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Differential pulse polarographic investigation of the antifungal drugs itraconazole, ketoconazole, fluconazole and voriconazole using a dropping mercury electrode

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Received January 4, 2012, accepted March 31, 2012

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Pharmazie 67: 987–990 (2012) *doi: 10.1691/ph.2012.2004*

The electrochemical reactions of the antifungal drugs itraconazole, ketoconazole, fluconazole and voriconazole have been investigated by differential pulse polarography (DPP) using a dropping mercury electrode (DME). All investigations were carried out in Britton-Robinson buffer solutions and methanol with varying pH values. Ketoconazole and itraconazole both showed a reduction peak with a potential between -1.5 V and -1.6 V. Stable and reproducible conditions for the determination of itraconazole ($c = 1 \times 10^{-7}$ M) were found within the pH range of 6.0 to 8.0 and for the determination of ketoconazole ($c = 5 \times 10^{-8}$ M) within pH 6.0 to 7.0. Voriconazole showed a reduction peak with a peak potential of -1.7 V ($c = 1 \times 10^{-5}$ M) within the pH range of 8.0 to 10.0. In the case of fluconazole no electrochemical activity was found.

1. Introduction

Azole antimycotics are used for the treatment of superficial and systemic mycoses and are tremendously important in clinical practice in particular for patients with hematologic and oncologic diseases. They have a broad spectrum of antifungal activity against dermatophytes, yeasts and moulds. The drugs inhibit the ergosterol synthesis in the fungal plasma membrane and therefore interrupt the integrity and function of the membrane. The main chemical characteristic of these antimycotics is the imidazole structure and in newer drugs it is the triazole structure, which is essential for their antifungal activity (Fig. 1).

There are few reports describing the polarographical reaction of ketoconazole. A voltammetric determination of ketoconazole is reported using a carbon paste electrode (Shamsipur and Farhadi 2000). There is a linear sweep voltammetric method for the determination of ketoconazole in tablets (Fijalek et al. 1992). Also, a method for the voltammetric determination of ketoconazole in biological fluids is described (Arranz et al. 2003).

The aim of the present study was to investigate the electrochemical reduction of itraconazole, ketoconazole, fluconazole and voriconazole by differential pulse polarography using a dropping mercury electrode. The influence of several parameters like different pH values or concentrations of the drugs were determined. The results are discussed depending on the different chemical structures of the antimycotics.

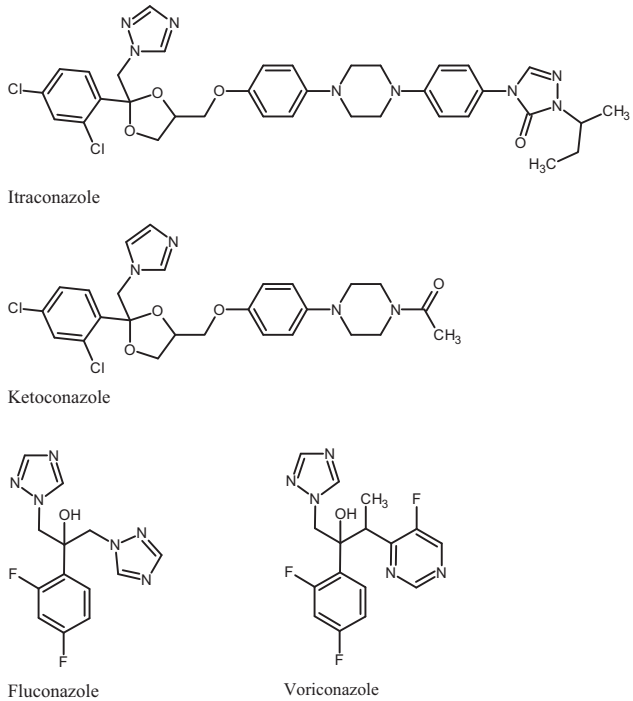

Fig. 1: Chemical structures of itraconazole, ketoconazole, fluconazole and voriconazole

2. Investigations and results

2.1. Peak profile

A 2×10^{-4} M standard solution of each drug was used for the measurements. An appropriate volume of each standard solution was added to the polarographic container for three times, taking three measurements after every addition (standard addition technique). Itraconazole and ketoconazole showed similar polarograms within the pH range from 5.5 to 9.0 (Fig. 2). In all the measurements the reduction peak currents fell off into the positive range with more negative potentials and led to

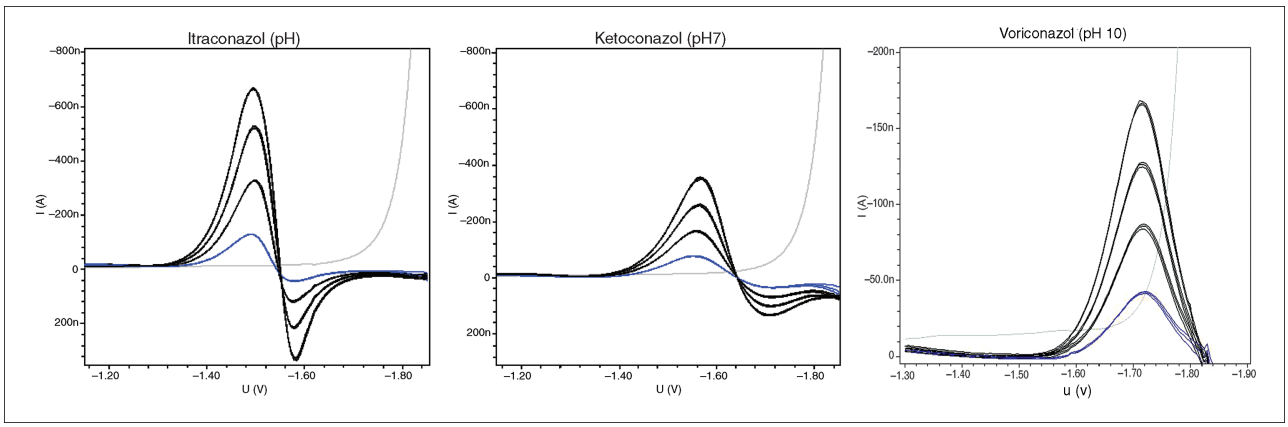

Fig. 2: Differential pulse polarograms of itraconazole (5×10^{-7} M–2×10^{-6} M) and ketoconazole (1×10^{-6} M–4×10^{-6} M) at pH 7.0 and voriconazole (1×10^{-5} M–4×10^{-5} M) at pH 10.0 in Britton-Robinson buffer solution

oxidation peaks. Therefore it was assumed that the process of reduction was not only controlled by diffusion but also by adsorptive effects on the electrode. Polarograms of voriconazole could only be analyzed within the pH range from 8.0 to 10.0. More concentrated solutions were required to receive peak currents comparable to those of itraconazole and ketoconazole. The polarograms of the fluconazole solutions did not show analyzable peaks.

2.2. *Influence of pH value*

The influence of the pH value on the reduction of ketoconazole ($c = 1 \times 10^{-6}$ M) and itraconazole ($c = 1 \times 10^{-6}$ M) was studied within the pH range of 4.0 to 9.0. With a pH of 4.0 to 5.5 it was impossible to evaluate the polarograms as peaks were not characteristic. It is assumed that the electrode was saturated within this pH range since peak currents did not rise with increasing concentrations of the drugs. At pH 6.0 the peak currents were -1383.3 nA for ketoconazole and −731.9 nA for the itraconazole solution. It was shown that the peak currents clearly decreased with increasing pH values. The ketoconazole solution showed a greater decrease in the peak current to −76.9 nA at pH 7.0 than itraconazole, which current decreased to −152.2 nA. Within the pH range of 8.0 to 9.0 the peak currents of both substances continued to decline. The detection limit for the ketoconazole solution was −5.1 nA at pH 8.0 and for itraconazole was −8.5 nA at pH 9.0. At higher pH values it was impossible to evaluate peaks as recorded peak currents were to small in relation to background signal. The peak potentials of ketoconazole shifted linearly to less negative values with increasing pH (70 mV/pH, $R^2 = 0{,}998$). Earlier studies led to similar results (Arranz et al. 2003). In contrast, peak potentials of itraconazole and voriconazole shifted lineary to more negative values with increasing pH (−10 mV/pH, $R^2 = 0{,}995$ and −22 mV/pH, $R^2 = 0{,}997$).

2.3. *Linear concentration range*

The influence of ketoconazole and itraconazole concentrations on the peak current was analyzed at pH 6.0 and 7.0. These conditions were found to be most appropriate for determination. A linear concentration range was investigated at pH 6.0 from 5×10^{-8} M to 2×10^{-7} M ($R^2 = 0.996$) for the ketoconazole solution and from 1×10^{-7} M to 4×10^{-7} M ($R^2 = 0.999$) for itraconazole. At pH 7.0 a linear concentration range was researched from 1×10^{-6} M to 4×10^{-6} M ($R^2 = 0.999$) for ketoconazole and from 5×10^{-7} M to 2×10^{-6} M ($R^2 = 0.997$) for the itraconazole solution.

Influence of the voriconazole concentration on the peak current was analyzed in the pH range from 8.0 to 10.0. Linearity was detected in the concentration range from 4×10^{-6} M to 4×10^{-5} M ($R^2 = 0{,}998$).

3. Discussion

The present study developed a practical method for the qualitative determination of the azole antimycotics itraconazole, ketoconazole and voriconazole by differential pulse polarography using a dropping mercury electrode. Fluconazole was not electrochemically detectable under the conditions used. The results could be used for further quantitative determinations of the drugs. In this instance itraconazole and ketoconazole should be determined in Britton-Robinson buffer solution at pH 7.0

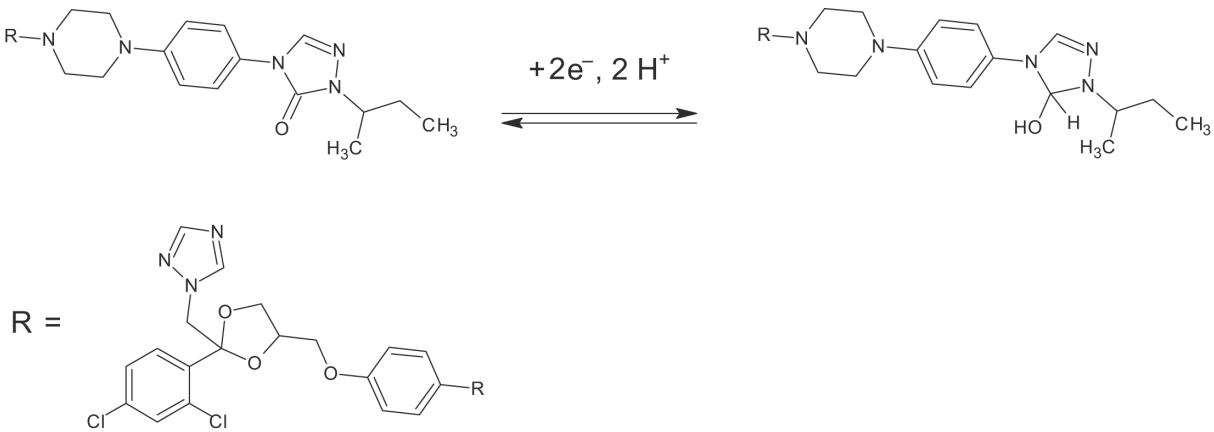

Fig. 3: Proposed mechanism for the reduction of itraconazole

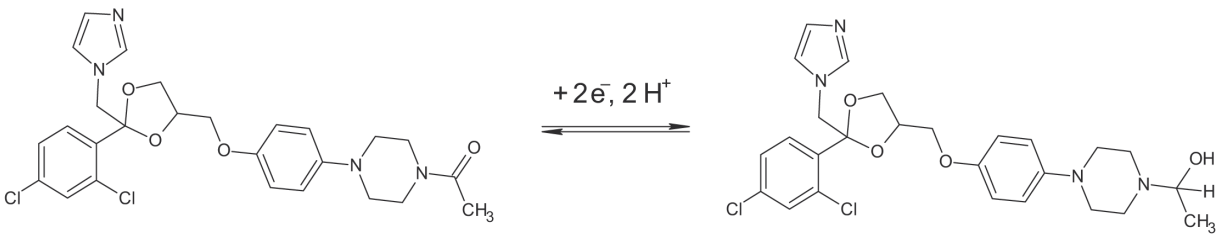

Fig. 4: Proposed mechanism for the reduction of ketoconazole

in the concentration range from 1×10^{-6} M to 4×10^{-6} M for ketoconazole and from 5×10^{-7} M to 2×10^{-6} M for itraconazole. Voriconazole should be quantified at pH 10.0 in the concentration range from 4×10^{-6} M to 4×10^{-5} M.

A parallel quantitative determination of itraconazole, ketoconazole and voriconazole could be developed for example using chromatographic analysis along with an electrochemical detector. Such a method has already been investigated for ketoconazole (Hoffman et al. 1988).

The mechanism of the electrochemical reduction of the azole antimycotics is hardly investigated and described. A study reported the mechanism of reduction of the antidepressant drug trazodone, which is chemically related to itraconazole (El-Enany et al. 2002). Trazodone and itraconazole both consist of a triazolone structure. According to the study the carbonyl group of the triazolone structure is supposed to be irreversibly reduced to the equivalent alcohol through uptake of two electrons and two protons. The proposed mechanism for itraconazole in line with trazodone is shown in Fig. 3. The triazole structure and the twice chlorinated phenyl group of itraconazole could also be responsible for the reduction at the dropping mercury electrode. However, triazole structures are described to be relatively resistant to oxidation and reduction reactions and in general, chlorinated aromatics are described to be hardly reducible (Beyer and Walter 2004; Henze 2001; Hoffmann 1972).

The carbonyl group of the terminal acid amide group of ketoconazole is supposed to be irreversibly reduced through uptake of two electrons and two protons (Fig. 4, Arranz et al. 2003). The contained imidazole structure is described not to have any polarographic activity (Henze 2001).

The fluorinated pyrimidine structure of voriconazole is supposed to be reducible as pyrimidines are polarographically active compounds in general (Henze 2001; Hoffmann 1972). It is assumed that the four electron-four proton reduction process is single-staged and consists of three steps. First, the nitrogen at position three of the fluorinated pyrimidine structure is being reduced through uptake of one electron and one proton forming a positive charged unstable intermediate. Then, the positive charged carbon at position four is being reduced through another uptake of one electron and one proton. At last, the second nitrogen and the carbon at positions one and two are being reduced through uptake of two electrons and two protons forming a tetrahydropyrimidine. The adapted mechanism is shown in Fig. 5.

In contrast to this, fluconazole shows no polarographic activity. As the substance has a triazole structure instead of the fluorinated pyrimidine structure of voriconazole, it is not electrochemically detectable.

Posaconazole is a most recent azole antimycotic, which was not investigated in this study. It is chemically related to itraconazole and also consists of a triazolone structure. Because of the results of the present determination it is proposed that posaconazole is also electrochemically detectable under the conditions described.

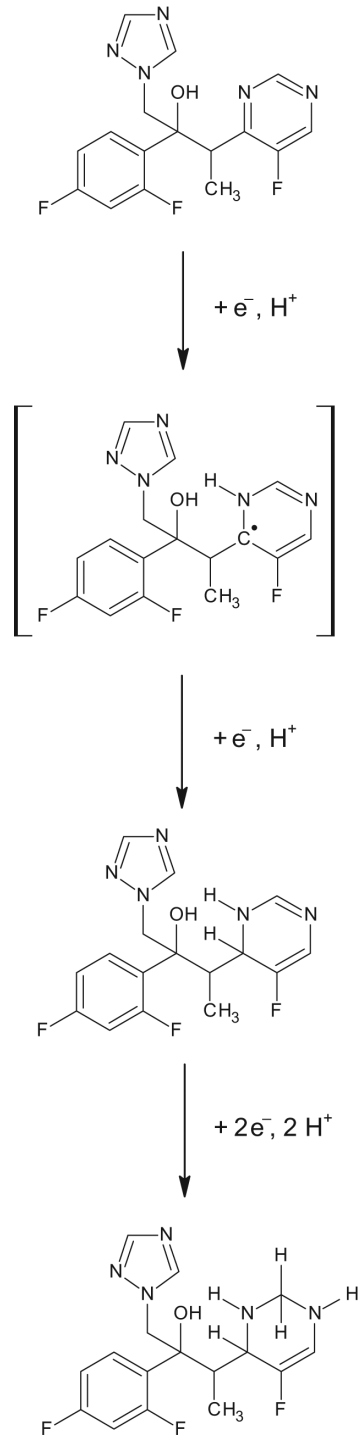

Fig. 5: Proposed mechanism for the reduction of voriconazole

4. Experimental

4.1. Chemicals and reagents

Itraconazole was obtained from Inresa (Freiburg/Germany), ketoconazole from Caesar & Loretz (Hilden/Germany) and voriconazole from Pfizer (New York/USA). All other chemicals were of analytical reagent grade. Stock solutions of the analytes were prepared using methanol (Merck, Darmstadt/Germany). Britton-Robinson buffer solutions were used as supporting electrolyte consisting 0.04 M each of phosphoric acid, acetic acid and boric acid (Merck, Darmstadt/Germany). Different pH values were adjusted with appropriate volumes of 1.0 M sodium hydroxide solution (Carl Roth, Karlsruhe/Germany). Distilled water was used for preparing the solutions. To run the electrode and deaerate the sample solutions, nitrogen gas with a reagent grade of 5.0 was obtained from Messer (Sulzbach/Germany). Mercury 99.9999 % (VWR International, Darmstadt/Germany) was used to fill the electrode.

4.2. Instruments and apparatus

Polarographic determinations were made using a computer controlled 797 VA Computrace analyser (Metrohm, Herisau/Switzerland) with a multimode electrode (MME). A dropping mercury electrode (DME) as working electrode, an auxiliary platinum electrode and an Ag/AgCl reference electrode (saturated with a 3.0 M KCl solution) completed the three electrode cells. The pH measurements were carried out with a digital WTW inoLab Level 2 pH meter (WTW, Weilheim/Germany).

4.3. Procedure

Equal volumes (5.0 ml) of Britton-Robinson buffer and methanol were placed into the polarographic container and then deaerated by nitrogen gas for 5 min. Background signal was recorded before adding an adequate volume of a 1×10^{-3} M stock solution from ketoconazole, voriconazole and fluconazole. A 5×10^{-4} M stock solution was determined for itraconazole as it is slightly soluble. Stock solutions were prepared daily by dissolving an appropriate amount of each analyte in methanol. After a second deaeration by nitrogen gas for 15 sec, an individual differential pulse polarogram of the analyte was obtained. The peak current was evaluated as the difference between each polarogram and the background signal. A scan rate of 14.9 mV/s, a pulse time of 0.04 s, a pulse amplitude of 50 mV, and a drop size of 0.29 mm^2 were chosen as operating parameters. All data was obtained at room temperature.

References

Arranz P, Arranz A, Moreda JM, Cid A, Arranz JF (2003) Stripping voltammetric and polarographic techniques for the determination of antifungal ketoconazole on the mercury electrode. J Pharm Biomed Anal 33: 589–596.

Beyer H, Walter W (2004) Lehrbuch der Organischen Chemie, S. Hirzel Verlag, 24. Auflage, Stuttgart.

El-Enany N, Belal F, Rizk MS (2002) Voltammetric analysis of trazodone HCl in pharmaceuticals and biological fluids. J Pharm Biomed Anal 30: 219–226.

Fijalek Z, Chodkowski J, Warowna M (1992) Polarographic examination of drugs derived from imidazol. I. Clotrimazole and ketoconazole. Acta Pol Pharm 49: 1–5.

Henze G (2001) Polarographie und Voltammetrie: Grundlagen und analytische Praxis, Springer Verlag, 1. Auflage, Berlin.

Hoffman DW, Jones-King KL, Ravaris CL, Edkins RD (1988) Electrochemical detection for high-performance liquid chromatography of ketoconazole in plasma and saliva. Anal Biochem 172: 495–498.

Hoffmann H (1972) Polarographische Arzneimittelanalyse. Pharm Uns Zeit 1: 151–157.

Shamsipur M, Farhadi K (2000) Adsorptive stripping voltammetric determination of ketoconazole in pharmaceutical preparations and urine using carbon paste electrodes. Analyst 125: 1639–1643.