Short Communications

FALSE-POSITIVE TDxFLx® URINE AMPHETAMINE/ METAMPHETAMINE II ASSAY FROM OFLOXACIN

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تستخدم طرق الفحص المناعي علي نطاق واسع في الكشف عن تعاطي العقاقير الممنوعة في عينات البول الخاصة بالمشتبه فيهم. ولقد وجد أن عقار اوفلوكساسين وعدد من الكينولات الأخرى لديها القدرة علي إحداث نتائج ايجابية كاذبة للأفيونات بطرق الفحص المناعي في عينات البول ، وهذا قد يؤدي إلى استنتاجات خاطئه في قضايا التعاطي والإدمان . من أجل ذلك كان هدف إجراء هذه الدراسة هو تقييم تأثير الجرعة العلاجية لعقار أوفلوكساسين –عند إعطائها للمتطوعين- في إحداث نتائج ايجابية كاذبة للعقاقير الممنوعة الشائعة في عينات البول عند فحصها بطرق الفحص المناعي. ولقد أجريت هذه الدراسة علي ستة من المتطوعين الذكور تتراوح أعمارهم بين 35- 45 سنه تم إعطاء كل واحد منهم جرعتين مقدار الواحدة منهما 400 مليجرام من اوفلوكساسين عن طريق الفم وبفاصل 12 ساعة بينهما وتم جمع عينات من البول من جميع المتطوعين قبل إعطاء العقار وبعد مضي 5 – 7.5 ساعات من الجرعة الثانية للأوفلوكساسين وتم اختبار العينات جميعا للكشف عن الأفيونات والأمفيتامين/ميتامفيتامين والكوكايين والحشيش وذلك بطريقة الفحص المناعي علي جهاز تي دي اكس اف ال اكس وبكواشفه الخاصة. وأثبتت الدراسة أن عقار اوفلوكساسين قد أدي إلي زيادة ذات دلاله إحصائية معنوية عالية جدا في متوسط مستوي الأفيونات والأمفيتامين/ ميتامفيتامين دون أي تأثير يذكر علي كل من الكوكايين والحشيش. ولقد ثبت إحداث الأوفلوكساسين لنتائج ايجابية كاذبة مرتفعه للأمفيتامين/ ميتامفيتامين في جميع المتطوعين. ونظرا لاتساع استخدام الأوفلوكساسين والمضادات الحيوية الكينوليه الأخرى في العلاج الطبي فإن الدراسة توصي بحتمية تأكيد نتائج الاختبارات التي يثبت إيجابيتها بطرق الفحص المناعي وذلك بإعادة فحصها بواسطة إحدى الطرق الأكثر خصوصية مثل طرق الفصل الكروماتوجرافي الغازي مع مقياس طيف الكتلة.

Immunoassays are widely used in testing urine for illicit drugs. Ofloxacin and a number of other quinolones were found to induce false-positive opiates (OP) urine immunoassays. This can result in misleading conclusions in the concept of drug abuse. The aim of the present study was to evaluate the effects of ofloxacin in therapeutic doses on the induction of false-positive urine immunoassays for common drugs of abuse in healthy male volunteers. The study was conducted on 6 male healthy human volunteers aging between 35-45 years. Two doses of 400 mg ofloxacin each, were given orally to each volunteer at 12-hours interval and urine samples were collected before ofloxacin administration and 5-7.5 hours after the second dose. Urine samples were subjected for OP, amphetamine/methamphetamine II (AM/MA II), cocaine and cannabinoids assays on TDxFLx® analyzer. Ofloxacin produced significant increase ($P< 0.001$) in OP and AM/MA II assays without any effect on cocaine and cannabinoids assays. False-positive results (>cutoff) for AM/MA II assay, were found in all volunteers after ofloxacin administration. The study recommends strongly the confirmation of positive urine immunoassay results for drugs of abuse by a more specific methodology e.g. gas chromatography / mass spectrometry (GC/MS).

Key words: Ofloxacin, Urine Immunoassays, Opiates, Amphetamine/Methamphetamine, False – positive.

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Introduction

Urine is widely used for illicit drug-testing because acquisition of urine sample is non-invasive and because most abused drugs can be detected in urine for a reasonable time after intake (1).

Testing urine for illicit drugs by immunoassay techniques has increased dramatically because such techniques are rapid, easy to automate and have analytic sensitivity in excess of that needed for the detection of overdose concentrations (2).

In general, immunoassays are considered a first step in screening for drugs of abuse, which must be confirmed by a second more specific methodology, gas chromatography with mass spectrometry (GC/MS). The pressure to reduce the cost of analytic testing makes it tempting to discontinue routine confirmation of urine specimens positive for drugs of abuse by immunoassays (3). In this situation, false positive or negative assays can result in serious problems especially under the public concerns as regards the use of illicit drugs.

Meatherall and Dai (1997) tested cross reactivity of four quinolone antibiotics – ofloxacin, norfloxacin, ciprofloxacin and nalidixic acid – toward the EMIT® II opiates enzyme immunoassay. They found that ofloxacin showed a potential for causing false-positive opiates immunoassay screening result (4).

Ofloxacin, a fluorinated quinolone, is a pyridine carboxylic acid derivative which exerts a broad spectrum antimicrobial effect. It is related to the older quinolone derivatives, nalidixic acid and oxolinic acid, and to the newer quinolone derivatives, norfloxacin and ciprofloxacin (5). Fluoroquinolones inhibit DNA gyrase (topoisomerase II) which is an essential bacterial enzyme required for bacterial DNA replication, transcription, repair, recombination and transposition. They are rapidly bactericidal in vitro and are considerably more potent against *E. coli* and various species of *Salmonella, Shigella*, *Enterobacter, Campylobacter* and *Neisseria* (6).

Ofloxacin is well absorbed from the gastrointestinal tract even when administered with food which causes only minor alterations in absorption. Its volume of distribution (Vd) is 2.4-3.5 L/Kg with a protein binding of 20%. It is excreted primarily unchanged in urine with elimination half-life of 5-7.5 hours (5).

As mentioned before, ofloxacin has a broad spectrum antimicrobial effect, but it is primarily used in the treatment of prostatitis due to E. coli and of some sexually transmitted diseases (STDs) with the exception of syphilis. It may be used as alternative therapy in patients with gonorrhea and it has some benefit in the treatment of skin and lower respiratory tract infections (7).

The wide use of immunoassays in illicit drug-testing and the wide use of quinolone antibiotics in clinical practice, together with the possibility of quinoloe-induced false-positive opiate immuno-assays, enforce strongly towards investigating the effects of therapeutic doses of quinolones on the induction of false-positive urine immunoassay results for drugs of abuse in humans. This is because of the medico-legal and social problems which may result in this respect, and the increasing interest about quality control and optimal laboratory use. These factors constituted the motive behind the present work.

The aim of the present study was to evaluate and compare the effects of oral administration of therapeutic doses of the fluorinated quinolone antibiotic, ofloxacin, in the induction of false-positive urine immunoassays for the common drugs of abuse in human volunteers. Urine samples from volunteers were examined before and after ofloxacin intake, for opiates, amphet-amines, cocaine and cannabinoids, using TDxFLx® system which utilizes Fluorescence Polarization Immunoassay (FPIA) technology.

Materials and Methods

Materials:

Ofloxacin:

Tarivid® 200 mg tablets (each tablet containing 200 mg ofloxacin) produced by Hoechst, Germany, was used in the study.

Human Volunteers:

Six healthy male human volunteers aged between 35─45 years were included in the study. They showed no history of drug abuse and none of them was under any regular medical treatment for any general disease. They were instructed not to take any medicine for one week before the onset of study.

Immunoassay Kits:

The TDx/TDxFLx® kits for urine opiates (OP), amphetamine / meth-amphetamine II (AM/MA II),

cocaine metabolite, and cannabinoids assays were used in the study. The kits are produced by ABBOTT laboratories, diagnostic division, Abbott park, IL 60064, USA. Each kit contains the following:

-Reagents Pack: Formed of wash solution (W), sheep antiserum specific for the drug tested (S), fluorescein tracer specific for each assay (T) and pretreatment solution (P).

-Calibrators Pack: Contains graded concentrations of the tested drug dissolved in human urine.

-Controls Pack: Contains known target concentrations (low, medium and high) for each assay dissolved in human urine.

Methods:

Ofloxacin dosing and samples collection:

A urine sample was collected from each volunteer before ofloxacin administration and stored at 4 ─ 8˚C for subsequent analysis. Then two doses of the maximum therapeutic dose of ofloxacin (400 mg) were given orally to each volunteer at 12 hours interval, then another urine sample was collected from each volunteer 5 ─ 7.5 hours (correspond to elimination half-life of ofloxacin) after the second dose and stored at 4 ─ 8˚C for subsequent analysis (5).

Urine immunoassay:

The TDx/TDxFLx® kits were run on automated TDxFLx® analyzer according to the instructions of the manufacturer. Each kit was first calibrated and control samples were run with each assay to assure accuracy of each assay. The urine samples were then subjected for OP, AM/MA II, cocaine and cannabinoids assays on TDxFLx® analyzer. These assays utilize FPIA technology which requires the use of a polarizing attachment to the fluorimeter, containing liquid crystal polarizing filters which are capable of measuring the emitted light in two planes at 90˚ to one another. Polarized light incident on a fluorophore- labeled molecule produces emission of plane polarized light. Binding of labeled drug molecule to the antibody (sheep antiserum) increases the intensity of polarized light emitted in a particular plane. This is used to measure drug concentration in a given sample (8).

Table 1: TDxFLX® urine assays of samples collected from volunteers before and after ofloxacin administration

Samples	OP ng/ml		AM/MA II ng/ml		Cocaine ng/ml		Cannabinoids ng/ml	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	8.41	115.85	15.4	2078.87	2.34	2.62	ND	ND
2	9.5	101.55	15.53	1855.62	0.42	4.46	ND	ND
3	9.22	87.3	11.51	1131.41	2.22	low	ND	ND
4	10.15	91.69	14.47	1352.07	2.61	1.7	ND	ND
5	10.8	142.64	23.26	2158.13	0.06	3.58	ND	ND
6	9.73	83.65	14.14	1959.89	0.64	2.56	ND	ND
X	9.635	103.78	15.818	1756	1.38	2.49	---	---
±SD	0.815	22.288	3.969	417.3	1.13	1.54	---	---
P	< 0.001		< 0.001		NS			

$\bar{X}$= Mean , ±SD= Standard deviation, OP= Opiates, AM/MA = Amphetamine/ Methamphetamine, ND=Not detected, NS=Not significant.

Statistical Analysis:

Data were statistically analyzed using the student "t" test (9).

Results

The results of the present study are shown in table 1. The urine samples collected from the six human male volunteers 5 ─ 7.5 hours after the second dose of ofloxacin, showed very highly significant increase ($p<0.001$) in the mean values of OP and AM/MA II assays, as compared to urine samples collected before ofloxacin treatment (103.78 ng/ml ±22.288 Vs. 9.635 ng/ml ±0.815 and 1756 ng/ml ±417.3 Vs. 15.718 ng/ml ±3.969 respectively). In spite of the very highly significant ofloxacin - induced OP urine immunoassay activity, none of the readings in all volunteers exceeded the cutoff (threshold concentration) stored on TDxFLx® analyzer as a positive result (200 ng/ml). The highest ofloxacin-induced OP activity was 142.64 ng/ml and the lowest was 83.65 ng/ml. On the other hand, all ofloxacin - induced urine AM/MA II assays were above the cutoff (300 ng/ml). The highest assay was 2158.13 ng/ml and the lowest was 1131.41 ng/ml.

As regards cannabinoids assay all the readings were configured as "low" and thus were below the sensitivity of the analyzer to detect. Cocaine assay showed very small figures unreliable to be statistically analyzed.

Discussion

The present study was conducted on six healthy male human volunteers to evaluate the ability of oral ofloxacin, in therapeutic dose, to induce false-positive urine TDx/TDxFLx® assays for common drugs of abuse. Ofloxacin was found to induce very highly significant increase in urine OP and AM/MA II assays without any effect on cocaine and cannabinoids assays.

There is a great controversy about the reliability of results of immunoassays. Some authors believe that immunoassays are extremely reliable and have relatively few false-positive results, and thus advocate that confirmation of such results is not necessary (10 & 11). Others believe that once a positive sample has been identified by immunoassays, a second more specific methodology e.g. GC/MS, should be done to confirm results (1 & 12).

Meatherall and Dai were the first to report false-positive OP urine immunoassays (EMIT II®) in urine samples spiked with ofloxacin and in urine samples obtained from 2 volunteers who each consumed a single 400 mg ofloxacin pill (4). Also, Baden *et al*. tested the reactivity of 13 quinolones in 5 commercial OP screening assays. They found that levofloxacin, ofloxacin and perfloxacin were the most likely to lead to false-positive OP results. In 6 healthy volunteers, they confirmed the cross-reactivity of levofloxacin or ofloxacin with OP screening assays and found false-positive results in urine samples collected from all volunteers (13).

In the present work, no ofloxacin-induced OP activity exceeded the cutoff (200ng/ml), thus could not be considered false-positive results. This may not agree with the results of Meatherall & Dai and Baden et al. in which OP cutoff was exceeded, but it must be realized that different immunoreactive compounds can have an additive effect on reaching the threshold for a given assay. A given quinolone together with another licit drug with mild OP immunoreactivity, can give false-positive OP urine assay, although the threshold might not be reached if either product taken alone (13 &14).

As regards AM/MA II assay, ofloxacin produced clearly false-positive urine assays in all volunteers. The lowest reading (1131.41 ng/ml) was nearly 4 times the cutoff (300 ng/ml). To the extent of our limited knowledge and after an internet search (Medline), no previous studies reported ofloxacin-induced false-positive AM/MA urine assays and the present study may be the first to report this finding.

Several studies reported false-positive AM/MA urine immunoassays with many licit drugs including some psycho-tropic drugs like chlorpromazine (15 & 16), the peripheral vasodilator buflomedil (17), the H_2 – antagonist ranitidine (18, 19 & 20) and the ß-blocker labetalol (21).

The results of the present study are of particular importance because of the wide-spread use of ofloxacin and other quinolones in clinical practice (22 & 23). This can be a reasonable source of induction of false-positive OP and AM/MA assays and consequently, results in many social and legal problems. Thus, it is strongly recommended to confirm any positive urine immunoassay for drugs of abuse, by another more specific methodology preferably GC/MS. An immunoassay should never be used alone as an evidence for drug abuse without confirmation.

Further studies are needed to investigate the effects of other quinolones on AM/MA immunoassays and to identify the possible mechanisms of such interaction.

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