomatis strain D is even destroyed by 0.113% PVP-I within 30 s by >5 log [7]. By contrast, AgNO₃ and erythromycin are...
without effect against the chlamydial stem in a period of 15 min (reduction 0.9 and 1.7 log, respectively) [8].

Conclusion
Hence 1.25% PVP-I for prophylaxis against ophthalmia neonatorum is a clearly more effective and efficient alternative to local antibiotics and likewise silver nitrate. For the same reasons, 1.25% PVP-I is the treatment of choice for preoperative eye antisepsis and 0.5% PVP-I with 5 min exposure for the prevention of infection in corneal transplants [9].

Nasal Cavity

Task
For two reasons, there is a need for an alternative to mupirocin, currently used as standard for MRSA carriers in nasal antisepsis:

because of the effect that is only microbiostatic, a failure of therapy in up to 27% is observed [10];

for about 10 years, an increase has been ascertained in methicillin-resistant S. aureus (MRSA) strains resistant to mupirocin [11].

Contrary to mupirocin, PVP-I has a microbicidal effect without the risk of developing resistance [12].

Prior to testing PVP-I for tolerance in the nasociliary epithelium, we checked its efficacy against MRSA in an ex vivo model on amputated skin, as requirement for the application of PVP-I to eliminate MRSA carriers.

Antiseptic Efficacy of PVP-I against MRSA on Human ex vivo Skin

Method. Testing was carried out on skin areas from freshly amputated skin of the thigh carried out for surgical indications. Otherwise the test was carried out in the same way as the testing of skin antiseptics in volunteer trials [13]. Nevertheless, instead of contaminating the resident flora as marker for efficacy, the amputated skin was contaminated with an MRSA patient strain [10^8 CFU/ml].

Results and Discussion. 10% PVP-I is significantly more effective than chlorhexidine and achieves practically the same effect as 2-propanol (fig. 1). Since PVP-I is also highly effective on oral and genital mucosa [14, 15], it would seem to be promising, in view of the evidence of tolerance, for the antisepsis of nasal cavities.

Tolerance for the Ciliated Epithelium Cells of the Nasal Mucosa

Method. Ciliated epithelium was taken out from the concha nasalis inferior with a bronchoscopic brush without application of local anesthetics. The brushes were washed out in 100 μl DMEM at 37°C. The activity of the cilia was measured with a photocell under a special microscope. The signals were analyzed with the PC program Turbolab [16].

Results and Discussion. No suppression of ciliary activity was caused by 1.25% PVP-I. By contrast, 0.2% chlorhexidine induced inhibition so that the activity of the cilia was reduced to below the range of the physiological minimum (fig. 2).

Since chlorhexidine – despite the lack of a tolerance test for ciliated epithelium – was used, albeit with unsatis-
factory results on the MRSA colonization [17], we used PVP-I on volunteers with S. aureus colonization. After twice daily application for 3 days, total eradication was achieved [18]. Hence PVP-I is a more effective alternative to mupirocin, with the same clinical tolerance as mupirocin [18].

**Cartilage**

**Task**

After Kallenberger et al. [19] had furnished evidence of inhibited growth with fetal cartilage and the release of glycosaminoglycan in the medium, iodophors were deemed to be unsuitable for use on the joint. Since, however, this model does not relate to the status of the adult cartilage, the issue should be taken up again with a sensitive relevant in vitro test.

**Method**

Sesamoid bones from metacarpophalangeal joints of adult animals were used [20]. Medial sesamoid bones were dissected within 2–4 h after slaughter under aseptic conditions. The cartilage from the medial sesamoid bones of a particular animal is uniform with respect to glycosaminoglycan synthesis, glycosaminoglycan content and proteoglycan composition.

The sesamoid bones were rinsed with 10 ml of sterile Ringer’s solution for 10 min, incubated, respectively, in 10 ml of sterile Ringer’s solution (control), 10% PVP-I, 1% PVP-I, 0.1% Octenisept® and 0.01% Octenisept in Ringer’s solution at 37°C for 2 h in a humidified atmosphere with 5% CO₂ and subsequently rinsed again for 5 × 5 min in 10 ml Ringer’s solution in each case. Thereafter sesamoid bones were incubated in 10 ml Ham’s F-12 medium supplemented with antibiotics for 7 days at 37°C in a humidified atmosphere with 5% CO₂ changing the medium daily. On day 6, 1.85 MBq ³⁵S-sulfate was added to the medium to label newly synthesized proteoglycans. After the labeling experiments, nonincorporated ³⁵S-sulfate was largely removed from the cartilage matrix by way of a 5-fold washing process of the sesamoid bones; 10 ml Ringer’s solution was used for each wash for 5 min.

Cartilage plugs of 2.8 mm in diameter were carefully removed and extracted thereafter in a stepwise manner [21, 22]. The first extraction was effected in 0.5 ml of 0.15 M Na-acetate buffer, pH 6.8, in the presence of protease inhibitors, by shaking 1.5-ml Eppendorf vials at 4°C for 24 h (iso-osmolar conditions). The second extraction of cartilage plugs was carried out in 0.5 ml of 4 M guanidinium hydrochloride, 50 mM Na-acetate buffer, pH 5.8, containing protease inhibitors for 2–3 days at room temperature (dissociative conditions). The doubly pre-extracted plug was digested in 0.5 ml tris(hydroxymethyl)aminomethane hydrochloride buffer, pH 8.0, containing 1 mg pronase per milliter for 24 h at 56°C (remaining proteoglycans).

The culture media of sesamoid bones and extracts of cartilage plugs were used for the quantitative determination of sulfated glycosaminoglycans by way of the dimethylmethylene blue method [23]. Extracts and extracts of cartilage plugs were used for the estimation of ³⁵S-sulfate incorporation into cartilage matrix by way of

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**Fig. 2.** Frequency (median) of cilia (100 cells/agent).
Table 4. Metabolism marker of cartilage after 2 h exposure with test substances and 7 days cultivation in vitro

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>Differences to the control (Ringer’s solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>proteoglycans µg/mg wet weight cartilage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COMP µg/mg wet weight cartilage</td>
</tr>
<tr>
<td>PVP-I</td>
<td>10</td>
<td>+2.62</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+0.25</td>
</tr>
<tr>
<td>Octenidine</td>
<td>0.1</td>
<td>-1.35</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>+1.02</td>
</tr>
</tbody>
</table>

* p < 0.01. COMP = Cartilage oligomeric matrix protein.

Table 5. Incorporation rate of 35S-sulfate into the proteoglycan fraction

<table>
<thead>
<tr>
<th>Agent</th>
<th>Differences of proteoglycans to the control (Ringer’s solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. iso. diss. remaining total cpm/mg cpm/mg cpm/mg cpm/mg cpm/mg</td>
</tr>
<tr>
<td>PVP-I</td>
<td>10 +53 +691 -384 +307*</td>
</tr>
<tr>
<td></td>
<td>1 -6 +1,013 -417 +597*</td>
</tr>
<tr>
<td>Octenidine</td>
<td>0.1 -25 -1,432 -1,950 -3,381*</td>
</tr>
<tr>
<td></td>
<td>0.01 -35 -1,167 -1,970 -3,137*</td>
</tr>
</tbody>
</table>

* p < 0.001. conc. = Concentration; iso. = extraction under iso-osmolar conditions; diss. = extraction under dissociative conditions.

scintillation β-counting. In the culture media of the sesamoid bones and in extraction solutions of the cartilage plugs, the content of the cartilage oligomeric matrix protein was determined by way of a quantitative ELISA method [24].

Results and Discussion

PVP-I as well as octenidine did not induce catabolic metabolism with an increased loss of proteoglycans (table 4). Under the influence of PVP-I, the incorporation rate of 35S-sulfate was increased by 10 and 20%, respectively (table 5). Until now, this phenomenon, the stimulation of proteoglycan synthesis, has not been reported in the literature for antimicrobials. By contrast, corresponding to the eluted amounts of proteoglycan and cartilage oligomeric matrix protein, octenidine nearly completely inhibits the synthesis of proteoglycan, depending on the concentration of octenidine (table 5).

In the meantime, the tolerability of 0.5% PVP-I is confirmed in the synovia and hyaline cartilage of the rabbit joint [25]. Since, in contrast to the comparatively tested mucosal antiseptics of chlorhexidine, polyhexanide, hexetidine and cetylpyridinium chloride, PVP-I loses none of its efficacy in vitro from 0.25% chondroitin sulfate [26], the requirements for efficacy in the joint are given.

Conclusion

In evaluating the test results and in light of the status of knowledge on PVP-I, the following new application fields can be seen for iodophors: the prevention of ophthalmia neonatorum, already empirically successful in Kenya [27], the antisepsis of nasal cavities, especially with MRSA colonization, and the antisepsis of joints.
References


