

Short communication

Determination of acid base balance and oil water partition coefficient of an atopy patch test of levofloxacin

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Abstract

Levofloxacin, a quinolone used in the treatment of an upper respiratory tract infection is involved in skin reactions. Patch tests are helpful for the aetiological diagnosis. Unfortunately, they are known for their lack of sensibility. A weak transcutaneous penetration due to the physicochemical characteristics of tested drugs is a well-known cause of false negative results but these characteristics are usually unknown. The aim of the present study was to evaluate physicochemical parameters by potentiometric method in order to optimize the transcutaneous penetration of a preparation for patch test. This method is applicable to a medium such as the preparation for patch test. In order to value the stability of the preparation, qualitative and quantitative analysis of the level of levofloxacin was effectuated. In our study, spectrophotometric and potentiometric data at T0 and six months later with the preparation showed the stability of the preparation. The proportions of non-ionized (45%) and ionized levofloxacin (55%) were not modified. Further experiments are needed to choose the minimal efficient dose of the suspected molecule able to detect a positive reaction in cases of allergic reaction. In addition, this method could be useful in qualitative and quantitative control of preparations made at hospital.

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1. Introduction

The skin is often the target of side effects of the drugs (25–30% of the cases). Antibiotics, which are widely prescribed at hospital, may cause allergic reactions [1]. Quinolones, potent antibacterial agents may induce hypersensitivity reactions in different organs [2]. Levofloxacin, a quinolone used in the treatment of an upper respiratory tract infection is also involved in skin reactions [3]. Skin testing with the suspected compound is helpful in the determination of the cause of cutaneous adverse drug reactions. Skin testing includes drug patches tests, prick tests and/or intradermal reactions. Among

these tests, patch tests are helpful for the aetiological diagnosis [4]. Unfortunately, patch tests are known for their lack of sensibility (false negative reaction). Low concentration of a drug can induce false negative reaction but other mechanisms are also involved. A weak transcutaneous penetration due to the physicochemical characteristics of tested drugs (oil/water partition coefficient, acid base balance, solubility) is a well-known cause of false negative results [5]. Unfortunately, these characteristics are usually unknown.

The aim of the present study was to evaluate physicochemical parameters by potentiometric method (PCA 200, Sirius[®]) in order to optimize the transcutaneous penetration of a preparation for patch test with levofloxacin. In addition, an evaluation of the preparation stability was performed by using potentiometric and spectrophotometric methods, six months

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later. These data might improve the preparation of patch test and then increase the sensibility of patch testing.

2. Materials and methods

2.1. Pharmaceutical preparation

Levofloxacin (Tavanic[®], Sanofi Aventis[®], France) was diluted in white petrolatum (30%), according to the guidelines for drug skin tests of the European Society of Contact. Petrolatum used in the preparation was Gilbert[®] Vaseline (Hérouville-Saint Clair, France). This petrolatum was prepared according to Pharmacopeia Guidelines and did not contain preservatives. According to guidelines, the level of levofloxacin in the preparation was lower than the therapeutic levels and therefore was safe. White petrolatum is widely used and recommended by ICDRG (International Contact Dermatitis Research Group) because of its occlusive effect and antioxidant characteristics [6].

2.2. Potentiometric analysis

Potentiometric determination of oil water partition coefficient (P) or $\log P$ was used for the assessment of pK_a . Potentiometry is a sensible technique for pK_a assessment indicating that the solubility of the substance is at least 10^{-4} M over a suitable pH range. Whether the substance is only soluble to the extent of 10^{-6} M and possesses an analytically useful chromophore, then spectroscopic methods can be applied. If the compound was water insoluble, then a mixed solvent approach (e.g. methanol–water) can be effectuated.

Analytic determination of pK_a by PCA 200 is not calculated from the determination of the equivalence point. Precision is due by the fact that each proton transferred in acidobasic reaction is taken for consideration [7]. This method is applicable to a medium such as the preparation for patch test.

Titration curve can be reproduced using a mathematical model in which some parameters are defined and kept constant (molecular weight of sample...) while some parameters are variable (sample concentration factors...).

Software is including indications of quantities of water, methanol, and sodium chlorate to add at the initial sample in order to keep constant ionic force and optimizing the determination.

Firstly, Levofloxacin had to be dissolved in chloroform. For a sample, 8.5 mg of the preparation was taken and dissolved in 200 μ l chloroform then 7.13 ml of a solvent (methanol/water 80/20% with 11.18 g/l NaCl) was added. Finally, distilled water was added in order to obtain a final volume of 10 ml.

In this study, potentiometric assessment was applied to the carboxylic function (C3) of levofloxacin.

2.3. Molecular spectrophotometry

In order to value the stability of the preparation, qualitative and quantitative analysis of the level of levofloxacin was effectuated in a new preparation, six months later. Uvikon 930[®]

Spectrophotometer (Kontron, Lyon, France) was used with quartz cuvettes (Kontron, Lyon, France) at 22 °C.

Twenty milligrams of the preparation for patch test were diluted in 5 ml chloroform. After filtration on a membrane (porosity 0.22 μ m, Millipore[®]) and dilution (1/100 in chloroform), spectrum from wavelengths 200–400 nm was performed.

3. Results

3.1. Potentiometric analysis

The concentration of levofloxacin, the proportions of ionized and non-ionized forms, pH and pK_a due to the carboxylic function (COOH) are shown in Table 1.

3.2. Molecular spectrophotometry

Two spectrums of patch test of levofloxacin preparation are shown in Fig. 1 (UV spectrophotometer). An increased absorption closed to 330 nm and two peaks of absorption at (229 nm and 300 nm, respectively) were observed. The spectrums were identical in the fresh preparation, six months after.

4. Discussion

Skin patch test is easy to perform and it enables to detect side effects of a drug. However, the sensitivity and specificity of this kind of test is not defined. Negative results with patch test do not exclude the role of the molecule in cutaneous adverse drug reactions. False negative reactions have been observed due to different mechanisms. The percentage of positive results in skin testing with penicillin derivatives has been included between 17% and 35% for example [8]. A weak transcutaneous penetration of the active molecule is most likely responsible for these discrepancies. Excipients may increase or decrease this penetration. Excipients can also lead to a false positive reaction through immunological reactions. This has been described for petrolatum, widely used in preparations for patch test. In the present study, our white petrolatum had not generated sensitization reactions. The knowledge of the different chemical species such as ionized and non-ionized form in the preparation makes it possible to avoid false negative reactions. pK_a of a molecule predicts the degree of ionization of a molecule at a fixed pH and therefore its absorption [9], and bioavailability. Moreover, the degree of ionization affects

Table 1
Proportions of ionized and non-ionized levofloxacin in the preparation at T0 and 6 months later

	Concentration, mg L ⁻¹	pH	pK_a (COOH)	%A	%AH
Patch test of levofloxacin at T0	10.27	7.47	7.092	54.81	45.19
Patch test of levofloxacin after six months	9.49	7.42	7.080	54.44	45.56

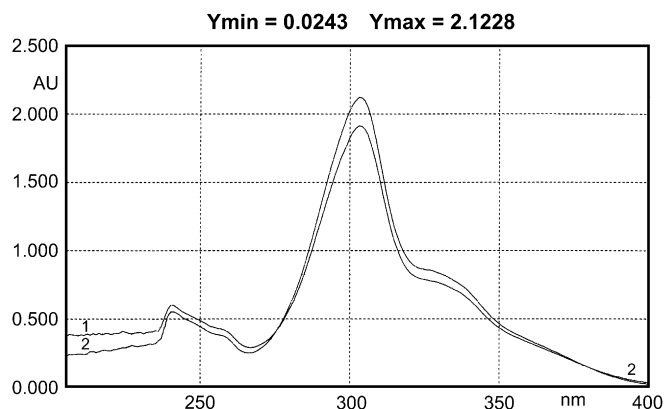


Fig. 1. Absorption spectrums of the preparation at T0 (curve 1) compared to 6 months later (curve 2).

the distribution of the molecule in the solution and also modifies the bioavailability of the molecule. Ionic charge modifies the solubility of the molecule in water. Usually the uncharged form of an organic molecule is able to enter through lipid cell membranes of organisms. The distribution of a molecule between a lipid and an aqueous solution is illustrated by the partition coefficient P . Moreover patch tests should be performed with commercialized forms of drugs [10].

Usually, pharmaceutical laboratories indicate one month of stability for their preparations for patch tests [6]. In our study, spectrophotometric and potentiometric data at T0 and six months later with the preparation (30% in Vaseline) showed the stability of the preparation: spectrums were identical. The proportions of non-ionized (45%) and ionized levofloxacin (55%) were not modified: chemical properties of the preparation were not altered in our conditions of storage six months later.

5. Conclusion

False negative and positive results are present with patch tests. Negative results with patch test do not exclude the role of the molecule in cutaneous adverse drug reactions.

The aim of the present study was to improve the sensitivity of patch tests through the knowledge of physical parameters of the molecule. Ionized and non-ionized forms of the tested drug were quantified in order to predict a good penetration of the molecule through the skin and to avoid false negative reactions.

Further experiments are needed to choose the minimal efficient dose of the suspected molecule able to detect a positive reaction with the suspected drug in cases of allergic reaction.

In addition, this method could be useful in a qualitative and quantitative control of preparations made at hospital.

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