Effect of African potato (Hypoxis hemerocallidea) on the pharmacokinetics of efavirenz

Seloi Mogatle, Michael Skinner, Edward Mills, Isadore Kanfer

**Purpose.** The purpose of this study was to evaluate the effect of the African potato (AP) on the pharmacokinetics of efavirenz.

**Methods.** A single-dose, two-phase sequential study was conducted over 31 days in 10 healthy volunteers. On day 1 of the study, volunteers were administered a 600 mg efavirenz tablet, and blood samples were collected before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 36 and 48 hours after dosing. From day 16, a traditionally prepared AP decoction was administered daily until day 30. On day 29, volunteers were administered a single 600 mg dose of efavirenz, as was done on day 1. Plasma samples were harvested immediately after blood sample collection and frozen at –80ºC until assayed. Plasma concentrations of efavirenz were determined by a validated high performance liquid chromatography (HPLC) method with UV detection, and pharmacokinetic parameters were calculated. Geometric mean ratios of $C_{\text{max}}$ and $AUC_{0-48}$ of efavirenz before and after co-administration of 14 successive daily doses of AP were compared.

**Results.** All subjects completed the study. The geometric mean ratios of $C_{\text{max}}$ and $AUC_{0-48}$ were 97.30 and 102.82 with corresponding 90% confidence intervals (CIs) of 78.81 - 120.14 and 89.04 - 118.80, respectively.

**Conclusion.** Pharmacokinetic data generated during this study indicated that AP did not significantly alter the pharmacokinetics of efavirenz. Hence, co-administration of AP is unlikely to affect the clinical usage of efavirenz.

The use of natural health products (NHPs) as traditional medicines (TMs) among people living with HIV/AIDS is widespread, although their effects on the pharmacokinetics of antiretroviral medicines (ARVs) have not been established. The African potato (AP) received strong support from the former Minister of Health for use as an immune booster for HIV-positive patients, and is purported to be one of the best-selling NHPs in South Africa. Those taking TMs believe that they are effective and have few or no side-effects compared with current ARVs. The use of TMs is high among indigenous people in South Africa, with 33.5% of those living with HIV/AIDS using them to manage HIV symptoms. Patients receiving ARVs commonly take NHPs concurrently with their therapy despite there being little or no published information on the effectiveness and possibility of inter-interactions. In the Eastern Cape province of South Africa, the highest selling NHP was AP, at 11 tons/year. The corn of the AP is traditionally used for the treatment of urinary infections among people living with AIDS, 64% of those who use TMs, use the AP. In vitro and in vivo data have suggested that the AP has immune-boosting properties.

Concomitant use of NHPs with orthodox medicines may affect the pharmacokinetics and pharmacodynamics through drug interaction mechanisms. NHPs can affect CYP450 enzymes and result in an increase or decrease in plasma concentrations of substrates which are metabolised by these enzyme systems. Possible effects of co-administration of NHPs and ARVs include increased side-effects of ARVs and losing the control of viral replication, which could lead to non-compliance and therapeutic failure, respectively.

Hypoxoside, the main component of the AP, is not absorbed through the gut but is extensively metabolised in the colon by β-glucosidase to rooperol, which is then absorbed into the bloodstream and undergoes further metabolism to glucuronides and sulphates. In vitro studies have shown that hypoxoside induces the transporter protein, p-glycoprotein (P-gp) in Caco-2 cells, and stigmasterol, which is a constituent of AP, and rooperol both exhibited high inhibition of CYP3A4, 3A5 and CYP19 enzymes. Ingestion of the AP may therefore have the potential to affect both transporters and enzymes and consequently interfere with the absorption and metabolism of concurrently administered therapeutic agents.

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which is effective against HIV-1 infection and is the backbone of highly active antiretroviral therapy (HAART).
in South Africa. Efavirenz is a substrate of both CYP3A4 and CYP2B6, and the metabolites are 7-hydroxyefavirenz, 8-hydroxyefavirenz and 8,14-dihydroxyefavirenz. It has a long half-life (of 52 - 76 hours) when administered as a single dose and 40 - 55 hours after multiple doses. The reduced half-life after multiple doses is due to metabolic auto-induction. Efavirenz is not a substrate of P-gp21 and therefore the absorption of efavirenz is unlikely to be affected by inducers or inhibitors of P-gp.

Since efavirenz and AP have been shown to be CYP450 enzyme substrates, the possibility exists of interaction between them when simultaneously administered. Reports have indicated that AP inhibits CYP3A4 in vitro. However, to our knowledge there is no published information that this occurs in vivo. We therefore investigated whether the concurrent administration of AP would affect the pharmacokinetics and subsequent clinical performance of efavirenz in human subjects.

**Materials and methods**

The HPLC system consisted of a Waters model 515 HPLC pump, a model 715 autosampler and a model 2995 PDA UV detector (Waters, Milford, MA, USA). Efavirenz was donated by Aspen Pharmacare (Port Elizabeth) and diclofenac sodium (DIC) was purchased from Sigma-Aldrich (Johannesburg). Acetonitrile and methanol of HPLC grade were purchased from Aspen Pharmacare (Port Elizabeth) and diclofenac sodium (DIC) was purchased from Sigma-Aldrich (Johannesburg). Water was purified by reverse osmosis and filtration through a Milli-Q purification system (Millipore, Milford, MA, USA). Human plasma with potassium EDTA as the anticoagulant (K-EDTA) as an anticoagulant was obtained from South African National Blood Services, Port Elizabeth.

**Study population**

Ten healthy, non-smoking, HIV-negative male subjects were enrolled after giving informed consent. Eligibility criteria included age between 18 and 55 years and a body mass index (BMI) between 19 and 30 kg/m². Pre-study medical screening was performed within a month of initiating the study, and volunteers who passed the physical, medical and laboratory screening tests were enrolled. Laboratory tests included tests for liver function, hepatitis B and C, HIV, blood biochemistry, urinalysis and drugs of abuse (including amphetamines, barbiturates, benzodiazepines, cocaine, methamphetamine, morphine, phencyclidine, THC and TCA).

Prescription and over-the-counter medicines were restricted from 1 week before each phase until the last blood sample had been taken at the end of the study. Consumption of alcohol was forbidden from 4 days before the study, and caffeine and grapefruit juice were restricted from 48 hours before the study. Volunteers were prohibited from strenuous exercise from 24 hours before the study.

**Study design**

Ethical approval was granted by the Rhodes University Ethical Standards Committee; the study was conducted according to the South African Good Clinical Trials guidelines and Declaration of Helsinki and its amendments.

A single-dose, two-phase sequential study was conducted in healthy male volunteers under fasting conditions. Phase 1 started on day 1 and phase 2 started on day 29, each phase lasting 3 days. The washout period between the administration of the efavirenz doses was 28 days. The night before the study (day 0) volunteers were checked into the clinic and tested for drugs of abuse and alcohol consumption and questioned to ensure that they had complied with the study restrictions. Volunteers fasted for 10 hours before they were administered a 600 mg efavirenz tablet with a 240 ml glass of water. Standard meals were provided until 24 hours after dosing, and the times at which meals were started and finished were recorded as well as the amounts consumed. Blood samples were collected in Vacutainers containing potassium EDTA as the anticoagulant at the following time intervals: before dosing (0) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 36 and 48 hours after dosing.

From day 16 until day 30 of the study, volunteers were administered a freshly prepared traditional AP decoction, at a dose of 15 mg/kg/day of hypoxoside, at the same time daily for 14 consecutive days. The AP decoction was prepared and assayed for hypoxoside content prior to administration. After receiving 12 consecutive daily doses of the AP decoction, phase 2 was initiated on day 28, when each of the volunteers received a 600 mg dose of efavirenz as at the beginning of the study. Blood samples were collected at the same time intervals as in phase 1.

Blood samples were centrifuged at 2 800 rpm for 10 minutes at 4°C, and plasma was harvested into two 2 ml polypropylene tubes (original and duplicate) and stored at –80°C until assayed.

**Safety and tolerability**

Baseline laboratory and medical data were documented before the study. During the study, volunteers were monitored for any adverse events, which were documented. Blood pressure, pulse and body temperature were taken before and 4 hours after dosing with efavirenz. During the study, volunteers were asked open questions about their wellbeing. Post-study laboratory and medical tests were conducted within 4 days of the end of phase 2 and the results compared with the baseline data.

**Preparation and analysis of AP**

The AP decoction was prepared in the traditional manner as follows: After removing the roots, the corms (~250 g) were washed with clean water, grated and weighed, boiled in 2.5 litres of water for 45 minutes, allowed to cool, and then mixed and strained with a clean muslin cloth before analysis. Two
10 ml samples of the AP preparation were placed in 20 ml Kimax centrifuge tubes and centrifuged in an IEC HN-SII centrifuge (Damon/IEC Division, Needham HTS, Massachusetts, USA) at 3 000 rpm for 10 minutes. The supernatant was filtered using a 0.44 µm filter and the internal standard, sulphasemizine, was added to the mixture, which was diluted by a factor of 500 and re-filtered. The samples were analysed according to a method reported by Nair.23

Analysis of plasma samples
Plasma samples were analysed by a validated HPLC method, and separation was achieved on a Phenomenex Luna C_{8}(2) (5 µm, 150 x 5 mm i.d.) column maintained at a temperature of 40±2°C. The mobile phase consisted of acetonitrile: 0.1M formic acid (pH 2.30): methanol (52:43:5 v/v/v) at a flow rate of 0.3 ml/min. The injection volume was 10 µl and the observed run time was less than 9 min. The eluent was monitored at λ = 247 nm and 275 nm for efavirenz and the internal standard, diclofenac sodium (DIC) respectively, using a photodiode array (PDA) detector.

An aliquot of 100 µl of plasma was precipitated with 200 µl DIC in mobile phase, the mixture was vortexed for 50 seconds and then centrifuged for 10 minutes at 13 500 rpm (10 000 g). About 100 µl of the supernatant was transferred to a micro-insert and injected into the HPLC system.

Pharmacokinetic analysis
The pharmacokinetic parameters of efavirenz before and after co-administration with AP were determined using a non-compartmental model.24 Exposure measures such as area under the curve (AUC) of the plasma concentration-time profiles from 0 hours to 48 hours (AUC_{0-48}), and peak plasma EVF concentrations (C_{max}) were used to assess the effect of AP on the pharmacokinetics of efavirenz. Other parameters that were monitored included the elimination half-life (t_{1/2}), time taken to reach peak plasma concentration (t_{max}) and the elimination rate constant (k). The AUC_{0-48} and C_{max} were calculated and the trapezoidal rule was used to estimate AUC_{0-48}.

Statistical analysis
ANOVA analysis was conducted on C_{max} and AUC_{0-48} using log-transformed data and the geometric mean ratios were calculated. The two one-sided t-test was used to determine the respective 90% CIs. An interaction would be concluded if the 90% CIs for C_{max} and /or AUC_{0-48} were found to be outside the limits of 80 - 125%.25

Results
Demographic characteristics
Of the 10 enrolled healthy male volunteers, 9 (90%) were black and 1 (10%) was white; their mean age was 23 (range 19 - 27 years) and their BMIs ranged from 19.42 to 27.90 kg/m.² All volunteers completed the study.

Pharmacokinetics of efavirenz
The mean plasma concentration/time profiles of efavirenz alone (phase 1) and efavirenz co-administered with AP (phase 2) are shown in Fig. 1. Visual inspection of the profiles suggests that plasma concentrations of efavirenz were not significantly affected by co-administration of AP. The geometric mean ratios of C_{max} and AUC_{0-48} were 97.30 and 102.82, and the corresponding 90% CIs were 78.81 - 120.14 and 89.04 - 118.80, respectively. Whereas the 90% CI of the C_{max} fell slightly outside the lower limit (78.81), the corresponding AUC_{0-48} values were within the limits of 80 - 125%. Hence, co-administration of AP with efavirenz did not appear to affect the pharmacokinetics of efavirenz. Table I summarises the pharmacokinetic parameters.

Safety and tolerability
Laboratory tests for all volunteers were normal; no serious adverse effects were reported or noted during the study. However, 2 subjects reported dizziness, euphoria and sleepiness during phase 2; they had peak plasma efavirenz concentrations of 3.26 and 3.34 µg/ml, respectively, while the other volunteers had peak plasma concentrations <3.05 µg/ml.

Discussion
Although the 90% CIs for C_{max} did not fall within the 80 - 125% interval, the acceptance limits for this parameter are generally considered to be less important than the AUC. Hence, the findings from this study suggest that interactions as a result of co-administration of AP and efavirenz are unlikely. There was no significant change in the measured pharmacokinetic parameters of efavirenz when administered alone or concomitantly with AP.

In vitro hypoxoside inhibits CYP3A4 enzymes and also induces P-gp.16 This differs in vivo since hypoxoside is rapidly metabolised to rooperol following oral administration in
isolated from Hypoxis and South Africa, respectively, and to our knowledge, this is the first report of the isolation of hypoxoside from Hypoxis latifolia. Hypoxis latifolia is native to South Africa and is renowned for its traditional medicinal use in the treatment of cancer and other diseases. Hypoxis latifolia contains various hypoxoside analogues, including hypoxoside, which is one of the major metabolites in the plant. In this study, we aimed to investigate the pharmacokinetic and pharmacodynamic properties of hypoxoside and its analogues in vivo to determine their potential as therapeutic agents. Our findings indicate that hypoxoside has promising therapeutic potential in the treatment of various diseases, including cancer and viral infections. Further studies are needed to investigate the safety and efficacy of hypoxoside in clinical trials.


