EXPERIMENTAL STUDY

Caffeine alters oxidative homeostasis in the body of BALB/c mice

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Abstract: Caffeine is a secondary metabolite known from some unrelated plants. Coffee plants such as Coffea arabica and C. canephora (1) and tea plants Camellia sinensis (2, 3) can be exemplified. Formerly, caffeine effect was known from drinking of tea and coffee or consumption of some sweets. Beside coffee and tea, caffeine is added to many drinks, energy drinks, food and drugs (4, 5). Caffeine is a drug, which is not legislatively regulated over the world comparing to the other excitation causing substances and despite some adverse effects when caffeine is taken in an excessive amount (6).

In the body, caffeine is targeted to many disparate macromolecular structures. The compound acts as a nonselective adenosine receptor antagonist (7). Though it is believed that the adenosine receptor is the major target, many other enzymes and receptors can meet caffeine. Caffeine can influence neurotransmission through acetylcholine, epinephrine, norepinephrine, serotonin, dopamine and glutamate pathways (8–12).

There is a link between oxidative balance and caffeine. It was proved that caffeine can increase low molecular weight antioxidants and increase enzymes involved in degradation of reactive oxygen species (13). In another experiment, caffeine was found suitable to protect against oxidative stress caused by pentylene-tetrazol-induced seizures (3). Caffeine was proved to be effective enough to control inflammatory response in the body during stress conditions as reported by Tauler and coworkers when they analyzed inflammatory cytokines in humans who underwent run competition (14). It should be emphasized that there is a close link between inflammation and oxidative stress (15). Though the effect of caffeine on oxidative stress was proved in the reported papers, no final conclusion owing to oxidative stress can be done. In this paper, a complex view on the oxidative balance after caffeine intake was done. The paper is aimed at finding of the most effected organs and scale of caffeine impact. Time intervals and lasting of the balance alteration were investigated as well. It is believed that this paper will allow answer how much, where and when the caffeine can alter oxidative balance.

Material and methods

Experiment on laboratory animals

The experiment was based on 400 female laboratory mice BALB/c (Velaz, Unetice, Czech Republic). In the beginning of the experiment, the mice weighted 19 ± 1 g and they were 8 weeks old. For the whole experiment, the mice were kept in an animal house with temperature adjusted up 22 ± 2 °C, humidity 50 ± 10 % and light/dark period each 12 hours. The mice had free access to food and water. Prior to the experiment beginning, plan of the project was permitted and then supervised by the ethical committee at Faculty of Military Health Sciences (Hradec Kralove, Czech Republic).

The animals were divided into 20 groups each 20 animals. They received a dose of caffeine 0 (saline) – 1 – 4 – 16 – 64 mg/kg given intramuscularly into rear limb. Caffeine was solved in saline and applied in amount 100 μl. The controls received saline 100 μl. The 20 animals per one dose were sacrificed after 4 hours and another 20 animals after one, two and three days. Sacrificing was
done under CO₂ anesthesia by cutting of jugular vein. The whole liver, kidney, heart, vastus lateralis muscle and brain were collected immediately. The organs were kept at –80 °C until assay, which was not later than three weeks after the experiment beginning.

**Assay of the markers**

The stored tissues were mechanically homogenized using Ultra-Turrax (IkaWerke, Staufen, Germany) mill device. 100 mg of the tested organ was cut from cortex region and thrown into 1 ml of phosphate buffered saline. The sample was processed by Ultra-Turrax for one minute. The homogenized samples were used immediately for the assays mentioned further.

Ferric Reducing Antioxidant Power (FRAP) is a method/value that respond to overall level of low molecular weight antioxidants (16, 17). It was measured using 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ; Sigma-Aldrich) as a chromogenic reagent. A solution containing 10 mmol/l TPTZ and 40 mmol/l HCl was prepared and poured in a ratio 1:1 with 20 mmol/l FeCl₃. An aliquot of the solution sized 5 ml was added into 25 ml of 0.1 mol/l acetate buffer pH 3.6 and incubated at 37 °C for 10 minutes. 30 μl of tissue homogenate was given to 200 μl of the aforementioned solution, diluted with 770 μl of deionized water, incubated for 10 minutes, and spin at 10,000 ×g for another 10 minutes. Absorbance was measured at 593 nm for the sample comparing to blank, which was saline solution assayed in the same way as the homogenate. FRAP value was calculated using extinction coefficient ε = 26,000 l/mol×cm. Interference of caffeine in the FRAP assay was neglected by a separate tests where its level up to 1 mmol/l was used.

Malondialdehyde is a marker responding to oxidative stress and oxidative damage of lipid membranes (18–20). The malondialdehyde was measured by Thiobarbituric Acid Reactive Substances (TBARS) assay. Firstly, a solution containing 67 mg of thiobarbituric acid (Sigma-Aldrich) in 1 ml of dimethylsulfoxide and 9 ml of deionized water was prepared shortly before use. 200 μl of homogenate prepared using the aforementioned protocol was purified from proteins by addition of 400 μl of 10 % trichloroacetic acid and incubated for 15 minutes on ice bath. After that, the mixture was centrifuged at 3,000 ×g for 15 minutes. 400 μl of supernatant or saline solution (blank) was mixed with another 400 μl of the solution containing thiobarbituric acid. In the final step, solution was heated at 99 ºC for 10 minutes. The molar value of TBARS was calculated using extinction coefficient ε = 156,000 l/mol×cm. Interference of caffeine in the TBARS assay was neglected by a separate test where its level up to 1 mmol/l was used.

Caspase 3 was chosen as a marker of apoptotic processes (19, 21). The marker was assayed using Caspase 3 Assay kit, Colorimetric (Sigma-Aldrich, Saint Louis, Missouri, USA). The kit was based on spectroscopic determination of caspase 3 enzymatic activity using standard 96 wells microplates and multichannel spectrophotometer. The assay was made in compliance with provided protocol.

**Statistics**

Origin 8 Pro (OriginLab Corporation, Northampton, MA, USA) software was used for statistical processing of experimental data. One-way ANOVA with Bonferroni test were used and significance of differences between the experimental groups was tested on probability levels p 0.05 and 0.01.

**Results**

In the animals, no significant behavioral manifestation of exposure to caffeine was observed. On the other hand, behavioral tests were not applied in this experiment so minor alteration in cognitive abilities could pass without notice. Owing to the oxidative stress markers, an unequal effect on the tested organs was found. While TBARS level was significantly increased in the kidney (Fig. 1), the brain level decreased (Fig. 2). No significant alteration in TBARS level was found in samples from liver, heart and vastus lateralis muscle.

**Fig. 1.** Thiobarbituric acid reactive substances (TBARS) found in the kidney of mice exposed to caffeine. Dose of caffeine in mg/kg is depicted in the Figure. Error bars indicate standard error of mean. * respective ** appoint at significance against the controls at probability level 0.05 respective 0.01.

**Fig. 2.** Thiobarbituric acid reactive substances (TBARS) found in the brain of mice exposed to caffeine. The overall description is same as in Figure 1 case.
Caffeine is able to regulate oxidative balance in the body. The effect is unequal to organs in the body. A positive effect in the brain can be considered as an explanation of the caffeine related effect on neurodegenerations.

Conclusions

Caffeine is able to regulate oxidative balance in the body. The effect is unequal to organs in the body. A positive effect in the brain and negative in the kidney was proved. The other organs seem to be resistant. The findings can explain known facts about coffee potency to ameliorate some neurodegenerative processes where central nervous system is a target of pathology.
References


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