



RESEARCH ARTICLE



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Spectrophotometric Determination of Chloroquine in the Hair of Mice

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Abstract

Background: Hair analysis for illicit, recreational and therapeutic drugs though an evolving science is easier, less invasive of privacy and there is less chance of tampering with the sample to affect results. Often, a combination of capillary gas chromatography with mass spectrometry, though with known short comings, is preferred for detection of drugs in hair specimen.

Objectives: This study aimed at determination of chloroquine in the hair of mice by Ultra-Violet spectrophotometry. It has the advantage of being easy to operate and sensitive to trace amount.

Methods: Twenty-five mice weighing about 25g each were administered chloroquine phosphate intramuscularly once a week for 4 weeks. Hair was harvested from the same spot before, during administration and at the end of dosing. The samples of hair were extracted with methanol and the resultant residue dissolved in 0.1M HCl. The resulting solutions were then run with the Ultra-Violet spectrophotometer to determine their concentrations using the calibration curve of the reference material.

Results: There was proportionate significant increase in the concentration of chloroquine phosphate in hair at the intervals of administration. The amount of chloroquine detected in hair samples increased from $2.98 \times 10^{-3}/10g$ of hair on the second week of administration to $4.88 \times 10^{-3}/10g$ of hair on the fourth week of administration.

Conclusion: The result from the present study showed that although a new innovation, hair testing using Ultra-Violet spectrophotometry is a valuable has been seen as a valuable tool for drug analysis.

Keywords: Ultra-Violet-Spectrophotometry, Hair, Chloroquine, Mice

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INTRODUCTION

Most analyses of drugs in the human body involve the use of blood or urine samples. Some of these conventional procedures are invasive and prone to transmission of infectious disease. Furthermore only substances that have been taken quite recently can be detected in these samples. An evolving analytical method is the use of hair samples [1]. Trace elements, in particular, are accumulated in the hair at concentrations that are generally at least ten times higher than those present in blood, serum or urine and may provide a continuous record of nutrition status and exposure to heavy metal pollutant [2].

Analysis of drugs in hair has been described as retrospective investigation. It is often employed to detect many therapeutic drugs especially chronic drug abuse, recreational drugs and intentional/unintentional poisoning.

It's easier, less invasive of privacy, and there is less chance of tampering with sample to affect results. Hair analysis is also used for the detection of many therapeutic drugs and recreational drugs [3], [4].

In this context, it has been reliably used to determine compliance with therapeutic drug regimens or to check the accuracy of a witness statement that an illicit drug has not been taken. When a drug is ingested or injected, it rapidly enters body fluids and tissues including blood, sweat and saliva. It is also incorporated into growing hair via the follicles; as the hair grows, the drug, locked inside moves with it. This is thought to be stable for a great many years, even post-mortem; meaning it can be detected many months later. Furthermore, hair analysis can provide information about the patterns of drug use, giving approximate time periods when different drugs were taken. This analysis, however, cannot show the quantity of drugs that were taken on a particular occasion [5]. Often, a combination of capillary gas chromatography with mass spectrometry, though with known shortcomings, is preferred for detection of illicit drugs or of specific toxic substances in hair specimens [6].

This method has been applied to hair samples obtained from a variety of animal species and human subjects [2]. For instance Cocaine and metabolites have been detected in the hair of laboratory animals following cocaine administration [2]. The species studied to date include sheep, mice, guinea pigs and rats [7]. In general, the drug concentrations in their hair are similar to those found in human hair, and the ratios of cocaine to its metabolites appear to be similar as well [2].

Chloroquine (7-chloro-4-diethylamino-1-methylbutylamino quinoline), once a drug of choice in treating malaria fever, now finds use only in ACT

(Artemisin Combination Therapy) [8]. Chloroquine has the tendency especially in high dosages to cause blurring of vision, bleaching of hair and mild skin eruptions. The observed adverse effects are attributed to its cumulative effect, and drug binding to melanin and drug retention in pigment cells for long periods. This study aimed at determination of chloroquine in the hair of mice by Ultra-Violet spectrophotometry. It has the advantage of sensitivity to trace amount. Ultra-violet light, albeit in some instances of very short wavelength and the absorption of Ultra-Violet / visible radiation by a molecule, leads to transition among the electronic energy levels of the molecule. The outcome of this study will serve as a base for future studies on the pharmacokinetics and patient compliance of chloroquine and related drugs. This will improve treatment success and reduce drug resistance.

METHODS

Drug administration and hair samples collection

Twenty five mice weighing about 25g each were procured from Animal House Unit of the University of Ibadan, Nigeria and acclimatized for seven days. The mice were distributed into five groups, each group was handled the same way throughout the experiment. Hair was clipped from the middle part of the back of each mouse at the beginning of the study. This served as the control sample. Twenty four hours afterwards, chloroquine phosphate 10mg/kg was administered intramuscularly to each mouse once a week for 4 weeks. Hair was harvested from the same spot as the control samples in the second and fourth week of the study. Harvesting of the hair was done twenty four hours after drug administration to give room for drug absorption.

Hair samples preparation

Each sample collected was washed separately with 1 ml of liquid detergent to remove external contaminants and rinsed thoroughly with distilled water. The samples were air-dried and pulverized with mortar and pestle. Trapped chloroquine in pulverized hair sample was extracted by heating 10 mg of each sample in methanol for 2 hrs at a temperature of 40°C. This was centrifuged at 1500rpm for 30 minutes and filtered using Whatman filter paper No 2. The filtrate was concentrated over-water bath at a temperature of 50°C. The resultant concentrate was reconstituted in 10ml of 0.1M HCl. The absorbance of the solution was measured with Ultra-Violet spectrophotometer at a wavelength of 221nm. The control hair samples were also prepared as the test samples. A portion of hair obtained from the mice before drug administration was dipped in 5ml of chloroquine phosphate and served as reference sample (positive control). The solution from

this reference sample was used for the calibration curve (figure 1) so that the absorbance of all other samples could be traced on the calibration curve and the concentration read.

Absorbance	Concentration
0.2	0.086
0.4	0.164
0.6	0.267
0.8	0.386
1	0.396
1.2	0.479

Equation: $y = mx + c$
Slope = 0.3971

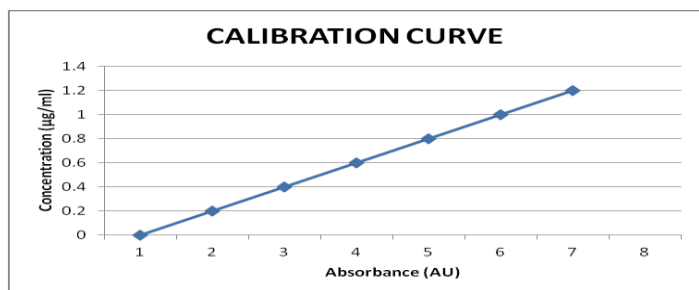


Figure 1: calibration curve of chloroquine phosphate

Statistical analysis was done using ANOVA and results are expressed as mean±standard error of mean

RESULTS

Table 1 shows the concentration of chloroquine phosphate in hair before drug administration to be 0.000 µg/ml and the amount of chloroquine phosphate in hair to be 0.000 µg/10 mg with an absorbance of 0.206AU. Two weeks into drug administration the concentration of chloroquine phosphate in hair was 0.120 µg/ml and the amount of chloroquine phosphate in hair 0.003 µg/10 mg with an absorbance of 0.253AU. Four weeks into drug administration the concentration of chloroquine phosphate in hair rose to 0.196 µg/ml and the amount of chloroquine phosphate in hair 0.005 µg/10 mg with the absorbance of 0.291AU. The results obtained after two weeks of administration of the drugs were statistically different from what was obtained before the drug was administered. While the results obtained four weeks into drug administration was statistically different from what was obtained before the drug was administered and two weeks into the drug administration.

DISCUSSION

The increases (in chloroquine concentration in hair samples in the present study) were time dependent. For instance before the administration of the drug the concentration in hair was 0.000 µg/ml which rose to 0.120 µg/ml when the drug had been administered for two weeks and there was a further increase to 0.196 µg/ml when it had been administered for four weeks.

SAMPLE	CONCENTRATION OF CHLOROQUINE IN HAIR µg/ml	AMOUNT OF CHLOROQUINE µg/ 10mg	ABSORBANCE(A U)
A	0.000 ± 0.0000	0.000 ± 0.0000	0.206 ± 0.0328
B	0.120 ± 0.0336 ^{a,c}	0.003 ± 0.0008 ^{a,c}	0.253 ± 0.0165 ^{a,c}
C	0.196 ± 0.022 ^{a,b}	0.005 ± 0.0005 ^{a,b}	0.291 ± 0.0093 ^{a,b}

Table 1: Concentration of Chloroquine in hair of mice over a period of four weeks

A = before drug administration

B = two weeks into drug administration

C = four weeks into drug administration

a = Statistical difference (p<0.05) exists between A and the group.

b = Statistical difference (p<0.05) exists between B and the group.

c = Statistical difference (p<0.05) exists between C and the group.

The result showed the probability of relative cumulative retention of chloroquine in hair. This is in line with previous observation that administered drugs can get locked in hair.

It can therefore be postulated that the amount/concentration retention of the administered drug will increase with time. The results from this study corroborates the findings of Kintz [9] who observed a comparable/proportionate increase the hair concentrations of buprenorphine and 7-aminoclonazepam after chronic administration using uv spectrometry.

The clinical significance of the outcome of this study cannot be overemphasized. It may be a useful tool in assay to determine the pharmacokinetics and patient adherence of the drug [10]. This is important especially in the face of resistance of malaria parasite to first generation drugs as a result of non-adherence to treatment regimen. Comparing the kinetics and patient compliance will reduce emergence of resistance strains and enhance success of treatment [11].

Another advantage of this method is the possibility of assessing different drugs without the likelihood of interference. It was observed that drug concentration could be read off at particular absorbance. This would be useful in the wake of advocacy of combination therapy to combat the malaria scourge. The kinetic of different component of the combined drugs could be assessed individually and compared to monitor synergism in hair sample. Compared with analysis of the

drug(s) in serum sample, hair analysis will be more stable and can be assayed several weeks after drug intake. This is evident from the cumulative nature of the drug under study. The result from the study showed that the drug concentration in the hair sample of experimental animals could be compared with that in human hair [2].The instrument used in this study combines the advantage of sensitivity to trace samples with ease of manipulation and affordability.

CONCLUSION

The result from the present study showed that although a new innovation, hair testing using Ultra-Violet spectrophotometry is a valuable has been seen as a valuable tool for drug analysis.

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