# Supplementary materials

## Materials and methods

*Pharmacokinetics of HOEC in rats after oral and intravenous administration*

Ten-week-old male rats, weighing 260±20 g, were purchased from Slac Laboratory Animal (Shanghai, China). The rats were housed in a temperature (22±1°C) - and humidity (55%) - controlled room with a 12-hour light/dark cycle. Water and food were provided ad libitum. The experimental procedures involved in this study conformed to animal ethics and the guidelines of Care and Use of Laboratory Animals of China for animal experimentation.

Rats were divided into two treatment groups: IV (1 mg/kg) and PO (10 mg/kg), 3 rats per group. Before the experiment, rats fasted for 12 hours but could have water. Blood (0.2 mL) was drawn via jugular catheter from each rat at the following time points: 0, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h, and anticoagulant heparin sodium was added. Blood samples were centrifuged at 1000 × g, 4°C for 15 min to prepare plasma for detection. 50 μL plasma sample was pipetted to the centrifuge tube and 250 μL internal standard (200 ng/mL tolbutami methanol solution) was added. The mixture was then mixed thoroughly and centrifuged at 14000 rpm for 5 min. Calibration standard (STD) sample (1000, 750, 500, 100, 50, 10, 5 ng/mL) and quality control (QC) sample (800, 200, 8 ng/mL) of HOEC and caffeic acid were prepared.

A Shimadzu HPLC system (UFLC) (Shimadzu, Columbia, MD, USA), consisting of a LC-20AD pump, a DGU-20A3 degasser, a SIL-20A autosampler, a CBM-20Atemperature control chamber and a CTO-20A column oven, were used for all chromatography. An Applied Biosystems 4000 QTRAP hybrid linear ion trap-triple quadrupole instrument (Applied Biosystems, Inc., US) was connected. Chromatographic separations were acquired on a Waters XTerra MS C18 5 μ (50 mm × 2.10 mm) eluted with 15-85% gradient solvent B (0.1% formic acid in ACN) insolvent A (aqueous 0.1% formic acid) at a flow rate of 1 mL/min for 0.9-1.8 min.

## Results

*Pharmacokinetic data*

Since HOEC is very unstable in vivo, we only detected the main metabolite of HOEC, i.e. caffeic acid. The pharmacokinetic parameters (mean ± SD) of caffeic acid, a metabolite of 1 mg/kg HOEC given intravenously in rats as follows, $T_{max}$ = 0.139±0.096 hr, $C_{max}$ = 70.83±43.51 ng/mL, $t_{1/2}$ = 0.13 hr, $AUC_{(0-t)}$ = 13.59±6.89 ng/mL*hr, $AUC_{(0-\infty)}$ = 19.04 ng/mL*hr. The pharmacokinetic parameters (mean ± SD) of the metabolites of caffeic acid, a metabolite of 10 mg/kg HOEC given orally in rats as follows, $T_{max}$ = 0.194±0.096 hr, $C_{max}$ = 360.27±175.01 ng/mL, $t_{1/2}$ = 0.32~0.54 hr, $AUC_{(0-t)}$ = 213.81±34.21 ng/mL*hr, $AUC_{(0-\infty)}$ = 233.30±27.98 ng/mL*hr. The pharmacokinetic data of caffeic acid is shown in following tables.

**Table 1.** Calibration standard sample and quality control sample

| Caffeic Acid | | | | | | |
|---|---|---|---|---|---|---|
| Sample Name | Analyte Concentration (ng/mL) | Analyte Peak Area (counts) | Area Ratio | IS Peak Area (counts) | Calculated Concentration (ng/mL) | Accuracy (%) |
| Blank | 0 | 0.00E+00 | -7.00E+00 | 0.00E+00 | NA | NA |
| Blank+IS | 0 | 0.00E+00 | 0.00E+00 | 1.35E+06 | NA | NA |
| STD-1 | 5 | 5.72E+03 | 4.29E-03 | 1.33E+06 | 4.94352 | 98.8704 |
| STD-2 | 10 | 8.22E+03 | 6.19E-03 | 1.33E+06 | 10.1262 | 101.262 |
| STD-3 | 50 | 3.44E+04 | 2.59E-02 | 1.33E+06 | 63.8868 | 127.774 |
| STD-4 | 100 | 5.59E+04 | 4.33E-02 | 1.29E+06 | 111.442 | 111.442 |
| STD-5 | 500 | 2.40E+05 | 1.83E-01 | 1.31E+06 | 493.889 | 98.7778 |
| STD-6 | 750 | 3.23E+05 | 2.61E-01 | 1.24E+06 | 706.722 | 94.2295 |
| STD-7 | 1000 | 4.57E+05 | 3.52E-01 | 1.30E+06 | 954.184 | 95.4184 |
| QCL | 8 | 7.44E+03 | 5.50E-03 | 1.35E+06 | 8.25185 | 103.148 |
| QCM | 200 | 1.05E+05 | 7.90E-02 | 1.33E+06 | 209.095 | 104.547 |
| QCH | 800 | 3.64E+05 | 2.68E-01 | 1.36E+06 | 725.847 | 90.7309 |
| QCL | 8 | 6.19E+03 | 5.11E-03 | 1.21E+06 | 7.19061 | 89.8827 |
| QCM | 200 | 1.00E+05 | 8.16E-02 | 1.23E+06 | 216.318 | 108.159 |
| QCH | 800 | 3.50E+05 | 2.83E-01 | 1.24E+06 | 765.382 | 95.6728 |
| QCL | 8 | 7.05E+03 | 5.47E-03 | 1.29E+06 | 8.17799 | 102.225 |
| QCM | 200 | 9.90E+04 | 7.77E-02 | 1.27E+06 | 205.63 | 102.815 |
| QCH | 800 | 3.51E+05 | 2.64E-01 | 1.33E+06 | 715.572 | 89.4465 |

NA: None.

**Table 2.** Plasma concentrations of caffeic acid after oral and intravenous administration of HOEC

| Time (hr) | IV-1 mg/kg | | | | |
|---|---|---|---|---|---|
| | Plasma Concentration (ng/mL) | | | | |
| | 101 | 102 | 103 | Mean | SD |
| Pre-dose | BLQ | BLQ | BLQ | NA | NA |
| 0.083 | 8.89 | 100.50 | 91.11 | 66.83 | 50.40 |
| 0.25 | 20.88 | 28.86 | 10.05 | 19.93 | 9.44 |
| 0.5 | 6.74 | 11.52 | 7.55 | 8.60 | 2.56 |
| 1 | BLQ | BLQ | BLQ | NA | NA |
| 2 | BLQ | BLQ | BLQ | NA | NA |
| 4 | BLQ | BLQ | BLQ | NA | NA |
| 6 | BLQ | BLQ | BLQ | NA | NA |
| 8 | BLQ | BLQ | BLQ | NA | NA |
| 24 | BLQ | BLQ | BLQ | NA | NA |
| Time (hr) | PO-10 mg/kg | | | | |
| | Plasma Concentration (ng/mL) | | | | |
| | 201 | 202 | 203 | Mean | SD |
| Pre-dose | BLQ | BLQ | BLQ | NA | NA |
| 0.083 | 533.22 | 308.13 | 125.31 | 322.22 | 204.32 |
| 0.25 | 292.22 | 364.32 | 183.28 | 279.94 | 91.14 |
| 0.5 | 166.94 | 210.26 | 163.01 | 180.07 | 26.22 |
| 1 | 68.87 | 70.97 | 75.37 | 71.74 | 3.32 |
| 2 | BLQ | 8.44 | 23.44 | 15.94 | NA |
| 4 | BLQ | BLQ | BLQ | NA | NA |

| | | | | | |
|---|---|---|---|---|---|
| 6 | BLQ | BLQ | BLQ | NA | NA |
| 8 | BLQ | BLQ | BLQ | NA | NA |
| 24 | BLQ | BLQ | BLQ | NA | NA |

NA: None. BLQ: Below the lowest quantifiable measure.

**Table 3.** PK parameters for caffeic acid after oral and intravenous administration of HOEC

| Rat No. | $t_{1/2}$ | $T_{max}$ | $C_{max}$ | $AUC_{(0-t)}$ | $AUC_{(0-\infty)}$ | $MRT_{(0-\infty)}$ |
|---|---|---|---|---|---|---|
| | hr | hr | ng/mL | ng/mL*hr | ng/mL*hr | hr |
| IV-1 mg/kg | | | | | | |
| 1 | NA | 0.25 | 20.88 | 6.31 | NA | NA |
| 2 | 0.14 | 0.08 | 100.50 | 20.02 | 22.29 | 0.22 |
| 3 | 0.12 | 0.08 | 91.11 | 14.43 | 15.78 | 0.18 |
| Mean | 0.13 | 0.14 | 70.83 | 13.59 | 19.04 | 0.20 |
| S.D. | NA | 0.10 | 43.51 | 6.89 | NA | NA |
| PO-10 mg/kg | | | | | | |
| 4 | 0.36 | 0.08 | 533.22 | 207.40 | 243.56 | 0.51 |
| 5 | 0.32 | 0.25 | 364.32 | 250.77 | 254.70 | 0.52 |
| 6 | 0.54 | 0.25 | 183.28 | 183.25 | 201.64 | 0.86 |
| Mean | 0.41 | 0.19 | 360.27 | 213.81 | 233.30 | 0.63 |
| SD | 0.12 | 0.10 | 175.01 | 34.21 | 27.98 | 0.20 |