Pharmacokinetics of Caffeine in the Lens Capsule/Epithelium After Peroral Intake: A Pilot Randomized Controlled Study

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Purpose. To determine the pharmacokinetics of perorally administered caffeine, a widely consumed and potent dietary antioxidant, in the anterior lens capsule and lens epithelial cells, a crucial cell monolayer for cataract development.

Methods. Bilateral cataract patients were scheduled for cataract surgery with a caffeine abstinence of 1 week before surgery of each eye. At the day of surgery of the second eye patients were administered no drink (0-mg group) or coffee with 60-, 120-, or 180-mg caffeine. After capsulorhexis the lens capsule including lens epithelial cells was transferred to a test tube for analysis of caffeine concentration by gas chromatography–mass spectrometry (GC–MS/MS).

Results. Coffee consumption significantly (P < 0.05) increased caffeine levels of the lens capsule/epithelium in the 60-, 120-, and 180-mg group. Caffeine concentrations (caffeine ng/lens capsule/epithelium) measured as difference between 1st and 2nd eye were −0.52 ± 1.16 (0-mg group, n = 7), 1.88 ± 2.02 (60-mg group, n = 8), 2.09 ± 0.67 (120-mg group, n = 9), and 3.68 ± 1.86 (180-mg group, n = 9). The increase constant of caffeine in a linear regression model was estimated as a 95% CI 0.02 ± 0.0046 (degrees of freedom; 25; r = 0.85).

Conclusions. Peroral intake of coffee significantly increased caffeine concentrations in the lens capsule and lens epithelial cells in a dose-dependent manner. This information is important for further investigations on preventing cataract.

Keywords: caffeine, cataract, lens epithelium, lens capsule...
Caffeine is consumed worldwide, most often in form of coffee, tea, or chocolate-based food products. Interestingly, in coffee other antioxidants such as chlorogenic acids are destroyed during roasting of the raw beans. The reported average intake of caffeine in the United States is 200 mg/d in 80% of adults corresponding to the amount in two 5-oz cups of coffee or four sodas. Despite the evidence of an actogenetic effect of caffeine and its worldwide consumption, little is known about the pharmacokinetics of caffeine in the human lens after peroral intake. The present study aims to investigate if peroral caffeine intake leads to caffeine accumulation in human lens capsule and lens epithelial cells.

**Methods**

**Ethics Statement**

The local Ethics Committee of the city of Vienna approved this clinical prospective randomized controlled pilot study (protocol number: EK-15-204-1215). Research adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with the European Union - Good Clinical Practice (EU-GCP) and European Union - Good Laboratory Practice (EU-GLP). Written informed consent was obtained from all participants prior to enrollment at the Hanusch Hospital.

**Subjects**

The participants were recruited at the Hanusch Hospital, Vienna, outpatient clinic. Prestudy screening included an ophthalmologic examination with the slit lamp, visual acuity testing (Snellen), biometry (IOLMaster 700; Carl Zeiss Meditec AG, Oberkochen, Germany) of both eyes and blood pressure measurement. Additionally, patients were asked for their caffeine habits and the last caffeine intake was recorded. Inclusion criteria included bilateral cataract, patients older than 21 years, patients who chose sedoanalgesia technique for cataract surgery before the recruitment process, and written informed consent prior to surgery. Exclusion criteria included consumption of beverages containing caffeine (such as coffee, Coca Cola, energy drinks, black or green tea) and dark chocolate within 1 week before surgery of each eye, pseudoxfoliation syndrome of the lens, systolic hypertension of more than 160 mm Hg at the day of surgery, and pregnancy. Types of cataract were not recorded.

**Study Design**

This is a prospective randomized, controlled, observer-blinded pilot study. Caffeine levels in human capsule and adherent lens epithelial cells were determined after peroral caffeine intake. All patients were randomly divided into three caffeine dosage groups and one control group (0-mg group). The randomization was performed in a 1:1:1:1 fashion to one of four groups using minimization for age (1 = 21–65 years; 2 = 66–75 years; 3 = 76–105 years), sex (1 = female; 2 = male) and weight (1 = 65 kg; 2 = 65 to 80 kg, 3 = 80 kg).

The interval between cataract surgery of the first eye and contralateral second eye was 1 week for all groups. In all groups the first eye was operated without caffeine consumption and kept as control. Coffee was then given in 60-, 120-, and 180-mg caffeine equivalents on the cataract surgery day of the second contralateral eye just before the surgery, respectively. The control group was kept caffeine abstinent for both eyes. The cataract surgeon and caffeine analysis lab were blinded and not informed about group allocation of the patient.

**Study Procedure**

One to three standardized cups with 40-mL espresso (i.e., 60 mg of caffeine, were given to the patient precataract surgery except for the control group and the contralateral eye). All patients received sedoanalgesia and were monitored for heart activity, blood pressure, and oxygen levels in the blood. Standby anesthesia was provided. To harvest the anterior lens capsule and adherent lens epithelial cells main incision and paracenteses were performed in a standardized way. Then, the anterior chamber was filled with ophthalmic viscosurgical device (OVD) and a capsulorhexis (aimed for 5.5 mm) was performed. Before continuing cataract surgery the lens capsule and adherent lens epithelial cells were taken with a forceps from the anterior chamber. Immediately after this procedure, the anterior lens capsule and adherent lens epithelial cells were transferred to sealed glass tubes and stored at 4°C prior to analysis. Each lens capsule and adherent lens epithelial cells were extracted with 0.25 mL of dichloromethane, evaporated to dryness, and dissolved in 0.05 mL of ethyl acetate. Four microliters were injected into a gas chromatography–mass spectrometry (GC–MS/MS) system, which consisted of a 7890B gas chromatograph coupled with a 7000C triple quad mass spectrometer (Agilent, Santa Clara, CA, USA). An autosampler AS 7693 was used for pulsed splitless injections onto a HP-5ms Ultra Inert capillary column (50 m, 0.25 mm internal diameter
(ID), film thickness 0.5 μm; Agilent). The injector temperature was set to 280°C, the carrier gas was helium at a flow rate of 1.6 mL/min. The oven temperature was set to 160°C, held at this temperature for 2 minutes, heated at a rate of 30°C/min to 230°C, held for 2 minutes, followed by a rate of 20°C/min to 290°C, and held for 5.7 minutes. The transfer line temperature was set to 300°C. After electron impact (EI)-ionization the mass spectrometer was operated in multiple reaction monitoring (MRM)-mode with a transition 194.0 to 109.0 m/z as quantifier and two further transitions as qualifiers. The whole procedure could achieve a limit of detection of 0.1-ng caffeine per lens capsule/epithelium. The reference standard for caffeine in this study was purchased from Fluka-Honeywell International, Inc. (Morristown, NJ, USA).

Statistical Parameters
The significance level and the confidence coefficients were set to 0.05 and 0.95, respectively, considering the sample size and the expected contrasts. Statistical analysis was performed with Microsoft Excel 2016 (Redmond, WA, USA), a StatPlus add-on for Excel and SPSS 25.0 (IBM, Armonk, NY, USA). Missing data were excluded from analysis.

RESULTS
Altogether, 80 eyes of 40 patients were recruited for the study. The first two patients had to be excluded because of labeling errors related to the test tubes. One patient was lost to follow-up. Four more patients were excluded because of protocol noncompliance. In total, 66 lens capsules including adherent lens epithelial cells taken from 33 patients were analyzed. Table 1 summarizes the characteristics of our study population. Time interval between coffee consumption and surgery is given in Table 2. Representative GC–MS/MS chromatograms from lens capsule/epithelium are shown in Figure 1.

Caffeine concentrations were normally distributed in all groups (Kolmogorov-Smirnov-test, $P = 0.2$) except for the 0-mg group (Kolmogorov-Smirnov-test, $P = 0.02$). Thus, nonparametric testing was used when comparing all four groups.

**FIGURE 1.** Total ion current chromatograms of extracts from a capsule/epithelium without caffeine (lower chromatogram) and from a capsule/epithelium with 1.15 ng of caffeine (upper chromatogram) at 4.5 minutes.

**FIGURE 2.** Lens capsule including adherent lens epithelial cells caffeine concentration in ipsilateral (=1st eye) and contralateral (=2nd eye) eye after peroral caffeine application plotted in a Tukey boxplot (x = extreme outlier, * = mild outlier; * = significance: $P < 0.05$, Wilcoxon test for paired samples).
Peroral caffeine intake (60, 120, and 180 mg) significantly increased caffeine levels in lens capsules including adherent lens epithelial cells, each $P < 0.05$, Wilcoxon tests for paired samples (Fig. 2). No difference of caffeine levels in the 0-mg caffeine group between ipsilateral and contralateral sample was found, $P = 0.29$, Wilcoxon test for paired samples (Fig. 2).

Overall, caffeine intake increased caffeine levels in the sample as indicated by a 95% CI for caffeine concentration between ipsilateral and contralateral sample ($-2.51 \pm 0.75$, degrees of freedom, 25). The amount of caffeine (ipsilateral minus contralateral sample) detected is displayed in Table 3 and plotted in Figure 3.

ANOVA analysis showed no difference between the three groups regarding time interval between coffee intake and surgery ($P = 0.47$). Significant differences in caffeine levels were observed between the four groups (Kruskal-Wallis test, $P < 0.01$). Contrasts of differences (ipsilateral minus contralateral sample) between groups were compared with orthogonal double-sided Mann-Whitney U tests according to the strategy in Table 4.

On the assumption of a linear increase of caffeine concentration, $C$ (caffeine ng/lens capsule/epithelium), with dose, $D$ (mg), with the increase constant, $k$, the caffeine concentration is expected to be 0 caffeine ng/lens capsule/epithelium at 0 mg peroral intake (Equation 1).

$$C = k \cdot D \quad (I)$$

The increase, $k$, was estimated as a 95% CI 0.02 $\pm$ 0.0046 (degrees of freedom, 25; $r = 0.85$).

### DISCUSSION

This study was designed to elucidate the pharmacokinetics of caffeine in the lens capsule and adherent lens epithelial cells after peroral intake. The accumulation of caffeine in the lens capsule and adherent lens epithelial cells was analyzed quantitatively. Moreover, the study provides information on the correlation between the amount of caffeine consumed and concentration achieved in the lens capsule including adherent lens epithelial cells.

In our study, participants were asked to drink coffee for caffeine pharmacokinetic analysis in order to simulate a common real world scenario. Peroral caffeine intake was defined as drinking one to three cups of coffee containing 60- to 180-mg caffeine. The US Food and Drug Administration advises a limit of 600 mg (4–7 cups of coffee) of caffeine per day. Taking into account that 80% of adults consume approximately 200-mg caffeine per day, the maximum dose of 180-mg caffeine in this study was considered as safe. We did not administer decaffeinated coffee for the control eye and control group because decaffeinated coffee might still contain some caffeine. Caffeine abstinence of 1 week before surgery of each eye was chosen because it is known from topical caffeine application that after a quick accumulation in the lens the washout phase of caffeine is slow. All our study participants had been drinking at least one coffee a day on a regular basis before inclusion in this study (Table 1). It is open to speculation whether caffeine levels in the 0-mg group and control eyes (Fig. 2) were the remains of caffeine levels before the study inclusion. Another explanation could be caffeine from other sources not known by the patient or simply noncompliance.

Time intervals in the study between administration of coffee and surgery varied with extreme outliers. The shortest interval in our study was 12 minutes in the 180-mg group (Table 2). Even at this short time interval, caffeine was detected in the lens capsule/epithelium sample. This suggests a very rapid accumulation of perorally taken caffeine in the lens. Our findings are supported by the fact that up to 99% of caffeine is gastrointestinally absorbed and that after peroral ingestion in...
Pharmacokinetics of Caffeine in the Lens

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