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Research Article
Population Pharmacokinetic Modeling and Simulation of Afloqualone to Predict Steady-state Exposure Levels

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Abstract

Background and Objective: Afloqualone (AFQ) is a quinazoline family GABAergic drug used as muscle relaxant. After oral administration of AFQ, greatly elevated exposure in some individuals were observed which might cause severe side effects. The aim of this study was to develop a population pharmacokinetic model of AFQ and search possible reason of high inter-individual variability (IIV) in the clinical study and further demonstrate its impact on exposure on AFQ through simulation. **Methodology:** To evaluate the exposure of AFQ and confirm the high difference between subjects, non-compartmental analysis was assessed. A Population PK model of AFQ was developed using healthy human AFQ PK data and simulation study was performed with final PK model. **Results:** A two-compartment model with first order absorption and elimination was used to explain the pharmacokinetics of AFQ. The high level of HIV in AFQ exposure was explained through assumption two subject group with high exposure group (HEG) or normal exposure group (NEG). Through simulations, it was proved that big difference of AFQ exposure between subjects could be observed in some individual and dose of AFQ needs to be reduced for such subjects in HEG. **Conclusion:** Population PK model of AFQ for explaining high IIV was successfully developed and exposure of AFQ between subjects was simulated. Finally, suggesting an appropriate dose of AFQ in HEG which could be had possibility of genetic polymorphism.

Key words: Afloqualone, pharmacokinetics, population analysis, inter-individual variability, high exposure group

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Afloqualone (AFQ), 6-Amino-2-fluoromethyl-3-(o-tolyl)-4-(3H)-quinazolinone, is a quinazoline family GABAergic drug and is an analog of methaqualone developed in 1976[1]. It has sedative and muscle-relaxant effects resulting from its agonist activity at the β-subtype of the GABAA receptors[2]. AFQ seems to be devoid of a hypnotic action and has different effects on the sleep-wakefulness cycle than those of both the hypnotics and the other muscle relaxants used[3]. The pharmacological properties (muscle relaxation and motor depression) of AFQ, as related to behavior (excitatory) differ from those of anti-anxiety drugs, hypnotics and stimulants (chlordiazepoxide, diazepam, meprobamate and pentobarbital-Na)[4].

AFQ has clinical uses such as neck-shoulder-arm syndrome, lumbago and spastic paralysis. It has minor side effects like headache, dizziness, drowsiness, lethargy, diarrhea, vomiting, abdominal pain and constipation[5]. Rarely it can cause skin problems such as dermatitis, pruritus and photosensitization that leads to discontinuation of the drug's use[6-8]. Ultraviolet light is the main action spectrum to elicit photosensitive skin reactions in patients medicated with AFQ. In addition to sunburn-like eruption and exudative erythema, AFQ also induces a skin eruption resembling lichen planus, a known immunologic skin disorder in which T-lymphocytes are involved[9].

The metabolic process of AFQ includes acetylation of the aromatic amino group, followed by hydroxylation at the methyl carbon of either the acetyl or 2-Methyl residue and direct conjugation of AFQ with glucuronic acid at the aromatic amino group. *In vitro* examination of commercially available recombinant UGT microsomes revealed that UGT1A3 and UGT1A4 possessed the highest AFQ N-glucuronosyltransferase activities among the UGT isoforms examined. Furthermore, inhibition studies and correlation analyses with typical substrates for UGT isoforms performed using human liver and jejunum microsomes indicated that UGT1A4 would mainly catalyze the AFQ N-glucuronidation in liver, whereas both UGT1A3 and UGT1A4 would catalyze the AFQ N-glucuronidation in intestine[10].

Moreover, clinical study has shown that when AFQ is given orally, the parent AFQ and its metabolites are excreted in urine (20% of dose), among which AFQ N-glucuronide is the major metabolite (8% of dose) in human urine[11]. In addition, systemic exposure of afloqualone in healthy human was observed with high inter-individual variability (IIV) and a few previous studies suggested, this phenomenon was related with AFQ N-glucuronidation because this type of conjugation is common in primary aromatic amines but the N-glucuronide of AFQ has not been seen in rats, dogs and monkeys[11,12].

Despite of potential possibility of over-exposure for AFQ, it is in use without dose adjustment in clinical situation because of lack of knowledge about AFQ. Therefore, this study was to develop the population PK model of AFQ and demonstrate difference of AFQ exposure between subject through population modeling and simulation techniques.

MATERIALS AND METHODS

Clinical study data: The clinical study data used in the analysis was collated data from three different clinical trials performed in 2006 at Kyungpook National University Hospital (KNUH; Daegu, Korea) re-evaluating AFQ in healthy subjects. In the study, 8 subjects were enrolled in each trial, with total of 24 subjects providing a total of 287 plasma concentrations. A single oral dose of AFQ 20 mg (Arobest) tablet with 240 mL of water was administered to each subject after an overnight fast. Blood samples (~6 mL) were taken before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after the dose for the determination of AFQ.

Inclusion criteria for participants to be involved in the study were age between 19-55 with no innate or chronic disease and non-pregnant females. Subjects involved in the study were healthy, which were confirmed by physicians after checkup before initiating the study. Demographics and laboratory data of the subjects are summarized in the Table 1. Studies were performed in compliance with the International Conference of Harmonization (ICH), guideline for good clinical practice[13], the declaration of Helsinki on the ethical conduct of medical research[14] and regulatory guidelines from the Institutional Review Board (IRB) of the trial center.

Table 1: Demographic characteristics and laboratory test values of study population

Demographic	Mean (Range)
Age (years)	23 (20-26)
Weight (kg)	70.67 (57-81.70)
Height (cm)	174.40 (167.20-182.00)
Aspartate transaminase (IU L^{-1})	21.38 (13.00-63.00)
Alanine transaminase (IU L^{-1})	18.00 (5.00-64.00)
Alkaline phosphatase (IU L^{-1})	73.21 (44.00-117.00)
Glucose (mg dL^{-1})	87.50 (78.00-99.00)
Total protein (g dL^{-1})	7.70 (6.90-8.40)
Albumin (g dL^{-1})	4.75 (4.40-5.00)
Total bilirubin (mg dL^{-1})	0.89 (0.51-1.49)
Cholesterol (mg dL^{-1})	157.08 (102.00-211.00)
Blood urea nitrogen (mg dL^{-1})	13.07 (6.60-21.00)
Serum creatinine (mg dL^{-1})	0.81 (0.68-0.97)

kg: Kilogram, cm: Centimeter, IU: International unit, L: Liter, mg: Milligram, dL: Deciliter, g: gram

Collected blood samples were centrifuged at 1360 rpm for 10 min and the resulting plasma samples were stored frozen at -80°C until analysis. Plasma AFQ concentrations were measured by fully validated methods at three different centers with different systems. API2000 and API4000 liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (Sciex Division of MDS, Canada) and acquity ultra-performance liquid chromatography (UPLC) system (Waters, USA) was used to analyze the samples. The lower limit of quantification of the assay was 0.5 ng mL^{-1} and the limit of detection was 0.1 ng mL^{-1} for all the methods used[15].

Pharmacokinetic analysis

Non-compartmental analysis: Non-compartmental analysis (NCA) of the clinical data was done in the Phoenix WinNonlin software (Pharsight, Palo Alto, CA). To determine the outliers among the 24 subjects in this study, a modified Z-score test (Iglewicz and Hoaglin's robust test for multiple outliers)[16] was applied on AUC and C_{max} values. Three out of 24 subjects were shown to be potential outliers for the test.

Population PK modeling

General modeling strategy: Model development was performed step by step process using NONMEM (ICON, Ellicott City, Maryland). At first step, a model with log-transformed plasma AFQ concentration data was developed. To describe the huge difference in the AUC and C_{max} among the individuals, those with outlier AUC and C_{max} were grouped as HEG and others as NEG. CL/F and K_a were separately computed for HEG and NEG in the model. Other available covariates were tested on the base model. The final covariate model was then determined after excluding implausible covariates and only retaining those with statistical significance and clinical or physiological relevance.

Base model development: Step by step modeling strategy followed for population PK modeling is illustrated in Fig. 1. Base model was developed in a step by step manner where the best model was chosen and was taken to the next step. At step 4, different relations were tested to compute CL/F in subjects with HEG and NEG on the best model from step 3. A parameter to account for the first pass effect (FPE) separately on each group was also incorporated in this step. This was conducted by specifying F1, the parameter represents the fraction absorbed from the absorption compartment. A separate k_a was also added for HEG and NEG.

Second level of random effects in PK model parameters was tested at step 5 with model carried forward from step 4.

Covariate modeling: The covariates available from the clinical study were age, weight, height, aspartate transaminase, alanine transaminase, alkaline phosphatase, glucose, total protein, albumin, total bilirubin, cholesterol, blood urea nitrogen and creatinine level. In step 6, potentially significant covariates were selected from the plots of the covariates versus the between subject variability (BSV) of the parameter estimates. Covariates were further evaluated for statistical significance using a stepwise covariate modeling (SCM) option in PsN, which uses forward addition ($p<0.05$) and backward elimination ($p<0.01$).

Model evaluation and simulation: The predictive performance and sufficiency of the model to characterize AFQ clinical data were evaluated from the visual predictive checks (VPC). Thousand data sets were generated with the final model based on the estimated parameter values. The observed concentrations and the median, 2.5th and 97.5th percentiles along with their corresponding 95% confidence interval of the observed concentrations were plotted. The plots were stratified based on their group to evaluate the model prediction separately for HEG and NEG subjects.

The final model of AFQ was used to simulate the time course of AFQ after single oral administration of AFQ tablet in subjects with HEG and NEG, for 24 h (n = 1,000).

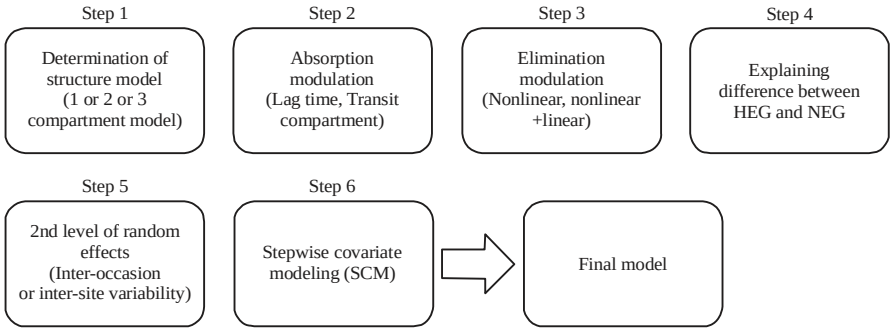

Fig. 1: Step by step modeling strategy for population PK model of AFQ

For further assessment of the AFQ exposure in clinical situations, simulations were performed with TID dose of AFQ for 7 days in subjects classified as HEG and NEG (n = 1,000).

A numeric predictive check was performed to determine the dose adjustment needed for the subjects in HEG. Comparison of average plasma concentration at the steady state C_{ss} and C_{max_ss} were done between the subjects in NEG at 20 mg AFQ with subjects in HEG at different level of dose (2-10 mg). A simulation with 1,000 subjects was done for the each dose tested for both HEG and NEG subjects. C_{ss} and C_{max} from NEG subjects were taken from 5th to 95th percentiles as a reference range to compare the difference dose of AFQ in HEG subjects.

RESULTS

Study population and pharmacokinetic data: The clinical data used for modeling incorporated 287 AFQ plasma samples from 24 healthy subjects. The concentrations that were observed ranged from 0.65-268.75 ng mL^{-1}. Only one concentration data were below the lower limit of quantification (LLOQ) and appeared at the start of the sampling period. Samples below the limit of quantification (BLQ) were excluded. As there was only one sample BLQ, it did not affect the parameter estimates and the AFQ data were unaffected by censoring.

Population pk modeling and simulations

Base model: The selected structural model was a two-compartment with first-order absorption model and random variability terms on CL/F and apparent central volume of distribution (V_2/F) parameters.

At step 4 the impact of exposure group was examined on CL/F. The model that was the best among the tested ones incorporated the separate CL/F for subjects classified as HEG and NEG and was carried forward to the next step. Additionally, inclusion of the parameter to specify the FPE and separate K_a parameter for HEG and NEG further reduced the dOFV and also improved the prediction of absorption phase in the HEG subjects. The models at step 5 were extended by the addition of inter-trial variability as a 2nd level of random effects in two ways as Between Occasion Variability (BOV) on random effects parameters and using $LEVEL to model inter-trial variability so that several subjects belong to a site. Addition of second level of random effect did not resulted in improvement of models, which suggests that the inter-site variability was not significantly larger than BSV. Therefore, no further changes were done to the model from step 4 and it was considered as the final model.

The final model included BSV on the CL/F and V_2/F. All final model parameters were reasonably precise (RSE%<38%, except V_3/F with RSE% = 55% and CL/F parameter for HEG with RSE% = 68%) and diagnostic plots showed that the model were able to describe the observed data well, with no systemic bias.

Covariate models: Besides the covariates used in base structural model, other potential significant covariate relationships assessed during covariate modeling building were age, body weight, height, cholesterol and glucose levels on both CL/F and V_2/F and aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein, albumin, total bilirubin, blood urea nitrogen and creatinine level on CL/F only. Addition of any of the covariates did not improved the model further.

Table 2 shows that, all estimated parameters were precise (except for the V_3/F and CL/F for HEG) and shrinkage of random parameters was below 12%. The goodness-of-fit plots (GOF) in Fig. 2 revealed that the data were equally dispersed about the lines with unit slope and no major systematic bias was seen. The observed versus individual predicted concentrations were closely dispersed about the identity line through the range of measured values, signifying that these data were sufficiently described by the model. Moreover, the conditional weighted residual did not show any trend over the time of observations.

Model evaluation and simulation: The visual predictive check (VPC) plots for AFQ are presented in Fig. 3. The VPC of AFQ revealed that the final model has good predictive performance for the observed plasma concentration profile. The time course of the median, 2.5th and 97.5th percentiles

Table 2: Population parameter estimates of AFQ from the final population PK model

Parameter	Population mean	RSE (%)
k_a for NEG (1 h^{-1})	0.24	26
k_a for HEG (1 h^{-1})	0.41	22
CL/F for NEG (L h^{-1})	34.20	23
CL/F for HEG (L h^{-1})	18.00	68
V_2/F (L)	71.80	46
Q/F (L h^{-1})	39.90	13
V_3/F (L)	975.00	55
BSV (CV% (shrinkage%)		
BSV-CL/F	39.10 (12.00)	33
BSV-V_2/F	81.40 (11.00)	26
Residual variability		
Proportional error (CV%)	45.30	13

k_a: Absorption rate constant, CL/F: Apparent clearance, V_2/F: Apparent central volume of distribution, Q/F: Apparent inter-compartmental clearance, V_3/F: Apparent peripheral volume of distribution, BSV: Between subject variability, CV: Coefficient of variation, RSE: Relative standard error

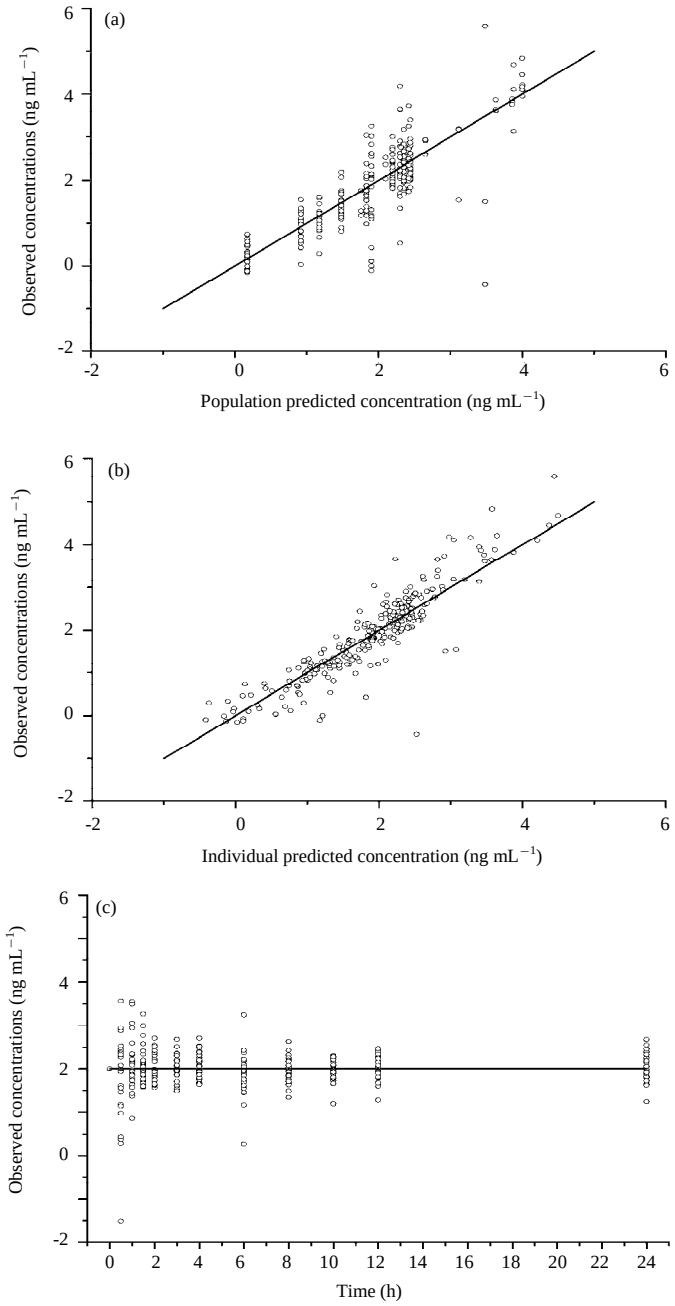

Fig. 2(a-c): Goodness-of-fit plots for the final model of AFQ (a) Observed concentrations (ng mL^{-1}) versus population predicted concentrations (ng mL^{-1}), (b) Observed concentrations (ng mL^{-1}) versus individual predicted concentrations (ng mL^{-1}) and (c) Conditional weighted residuals versus time. The solid line represents the line of unity

for the observed and 95% confidence interval (CI) of simulated-based concentrations were similar.

From the simulation with TID dose of 20 mg AFQ tablet, a significant difference in the exposure of AFQ in HEG and NEG subjects were observed. $AUC_{160\text{-}168}$ from last dosing time (160 h) till end of observation and C_{max} values in the HEG was significantly higher than that in the NEG ($p<0.05$). It can be seen in the plasma concentration versus time graph in Fig. 4 and Table 3, maximum observed concentration in NEG subjects was 35.11 ng mL^{-1}, where it was 137.35 ng mL^{-1} in HEG subjects i.e., 5 time greater in HEG subjects.

NPC (Numerical predictive check) results showed that the exposure of AFQ was very high in HEG subjects. Even at the half dose (10 mg) of AFQ, approximately only 1/3rd of the

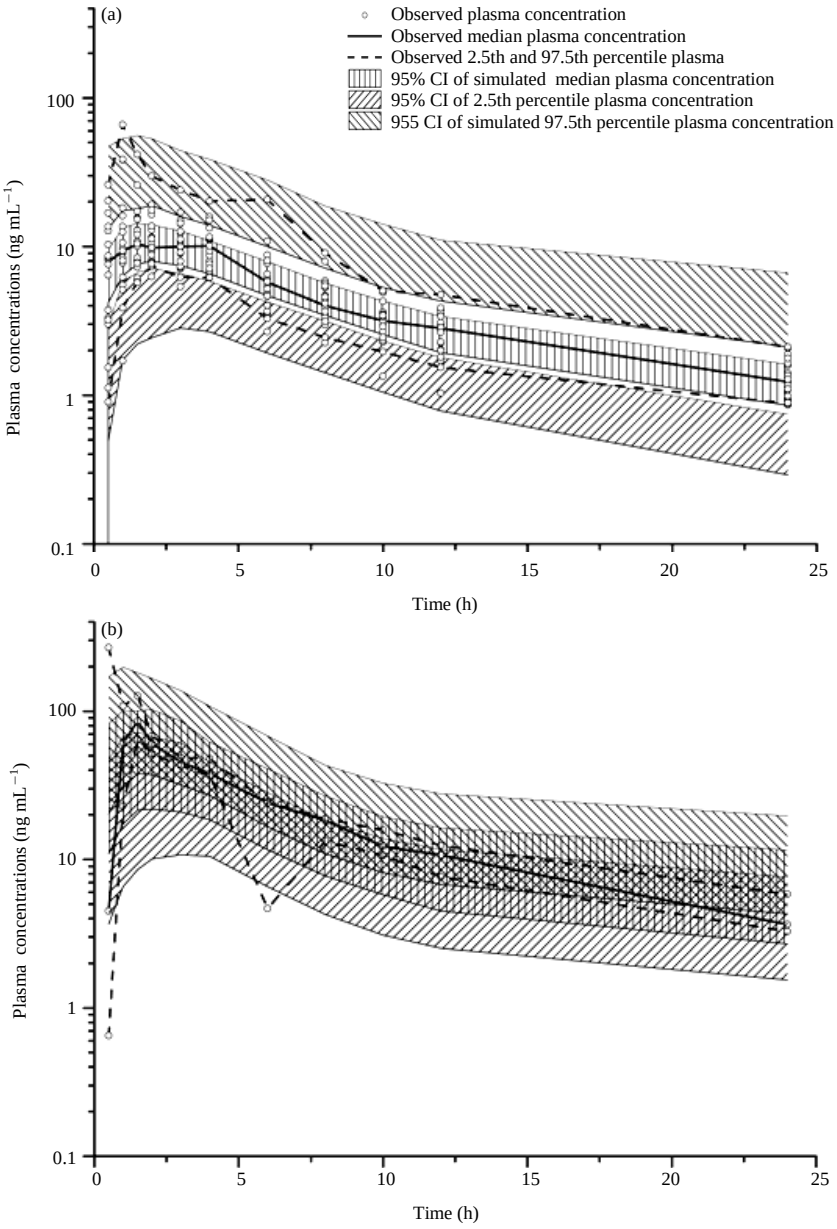

Fig. 3(a-b): Visual predictive checks of AFQ for single dose of 20 mg AFQ tablet for (a) NEG and (b) HEG subjects. Open circles represents observed AFQ concentrations. The solid line represents the median of the observed concentrations and the dashed line represents the 2.5th and 97.5th percentiles of the simulated concentrations. The region with diagonal lines represent the 95% confidence interval of the 2.5, 50 and 97.5th percentiles of the simulated concentrations

Table 3: $AUC_{160\text{-}168}$ and C_{max} computed from the population PK model after a week of 20 mg AFQ tablet TID dose in subjects with HEG and NEG

	Mean (2.5th and 97.5th percentile)	
Parameters	$AUC_{160\text{-}168}$ (ng h mL^{-1})	C_{max} (ng mL^{-1})
NEG	174.41 (74.44-322.75)	35.11 (14.03-67.99)
HEG	869.91 (402.53-1516.75)	175.35 (75.12-324.54)

$AUC_{160\text{-}168}$: AUC (Area under curve) from last dosing time (160 h) till end of observation

1000 subjects were attaining the C_{ss} and C_{max} within the reference range. The recommended dose from the NPC would be 4 mg of AFQ for subjects classified as HEG. The results of NPC are shown in Table 4.

DISCUSSION

A population PK model was developed for AFQ using single-dose oral data collected from three clinical trials with total of 24 healthy subjects. High IIV in exposure was observed among the subjects. Therefore, developing population PK

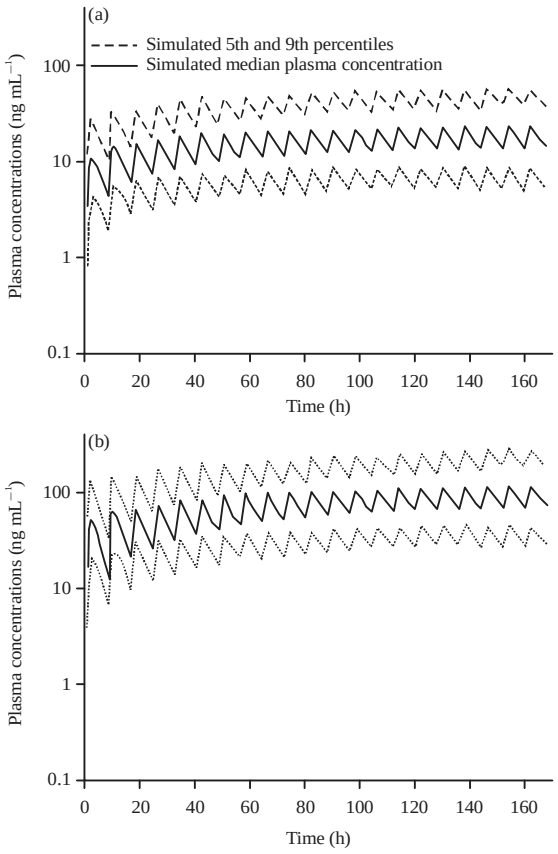

Fig. 4(a-b): Population PK model predicated AFQ concentrations after one week dose of 20 mg AFQ tablet TID in (a) NEG and (b) HEG subjects. The solid lines are the simulated median plasma concentration and dashed lines are the 5th and 95th percentiles.

Table 4: NPC results comparing C_{max} and C_{ss} from different dose of AFQ in HEG subjects

	Subjects within the reference range (%)	
Dose (mg)	C_{max} (9.17-40.37 ng mL^{-1})	C_{ss} (13.79-68.13 ng mL^{-1})
10	37.00	28.10
8	54.80	49.30
6	78.30	75.20
5	88.50	88.60
4	91.90	93.20
2	61.80	60.10

model of AFQ was performed with a focus on exploring the sources of observed high IIV in the clinical data. This study presents the first population PK model of AFQ with healthy subjects.

The final population PK model of AFQ consists of a two-compartment model with first-order oral absorption and elimination. The model used a separate elimination parameters for assumed HEG and NEG subjects, which allowed different elimination rates in 2 groups. This approach stabilized the model by accounting for source of variability in the levels of exposure and significantly reducing the BSV in CL/F and V_2/F. Further, including the parameter to account for the first pass effect in the model reduced the dOFV and improved the VPC as well.

Three Individuals which had high drug exposure than other individuals could be thought as an outlier and removed. But these individuals were classified as HEG because, since the study was conducted in healthy subjects under controlled environment, pathophysiological and food effect could not be source of high IIV.

This high variability could have occurred by many circumstances but it is assumed that this high difference between HEG and NEG caused by genetic polymorphism of metabolizing enzymes UDP-glucuronosyltransferases (UGT) 1A3 and 1A4. Previous *in vitro* studies suggested that AFQ is metabolized mainly by UGT1A4 in the liver and by UGT1A3 and 1A4 in the intestine[10]. Other studies have shown that genetic polymorphism of UGT1A3 and 1A4 have resulted in reduced glucuronidation activities. Furthermore, prevalence of UGT1A3 and 1A4 mutant variants in Asian population are reported to be <10%[17] and <12.5%[18], respectively, which approximately matches with prevalence of subjects with outlier AUC and C_{max} (3 out of 24 i.e., 12.5%) in our study sample. All these facts indicate toward the involvement of genetic polymorphism of metabolizing enzymes as a source of observed high exposure in HEG.

The present model provides strong evidence that the there was significant difference in the exposure of AFQ between 2 group which could be caused by polymorphism of UGT. From the simulation result of 1,000 subjects it was observed that the AUC_{0-24} and C_{max} are significantly different between the NEG and HEG subjects indicating the requirement of dosage adjustment. In clinical use, this difference is more prominent as the AFQ is used at TID dose for a week or more. Both $AUC_{160-168}$ and C_{max} after the last dosing time till the end of observation increases by 5 times in subjects in HEG. Extremely higher concentration observed by the end of 1 week dosing (175.35 ng mL^{-1}) in HEG subjects compared to maximum concentration observed in NEG subjects (35.11 ng mL^{-1}) can potentially lead to severe side effects. According to the NPC results, dose of AFQ was required to be reduced from 20-4 mg for the subjects having HEG to have C_{max} comparable to that of NEG.

The model presented here is suitable for the purpose of confirming the high difference of AFQ between 2 groups which assumed having genetic polymorphism of UGT.

Therefore, screening of the polymorphism of UGT should be performed in the studied subjects. Moreover, assessment of UGT1A3 polymorphism and determination of FPE caused by UGT1A3 as well as UGT1A4 are warranted for further refining the final model. Subsequently after this studies, the presented model can be refined to determine dose adjustment required for the patients with UGT1A4 and UGT1A3 polymorphism. This will help in preventing severe skin reactions such as dermatitis and pruritus and photosensitization that leads to discontinuation of the use of AFQ.

However, one should be careful in applying outcomes of the present study in clinical use of AFQ because of the lack of real pharmacogenetic data in this study, high variability in kinetics and inherent BSV in AFQ absorption as well as disposition. Another limitation of the present analysis is that the study was done on healthy subjects with normal physiological conditions but in clinical situations there may be patients with wide range of difference in physiological conditions and diseases affecting the absorption and disposition of AFQ. Therefore, at present the utility of present model cannot be justified for clinical use for dose adjustment of the AFQ in patients with any of medical conditions.

CONCLUSION

This study successfully developed the population pharmacokinetics model of AFQ and explained the big difference of AFQ exposure in some subjects and suggested an appropriate dose of AFQ in HEG which could have possibility of genetic polymorphism.

SIGNIFICANCE STATEMENT

This study discovered the possibility of UGT genetic polymorphism which can highly affect the total exposure of AFQ. This will help the researcher to uncover the critical areas of AFQ adverse effect and high exposure in some subjects that many researchers were not able to explore. Thus, a new theory on UGT polymorphism effect on AFQ may be arrived at.

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REFERENCES

1. Inoue, I., T. Oine, Y. Yamada, J. Tani, R. Ishida and T. Ochiai, 1976. 2-Fluoromethyl-3-o-tolyl-6-amino-4(3H)-quinazolinone. US Patent 3966731. https://www.google.com/patents/US3966731
2. Ochiai, T. and R. Ishida, 1982. Pharmacological studies on 6-amino-2-fluoromethyl-3-(O-tolyl)-4(3H)-quinazolinone (afloqualone), a new centrally acting muscle relaxant. (II) Effects on the spinal reflex potential and the rigidity. Jpn. J. Pharmacol., 32: 427-438.
3. Kojima, M., Y. Kudo and R. Ishida, 1981. [Effects of afloqualone, a centrally acting muscle relaxant, on the sleep-wakefulness cycle in cats with chronically implanted electrodes]. Nihon Yakurigaku Zasshi, 78: 471-482, (In Japanese).
4. Yamamura, M., T. Ochiai and R. Ishida, 1981. [Effects of afloqualone, a new centrally acting muscle relaxant, on DRL response and CER in rats]. Nihon Yakurigaku Zasshi, 78: 381-392, (In Japanese).
5. Hur, G.Y., E.K. Hwang, J.Y. Moon, Y.M. Ye, J.J. Shim, H.S. Park and K.H. Kang, 2012. Oral muscle relaxant may induce immediate allergic reactions. Yonsei Med. J., 53: 863-865.
6. Takeshi, I., K. Ryoichi and N. Michihito, 1994. Photoleukomelanodermatitis (Kobori) induced by afloqualone. J. Dermatol., 21: 430-433.
7. Takeuchi, A., Y. Kuroki, F. Iitsuka and Y. Toshihar, 2011. [Survey of the current conditions in acute poisoning due to antispasmodic agents-special reference to eperisone hydrochloride and afloqualone]. Chudoku Kenkyu, 24: 259-264, (In Japanese).
8. Zuba, E.B., S. Koronowska, A. Osmola-Mankowska and D. Jenerowicz, 2016. Drug-induced photosensitivity. Acta Dermatovenerol. Croat., 24: 55-64.
9. Tokura, Y., M. Ogai, H. Yagi and M. Takigawa, 1994. Afloqualone photosensitivity. Immunogenicity of afloqualone-photomodified epidermal cells. Photochem. Photobiol., 60: 262-267.
10. Kaji, H. and T. Kume, 2005. Characterization of afloqualone N-glucuronidation: Species differences and identification of human UDP-glucuronosyltransferase isoform(s). Drug Metab. Dispos., 33: 60-67.
11. Minezo, O., S. Furuuchi, S. Usuki, S. Nitta and S. Harigaya, 1983. Metabolism of afloqualone, a new centrally acting muscle relaxant, in monkeys and dogs. J. Pharmacobiodyn., 6: 708-720.

12. Minezo, O., T. Kurozumi, S. Furuuchi, S. Usuki, K. Kotera and S. Harigaya, 1983. Metabolism of afloqualone, a new centrally acting muscle relaxant, in the rat. Chem. Pharm. Bull., 31: 2438-2450.
13. ICH Harmonised Tripartite Guideline, 1996. Guideline for good clinical practice E6(R1). ICH Harmonised Tripartite Guideline, pp: 1-53. https://www.ich.org/fileadmin/ Public_ Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf
14. Spiegel, R., 2003. Psychopharmacology: An Introduction. 4th Edn., John Wiley and Sons, New York, ISBN: 9780470869468, pp: 3-4.
15. Yun, H.Y., S.P. Lee, H.H. Jeong, Y.R. Yoon and S.J. Sohn *et al*., 2007. Determination of afloqualone in human plasma using liquid chromatography/tandem mass spectrometry: Application to pharmacokinetic studies in humans. Talanta, 73: 635-643.
16. Lglewicz, B. and H.C. David, 1993. How to Detect and Handle Outliers. ASQC Quality Press, USA., ISBN: 9780873892476, Pages: 87.
17. Chen, Y., S. Chen, X. Li, X. Wang and S. Zeng, 2006. Genetic variants of human UGT1A3: Functional characterization and frequency distribution in a Chinese Han population. Drug Metab. Dispos., 34: 1462-1467.
18. Saeki, M., Y. Saito, H. Jinno, K. Sai and A. Hachisuka *et al*., 2005. Genetic variations and haplotypes of UGT1A4 in a Japanese population. Drug Metab. Pharmacokinet., 20: 144-151.