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Antifungal efficacy of oxythiamine – antivitamin derivative of vitamin B_1

Antygrzybicza aktywność oksytiaminy – antywitaminowej pochodnej witaminy B_1

SUMMARY

The *in vitro* antifungal activity (MIC, MFC) of oxythiamine was assessed against 15 dermatophyte strains, 9 moulds and 24 yeast-like fungi. High activity of oxythiamine was determined towards *Malassezia pachydermatis* strains (n = 10); in this case MIC was found within 1.25–2.5 μg ml^{-1}. The biocidal efficacy (MFC) of oxythiamine was not recorded in any drug dose applied.

Key words: oxythiamine, antifungal efficacy, MIC, MFC

INTRODUCTION

Some water-soluble vitamins are known to be the precursors of coenzymes making up the key metabolic enzymes. However, the structurally modified vitamins as the antivitamin derivatives have aroused the scientists' interest only recently in their efforts to find some potent means for the selective inhibition of particular enzymes and, as consequence, metabolic paths [Moszczyński and Pyć 1998]. This attitude yielded the elaboration of some highly efficient pharmacological preparations, e.g. folic acid analogs with antibacterial and antineoplastic activity [Stryer 1997].

The research work on new antifungal means drew attention to the antivitamin derivative of B1 vitamin – oxythiamine. In the pyrimidine ring this compound does not contain an amino group but a hydroxyl one, thus being unable to participate in catalysis of such enzymes like pyruvate dehydrogenase, 2-oxoglutare dehydrogenase and transketolase [Strumiło *et al*. 1995]. In the cells, oxythiamine undergoes the phosphorylation process to oxythiamine diphosphate (just like thiamin to thiamin diphosphate) and blocks the active centers of the above mentioned key metabolic enzymes [Schellenberger 1998]. Numerous studies on the enzymes from animal tissues revealed strong inhibitory activity of oxythiamine [Strumiło *et al*. 1984, Zimatkina *et al*. 1996]. Recent research showed that thiamin-dependent enzymes in *Saccharomyces cerevisiae* are also inhibited after the medium supplementation with oxythiamine which induces the growth inhibition of these

cells [Tylicki *et al*. 2003]. The objective of the following work was to check a potential inhibitory activity of oxythiamine against selected fungus species being pathogens or potential pathogens for man and animal.

MATERIAL AND METHODS

Strains

The research covered totally 43 strains of various fungi species constituting the clinical isolates. There were included *Microsporum canis* (n = 5), *Trichophyton verrucosum* (n = 5), *Trichophyton mentagrophytes* (n = 5), *Aspergillus fumigatus* (n = 3), *Penicillum* spp. (n = 3), *Mucor* spp. (n = 3), *Candida albicans* (n = 5), *C. famata* (n = 2), *C. glabrata* (n = 3), *C. crusei* (n = 2), *Saccharomyces* spp. (n = 2), *Malassezia pachydermatis* spp. (n = 10).

Inoculum

a) Yeast-like fungi were incubated at 37°C in the Sabouraud solid medium for 2 days (*Candida*) or 3 days (*Malassezia*). The collected material was suspended in the physiologic saline and the suspension density was fixed 10^4 cfu ml^{-1}.

b) Filamentous fungi were multiplied on the Sabouraud solid medium for 14 days (*T. verrucosum*, *T. mentagrophytes*, *M. canis*), 4 days (*A. fumigatus*, *Penicillum* spp.) or 2 days (*Mucor* spp.) at 25°C temperature (*M. canis*, *Penicillum* spp., *Mucor* spp.) or at 37°C (*T. mentagrophytes*, *T. verrucosum*, *A. fumigatus*).

An inoculum of each fungus was made by agarose cylinders of 5 mm diameter covered with homogenous microculture of fungus. The cylinders were cut out from the sites off the same distance from the colony center, which assured the inoculum standardization (equal number of fungus elements in respect of quality and quantity).

MIC (minimal inhibitory concentration) determination

a) Yeast-like fungi – the investigation performed after the reference macromethod defined by NCCLS M27A (National Committee for Clinical Laboratory Standards 1997). A fungal suspension of 10^4 cfu ml^{-1} density was introduced to 5 ml Sabouraud medium with a diluted drug. The inhibitory activity of the drug was determined for the doses ranging from 160 μg ml^{-1} to 0.3 μg ml^{-1}.

b) Filamentous fungi – the research made after the cylinder dilution method [Wawrzkiewicz *et al*. 2000) there were placed 3 cylinders of the examined fungus species. Into each tube containing 50 ml Sabouraud medium with fixed drug concentration (from 160 μg ml^{-1} to 0.3 μg ml^{-1}). The cultures were incubated at 25°C or 37°C for 3 and 7 days and the results were assessed macroscopically.

The drug concentration at which no fungal growth was recorded after 7 incubation days was defined as an inhibitory dose of drug. The fungal growth assessment was performed with reference to the culture parallel to the control, that is 50 ml Sabouraud medium (no drug) with a fungal inoculum analogicous to the studied sample. Each investigation was performed at three replications at different time.

MFC (minimal fungicidal concentration) determination

A fungal inoculum from the samples for MFC determination that did not show any growth in the Sabouraud medium at a definite drug concentration was transported to the Sabouraud liquid medium without a drug. The culture incubation was carried out for 7 days and the MFC was defined as a drug concentration that resulted in complete inhibition of fungal growth (macroscopic and microscopic evaluation).

RESULTS AND DISCUSSION

An increase of mycotic oportunistic infections recorded recently that often lead to patients' death as well as an evident rise of mycotic pathogen resistance to the available therapeutic and disinfecting preparations make a serious problem in both human and veterinary medicine.

Intensive research work aiming at the elaboration of specific and highly effective chemotherapeutic agents has been still insufficient despite its significant advancements, e.g. introduction of novel preparations (voriconazol) from an azole group [De Pauw 2000] or echinocandin – antibiotics of lipopeptide character [Odds *et al*. 2003].

Oxythiamine as a substance blocking active centers of the metabolic enzymes and, as a consequence, inhibiting the eukaryotic cell growth [Tylicki *et al*. 2003] underwent the preliminary assessment to check its therapeutic usefulness, that is to determine its antimycotic activity *in vitro*. Moreover, the MIC and MFC of oxythiamine (Sigma) were established against the dermatophyte strains isolated directly from the clinical material (n = 15), moulds (n = 9) and yeast-like fungi (n = 24). The obtained results are shown in Tables 1 and 2.

It was found that irrespective of a strain and species, the filamentous fungi (dermatophytes and moulds) appeared to be insusceptible to the preparation doses applied (Tab. 1).

Table 1. MIC values of oxythiamine for filamentous fungi
Tabela 1. Wartości MIC oksytiaminy dla grzybów strzępkowych

Species Gatunki	Number of strains Liczba szczepów	MIC μg ml^{-1}
M. canis	5	>160
T. mentagrophytes	5	>160
T. verrucosum	5	>160
A. fumigatus	3	>160
Penicillum spp.	3	>160
Mucor spp.	3	>160

Table 2. MIC and MFC values of the oxythiamine for yeast-like fungi
Tabela 2. Wartości MIC i MFC oksytiaminy dla grzybów drożdżopodobnych

Species Gatunki	Number of strains Liczba szczepów	MIC μg ml^{-1}	MFC
C. albicans	5	>160	Nb
C. famata	2	>160	Nb
C. glabrata	3	>160	Nb
C. crusei	2	>160	Nb
Saccharomyces spp.	2	>160	Nb
M. pachydermatis	10	1.25–2.5	>160

Among the yeast-like fungi, only *Malassezia pachydermatis* demonstrates high sensitivity to oxythiamine. The MIC values of oxythiamine for this species ranged from 1.25 to 2.5 μg ml^{-1}, subject to a strain. Attempts at the MFC determination were unsuccessful, the maximal preparation concentration applied in the studies, that is 160 μg ml^{-1}, did not provide a biocidal effect (Tab. 2).

High susceptibility of *Malassezia* strains *in vitro* to oxythiamine is likely to be associated with the unique structure of a cell wall of this fungus. This thick (up to 0.25 μm) wall, compared to other anascogenic yeasts and multistratified cell wall [Mittag 1995], constitutes about 26–37% of cell volume [Keddie and Barajas 1972], making a natural barrier, for many antifungal preparations and at the same time showing a strong expression of the metabolic enzymes. Some part of them, e.g. lipases are connected with cell membrane, while the proteolytic enzymes and those responsible for the growth and multiplication of yeast-like fungi *Malassezia* strain, are exposed on the cell wall area or secreted to the environment [Mathieson *et al*. 1998]. Oxythiamine, a preparation blocking active centers of the metabolic enzymes being in direct contact with fungal cells can inhibit efficiently their growth.

An increased number of *Malassezia* strains resistant to the therapeutics applied [Kontoyiannis and Lewis 2002, Sanglard 2002] as well as the appearance of side effects like anorexia, nausea, vomiting, diarrhea or liver damage accompanying antifungal general treatment needs elaboration of a new generation of highly-specific, efficient drugs of low harmfulness.

High antimycotic efficacy of oxythiamine *in vitro* is a quite an encouraging premise to take up new studies that would evaluate the usability of the preparation under the clinical conditions.

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STRESZCZENIE

Aktywność przeciwgrzybiczą (MIC, MFC) oksytiaminy określono *in vitro* w stosunku do 15 szczepów dermatofitów, 9 szczepów grzybów pleśniowych oraz 24 szczepów grzybów drożdżopodobnych. Wykazano wysoką aktywność oksytiaminy w stosunku do szczepów *Malassezia pachydermatis* (n = 10); MIC w tym przypadku zawierał się w przedziale 1,25–2,5 μg ml^{-1}. Działania bójczego (MFC) oksytiaminy nie wykazano w przypadku żadnej z zastosowanych dawek leku.

Słowa kluczowe: oksytiamina, aktywność antygrzybicza, MIC, MFC