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

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Caffeine (coffee) is in the spotlight of preventing the onset of cataract. Several studies, experimentally and epidemiologically, have shown that caffeine inhibits cataract development.1–11 Varma et al.11 found that the incidence of cataract blindness in humans was significantly lower in groups consuming higher amounts of coffee in comparison to the groups with lower coffee intake. Moreover, Rautianen et al.7 showed that the dietary total antioxidant capacity including coffee of middle-aged and elderly women was inversely associated with the risk of age-dependent cataract.

One of the major risk factors for age-dependent cataract development is ultraviolet radiation B (UVR-B).12 UVR-B radiation is absorbed by proteins in the lens epithelium and underlying lens fibers damaging those structures by the photochemical formation of reactive oxygen species (ROS),13,14 including superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. The lens epithelium is crucial for keeping the lens transparent.15 As a consequence of inflicting the lens epithelium by oxidative stress, lens transparency is disturbed leading to cataract.16 The importance of oxidative stress has been demonstrated in vivo and in the protective effect of ROS scavengers against cataract formation in experimental animal models,17–21 as well as in human studies with antioxidant vitamins.22–25 Thus, the absence or presence of antioxidant agents significantly modulate cataractogenesis.

Caffeine effectively scavenges ROS.26–28 An electron spin resonance study revealed that the antioxidant effect of caffeine was similar to glutathione and significantly higher than that of ascorbic acid.28 These data are supported by our recent study on the protective effect of topically applied caffeine.6 To compare candidate molecules for prevention of experimentally UVR-B induced cataract, we developed the concept of the protection factor (PF).29 The PF is identical to the PF of sunscreens: the ratio between the threshold dose of toxic agent in vivo and the threshold dose without the toxic agent in vivo. The PF observed for caffeine (PF: 1.23)6 is higher than the PF for perorally administered vitamin E (PF: 1.14) (Soderberg PG, et al. IOVS 2012;53:ARVO E-Abstract 2283) and vitamin C (PF: 1.0).30 Only the glutaredoxin gene provides a higher PF (PF: 1.3).29 Thus, caffeine gives a better protection against cataract formation than candidate molecules vitamins E and C.

Almost all of perorally administered caffeine is absorbed gastrointestinally.31,32 Peak plasma levels of caffeine in blood are detected 15 to 120 minutes after peroral consumption.33 Its chemical properties allow caffeine to pass the blood–brain barrier and all biological membranes.34 Caffeine is metabolized in the liver by hepatic enzymes belonging to the cytochrome P-
The antioxidant activities of paraxanthine, theobromine, theophylline, and 1-methylxanthine are comparable to that of caffeine and the activity of 1-methyluric acid is actually higher than that of caffeine. Thus, caffeine keeps its antioxidant activity when metabolized.

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 anticyataractogenic 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. 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.

**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 abstenient 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. Stand-by 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 mass spectrometer (Agilent, Santa Clara, CA, USA). An autosampler AS 7695 was used for pulsed splitless injections onto a HP-5ms Ultra Inert capillary column (50 m, 0.25 mm internal diameter.

**Table 1.** Characteristics of Study Population

<table>
<thead>
<tr>
<th>Caffeine Dose Group</th>
<th>0 mg</th>
<th>60 mg</th>
<th>120 mg</th>
<th>180 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Age ± SD, y</td>
<td>72.6 ± 5.6</td>
<td>78 ± 4.6</td>
<td>75.1 ± 10.2</td>
<td>73 ± 6.9</td>
</tr>
<tr>
<td>Weight ± SD, kg</td>
<td>77.6 ± 12.3</td>
<td>78.4 ± 8.9</td>
<td>79.8 ± 15</td>
<td>79.2 ± 10.9</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Average cups of coffee/day ± SD</td>
<td>1.6 ± 0.5</td>
<td>2.3 ± 0.7</td>
<td>1.9 ± 1.1</td>
<td>1.9 ± 0.8</td>
</tr>
</tbody>
</table>

**Table 2.** Time Interval Between Coffee Consumption and Surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Average ± SD, min</th>
<th>Minimum; Median; Maximum, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mg</td>
<td>169.4 ± 69.8</td>
<td>88; 146; 296</td>
</tr>
<tr>
<td>120 mg</td>
<td>191.3 ± 70</td>
<td>96; 187; 295</td>
</tr>
<tr>
<td>180 mg</td>
<td>144.1 ± 81.6</td>
<td>12; 149; 295</td>
</tr>
</tbody>
</table>

450 family, mainly CYP1A2, to paraxanthine, theobromine, theophylline, 1-methylxanthine, and 1-methyluric acid. The antioxidant activities of paraxanthine, theobromine, theophylline, and 1-methylxanthine are comparable to that of caffeine and the activity of 1-methyluric acid is actually higher than that of caffeine. Thus, caffeine keeps its antioxidant activity when metabolized.
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.
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 ± 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).

\[
C = k \cdot D \tag{1}
\]

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

**Table 3.** Difference of Caffeine Concentration Between Ipsilateral (=1st eye) and Contralateral (=2nd eye) Lens Capsule Including Adherent Lens Epithelial Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Average ± SD (Caffeine ng/Lens Capsule/Epithelium)</th>
<th>Minimum; Median; Maximum (Caffeine ng/Lens Capsule/Epithelium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg</td>
<td>−0.52 ± 1.16</td>
<td>−2.88; 0; 0.46</td>
</tr>
<tr>
<td>60 mg</td>
<td>1.88 ± 2.02</td>
<td>0; 1.24; 6.26</td>
</tr>
<tr>
<td>120 mg</td>
<td>2.09 ± 0.67</td>
<td>1.15; 2.05; 3.17</td>
</tr>
<tr>
<td>180 mg</td>
<td>3.68 ± 1.86</td>
<td>1.29; 3.41; 7.51</td>
</tr>
</tbody>
</table>

**Figure 3.** Difference of caffeine concentration between ipsilateral (=1st eye) and contralateral (=2nd eye) lens capsule including adherent lens epithelial cells plotted in a Tukey boxplot (\( \times \) = extreme outlier, \( \ast \) = mild outlier, \( * \) = significance; \( P < 0.05 \), orthogonal double-sided Mann-Whitney \( U \) tests according to the strategy in Table 4).

**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.\(^7\) Taking into account that 80% of adults consume approximately 200-mg caffeine per day,\(^7\) 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.\(^8\) 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.\(^9\) 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

**Table 4.** Contrasts of Differences Between Ipsilateral (=1st eye) and Contralateral (=2nd eye) Lens Capsule Including Adherent Lens Epithelial Cells

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>( n )</th>
<th>( U )</th>
<th>( U^* )</th>
<th>Significant Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 vs. 60 mg</td>
<td>15</td>
<td>52(^*)</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>0 vs. 60 and 120 mg</td>
<td>24</td>
<td>115(^*)</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>0 vs. 60, 120, and 180 mg</td>
<td>33</td>
<td>180(\uparrow)</td>
<td>2</td>
<td>136</td>
</tr>
</tbody>
</table>

\( ^* \) Mann-Whitney two sample rank test.
\( \uparrow \) Significance (\( P < 0.05 \)).
humans caffeine quickly reaches peak plasma concentrations.\textsuperscript{3,15}

We were able to increase lens caffeine concentration to 3.68 ng/lens capsule/epithelium (mean) by consumption of 180-mg caffeine. It is unclear if this level of caffeine concentration is sufficient for acute protection of the lens against ultraviolet radiation or ionizing radiation (e.g., from medical treatments). However, using a similar caffeine dose for peroral intake, Natella et al.\textsuperscript{10} demonstrated that acute peroral caffeine consumption increases the antioxidant capacity of plasma. Besides evidence of the protective effect of caffeine against cataract development in experimental studies,\textsuperscript{1,6,8,10} epidemiologic studies support that coffee consumption prevents cataract.\textsuperscript{7,11}

Despite regular caffeine consumption (Table 1) all patients analyzed in this study had developed cataract. We would have expected a delay in onset of cataract. The average age of 74.7 at cataract surgery in our study is minimal above the age at cataract surgery in Denmark (average age between 73 and 73.5).\textsuperscript{11} However, study design and sample size in our study was chosen to investigate pharmacokinetics and other risk factors for cataractogenesis were not assessed. Further, a control group with no regular caffeine consumption is lacking in our study to quantify the effect of cataractogenesis inhibition.

In conclusion, we demonstrated the pharmacokinetic of perorally administered caffeine, one of the most widely consumed and most relevant sources of a dietary antioxidant compound. Perorally absorbed caffeine quickly accumulates in the lens capsule including lens epithelial cells in a dose-dependent manner. The currently presented information forms a basis for further investigations of inhibiting cataractogenesis.

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References


