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# *Antifungal efficacy of a novel silica-based iodophor*

Antygrzybicza aktywność nowego preparatu jodoforowego

## SUMMARY

The purpose of this investigation was to assess the *in vitro* antifungal efficacy of a silica-based iodophor containing 12 per cent of active iodine. The susceptibility of dermatophytes ( n = 26), moulds (n = 16) and yeast-like fungi (n = 38) to the iodophor was estimated. The last-named fungi turned out to be least resistant to the iodophor, their MIC and MFC values ranging from 0.5 to 0.3 mg $ml^{-1}$, whereas those belonging to dermatophytes exhibited a higher resistance with MIC and MFC values of 1.2 mg $ml^{-1}$. The difference in susceptibility of the pathogens could be attributed to specific structure of the cell walls of yeast-like and filamentous fungi.

**Key words**: iodophor; iodine; silica, antifungal efficacy, MIC, MFC

## INTRODUCTION

Elemental iodine has long been known for its strong biocidal efficacy. One of the earliest preparations used for disinfection of small wounds was iodine tincture. Later, Lugol's iodine was developed for disinfection of naso-pharyngeal and bucco-pharyngeal mucosa as well as a thyreostaticum.

A salient feature of the iodine tincture is its effectiveness against a variety of pathogens including both Gram-positive and Gram-negative bacterial strains, viruses, spores, protozoa, and fungi [Leitmanova *et al.* 1987]. Some disadvantages of this formulation are irritancy which also impedes wound healing, and staining of the skin. This was the reason for gradual replacement of the iodine tincture by synthetic organic preparations, for instance acridine and phenothiazine dyes, on

the one hand, and for stimulation of efforts focused on elimination of the disadvantages of the tincture, on the other. In the latter instance, through interdisciplinary efforts of many research workers, preparations currently known as iodophors have been developed. These are solid or liquid formulations consisting of elemental iodine deposited on natural or synthetic carriers. Particulars on their preparation, composition, antimicrobial activity, application areas, and side effects can be found in an excellent review by Leitmanova *et al.* [1987].

One of the most widely used iodophors is Povidone Iodine (PVP-iodine; PVP-I; Betadine®; Isodine; Dermadine) in which iodine is complexed with a poly(vinylpyrrolidinone) carrier. Its germicidal efficacy has been reported in many papers [Leitmanov *et al.* 1987, Ghogawala and Furtado 1990, El-Sayed *et al.* 1993, Isenberg *et al.* 1995, Fleischer and Reiner 1997, Reimer *et al.* 2002, Wewalka *et al.* 2002, Zhou *et al.* 2002]. The application areas of PVP-I encompass the treatment of skin infections, abrasions, cuts, wounds, not too-extensive burns, mouth ulcers, bed sores and postoperative wounds. It has also found application for pre-operative preparation of the skin and mucous membranes.

Recently, a novel iodophor with an amorphous silica carrier has been patented by two of the present authors [Piękoś and Teodorczyk 2001]. The main advantages of the iodophor is the wound repair action of the carrier itself, its simple preparation method by using the sol-gel technique and cost-effectiveness. The primary objective of the contribution was to assess the antifungal efficacy of the novel iodophor.

## MATERIAL AND METHODS

### Preparation of the iodophor

The iodophor was prepared by a slightly modified patented procedure [Piękoś and Teodorczyk 2001] as follows. A solution of technical water glass R-145 (manufactured by Enterprise WAMA, Lębork, Poland) was 5-fold diluted with distilled water and applied on top of a glass column (500 × 40 mm) packed with Amberlite IR 120 ion exchange resin (grain size 0.074 to 0.149 mm) in the $H^+$ form. To approx. 584 ml of the eluate, containing silicic acids, a triturated mixture of 120 g of elemental iodine and 296 g of potassium iodide was added and the solution was left for aging. The onset of gelation of the solution was noted after 15 minutes. In this way a 1-kg batch of the iodophor containing 12 per cent of active iodine was prepared.

### Species and strains

The *in vitro* antimycotic activity of the iodophor was evaluated against selected species of pathogenic and opportunistic fungi. The strains came from the collection of the Department of Veterinary Microbiology, Agricultural University of Lublin, Poland. The following strains were used in the study: *Candida albicans* (n = 6); *C. parapsilosis* (n = 2); *C. crusei* (n = 3); *C. famata* (n = 2); *C. glabrata* (n = 3); *C. kefyr* (n =1); *Saccharomyces cerevisiae* (n = 2); *Cryptococcus neoformans* (n = 2); *Malassezia pachydermatis* (n = 10); *Geotrichum candidum* (n = 5); *Nocardia spp.* (n = 2); *Trichophyton mantagrophytes v. mantagrophytes* (n = 6); *T. mentagrophytes v. granulosum* (n = 5); *T. verrucosum* (n = 5); *Microsporum canis* (n = 10), *Aspergillus fumigatus* (n = 7); *Penicillium spp.* (n = 5); *Mucor spp.* (n = 6).

The *Candida spp., Malassezia spp.,* moulds and dermatophyte strains were multiplied on Sabouraud agar for 48 h, 3 days, 5 days and 14 days, respectively.

**Antimycotic activity**

The activity of the iodophor against the strains of the fungi was evaluated by macrodilution test in the Sabouraud glucose liquid medium [Clayton 1989]. The concentration of antimycotic preparations in the medium ranged from 1.2 mg $ml^{-1}$ to 0.0012 mg $ml^{-1}$. As inoculum, a fungi spore suspension was used at a concentration of 0.1 ml per ml of the liquid medium. The density of each suspension was around $2 \cdot 10^5$ cfu $ml^{-1}$. The cultures were incubated at 37°C up to 3 days in the case of yeast-like fungi and up to 7 days with the remaining fungi. The minimal inhibitory concentration (MIC), defined as the lowest concentration of the iodophor that resulted in complete inhibition of fungal growth, was assessed after 3 and 7 days, respectively.

The minimal fungicidal concentrations (MFC) were determined by plating 200 µl of the test medium from dilution tubes containing a 3-or 7-days MIC into tubes containing only Sabouraud liquid medium. The cultures were incubated, as in the case of MIC determination, for 3 and 7 days at 37°C.

## RESULTS AND DISCUSSION

The results of evaluation of the susceptibility of dermatophytes (n = 26), moulds (n = 16) and yeast-like (n = 13) fungi to the iodophor are presented in Tables 1 and 2. The highest activity of the iodophor was exhibited against yeast-like fungi. The MIC and MFC values for these organisms ranged from 0.5 to 0.3 mg $ml^{-1}$ (Table 1). The fungi belonging to dermathophytes, *i.e. T. mentagrophytes, T. verrucosum* and *M. canis*, as well as moulds *Aspergillus spp.*, *Penicillium* and *Mucor spp.*, demonstrated higher resistance to the preparations studied. Both the MIC and MFC values for these organisms were 1.2 mg $ml^{-1}$ on the whole (Table 1).

Tab. 1. The MIC and MFC values of the iodophor for filamentous fungi
Wartości MIC I MFC preparatu jodoforowego dla grzybów strzępkowych

| Species<br>Gatunki | Number of strains<br>Liczba szczepów | MIC<br>mg · $ml^{-1}$ | MFC<br>mg · $ml^{-1}$ |
|---|---|---|---|
| *T. mentagrophytes v. mentagrophytes* | 6 | 1.2 | 1.2 |
| *T. mentagrophytes v. granulosum* | 5 | 1.2 | 1.2 |
| *T. verrucosum* | 5 | 1.2 | 1.2 |
| *Microsporum canis* | 10 | 0.5 | 0.9 |
| *Aspergillus fumigatus* | 7 | 1.2 | 1.2 |
| *Penicillium spp.* | 5 | 1.2 | 1.2 |
| *Mucor spp.* | 6 | 1.2 | 1.2 |

Tab. 2. The MIC and MFC values of the iodophor for yeast-like fungi
Wartości MIC I MFC preparatu jodoforowego dla grzybów drożdżopodobnych

| Species<br>Gatunki | Number of strains<br>Liczba szczepów | MIC<br>mg $ml^{-1}$ | MFC<br>mg $ml^{-1}$ |
|---|---|---|---|
| *Candida albicans* | 6 | 0.5 | 0.5 |
| *C. parapsilosis* | 2 | 0.5 | 0.5 |
| *C. krusei* | 3 | 0.3 | 0.5 |
| *C. famata* | 2 | 0.5 | 0.5 |
| *C. glabrata* | 3 | 0.5 | 0.5 |
| *C. kefyr* | 1 | 0.3 | 0.3 |
| *Saccharomyces cerevisiae* | 2 | 0.5 | 0.5 |
| *Cryptococcus neoformans* | 2 | 0.3 | 0.3 |
| *Geotrichum candidum* | 5 | 0.5 | 0.5 |
| *Nocardia spp.* | 2 | 0.3 | 0.3 |
| *Malassezia pachydermatis* | 10 | 0.3 | 0.3 |

The differences in the susceptibility of the microorganisms to the iodophor seem to correlate not so much with species or strain of the fungi under study, but rather with their affiliation either to the yeast-like or filamentous fungi, as has been reported by other authors [McDonnel and Russell 1999], being generally attributed to specific structure of their cell walls which is likely to impede biocide penetration into the cell [Hector 1993].

The absence of irritative activity towards tissues [Boddie *et al.* 2000], weak allergising properties [Zamora 1986], a low percentage of iodine-resistant strains [Lacey and Catto 1993, Russel 2002], as well as the capability of some iodophors to produce water-resistant films at the surface, increase their *in vivo* efficacy [Jeng 2001] that contributed to widespread clinical application of this class of biocides. Taking into account the sensitivity of *Malassezia pachydermatis* strains to the *in vitro* fungicidal activity of the iodophor, preliminary *in vivo* examinations have been carried out by the present authors. The iodophor preparation was applied to the dogs with clinical symptoms of *otitis externa* and confirmed infection (culture examination) with *M. pachydermatis.* The results (to be published) were quite encouraging as demonstrated by regression of clinical symptoms in the animals with a benign form of the disease and much quicker recovery in more acute cases.

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STRESZCZENIE

Badanie aktywności przeciwgrzybiczej (MIC, MFC) nowego preparatu jodoforowego na bazie krzemu, zawierającego 12% aktywnego jodu przeprowadzono *in vitro* w stosunku do 26 szczepów dermatofitów, 18 szczepów grzybów pleśniowych oraz 38 szczepów grzybów drożdżopodobnych. Najwyższą aktywność preparat jodoforowy wykazywał w stosunku do grzybów drożdżopodobnych; wartości MIC i MFC wahały się od 0,5 do 0,3 mg $ml^{-1}$ . Dermatofity oraz grzyby pleśniowe charakteryzowały się wyższą opornością na działanie jodoforu; zarówno MIC jak i MFC wynosiły w tym przypadku około 1,2 mg $ml^{-1}$. Różnice we wrażliwości różnych gatunków grzybów związane są prawdopodobnie z odmienną budową ich ściany komórkowej.

**Słowa kluczowe**: jod, jodofor, krzem, antygrzybicza aktywność, MIC, MFC