CAFFEINE DOES NOT MODULATE NUTRITIVE BLOOD FLOW TO RAT GASTRIC SUBMUCOSA – A MICRODIALYSIS STUDY

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Received: February 20, 2008; Accepted (with revisions): April 15, 2008

Key words: Caffeine/Microcirculation/Nitric oxide/Microdialysis/Rat

Background and Aims: Coffee irritates the gastric mucosa disrupting its barrier and increasing the risk of peptic ulcers. However, caffeine's contribution to these effects has not yet been elucidated. In this study we looked at the local effect of caffeine on the microcirculation and nitric oxide production in rats together with systemic marker of oxidative stress malondialdehyde as possible mechanisms whereby caffeine might participate in mucosal barrier impairment.

Materials and Methods: Four groups of rats were anesthetized and administered as a bolus four different intraperitoneal doses of caffeine (0, 1, 10 and 50 mg kg⁻¹ b.wt.). The gastric submucosal microcirculation and nitric oxide production were then recorded for 2.5 hours by *in situ* microdialysis using the flow marker ethanol. At the completion of the experiments, plasma caffeine and malondialdehyde levels as well as morphological mucosal injury were determined.

Results: There were no major differences in the macro- or microscopic pictures of the mucosa among the groups. Local microcirculatory (ethanol out/in ratio) and nitric oxide monitoring failed to demonstrate statistically significant changes as did measurement of plasma malondialdehyde in response to caffeine injections.

Conclusions: Caffeine *per se* seems unlikely to contribute to the gastric mucosal barrier injury associated with coffee consumption by alterations in nutritive blood flow, nitric oxide production or aggravation of systemic oxidative stress. This information is relevant for better understanding of the mechanisms involved in caffeine-mediated influences on gastric physiology in relation to the irritant effects of coffee.

INTRODUCTION

Caffeine (contained in coffee, tea, caffeinated beverages, cocoa, chocolate etc.) is the most consumed stimulant drug of abuse worldwide. With regard to coffee, epidemiological data support its irritant role in stomach and esophagus in association with gastroesophageal reflux leading to enhanced risk of ulcers and cancer in the afflicted areas^{1,2}. Recently, this irritant effect was also confirmed in young asymptomatic individuals³. The beverage is a complex mixture of possibly antagonistic substances comprising, apart from caffeine, isoflavones, polyphenols, diterpenes etc. exhibiting effects according to the type of coffee bean processing^{4,5}. To elucidate the cause of the irritant nature of coffee to the stomach, focusing on the effects of isolated and purified (pharmacologically) active components of coffee would lead to more information. Of these components caffeine is the most studied.

Maintenance of the gastric mucosal barrier integrity is dependent on the balance between aggressive and protective factors represented by hydrochloric acid on one hand and adequate mucosal blood flow with sufficient mucus production on the other. Caffeine, a known acid

secretagogue, has long been suspected of causing mucosal hypoperfusion due to (micro)vascular impairment⁶. This hypothesis was later supported by electron microscopy⁷. More recent observations report suppressed acetylcholine (ACh)-induced mucus production⁸ by caffeine and gastric mucosal transmembrane potential difference⁹, completing the barrier-braking mosaic picture of caffeine. However, the aforementioned notion has been challenged by experimental observations showing enhanced mucosal blood flow by caffeine¹⁰ and a protective influence of this drug on mucosal barrier integrity¹¹ suggesting actually a preventive role for caffeine in gastric mucosal injury¹².

Caffeine is a methylxanthine with pluripotent and possibly opposing pharmacological actions. It is a non-selective adenosine receptor antagonist, phosphodiesterase inhibitor, ryanodine-sensitive Ca²⁺ channel activator and soluble guanylate cyclase (GC) inhibitor. As a consequence, these actions may interfere with nitric oxide (NO) production and/or its second messenger cyclic guanosine monophosphate (cGMP) pathway leading to modulation of a wide spectrum of mucosal barrier-related (patho)physiological effects exerted by NO including vascular tone regulation or modulation of oxidative stress

level¹³. The literature, to the best of our knowledge, provides rather limited data on caffeine's effect on gastric (sub)mucosal microcirculation and has not included parallel monitoring of local NO release (using microdialysis), so far. Similarly, the putative effect of caffeine on oxidative stress necessitates clarification. The aim of the present study was twofold: first, to evaluate the possible impact of caffeine on gastric submucosal microcirculation, nitric oxide production and morphology, and second, to measure plasma malondialdehyde (MDA) as a marker of oxidative stress (lipid peroxidation).

MATERIALS AND METHODS

Animals

Conventionally bred adult white male Wistar rats (Biotest s.r.o., Konárovice, Czech Republic) weighing 300–400 g, were used. The animals were housed in the animal quarters for at least 7 days prior to experiments under controlled environmental conditions. The rats had free access to standard pellets (ST 1-TOP, Velas, Prague, Czech Republic) except for the 16–18 h before experiments, when they were fasted. Tap water was provided *ad libitum* until the day of the experiment. All animals received humane care in accordance with the guidelines set by the institutional Animal Use and Care Committee of the Charles University, Prague, Faculty of Medicine in Hradec Králové, Czech Republic. All experimental procedures were approved by the same committee.

Animal preparation

All animals were anesthetized with single i.p. dose of pentobarbital (50 mg kg⁻¹, Nembutal®, Abbott Laboratories, North Chicago, USA) and placed in a supine position on an unheated bed. They were kept under general anesthesia by cyclic i.p. administration of Nembutal® (15 mg kg⁻¹ h⁻¹). Body temperature was monitored using a rectal thermometer probe (Ama-digit ad 15th, ApreCISION, Germany) and maintained at 37.5–38.0 °C by means of a heating lamp. The trachea was cannulated with a short polyethylene catheter to ensure patent airways.

Substances

For microdialysis, ethanol-enriched normal 0.9 % saline (final concentration 50 mmol l⁻¹)¹⁴ was used as a perfusion medium. Caffeine (purchased from Sigma-Aldrich, St. Louis, MO, USA) was dissolved in normal saline to obtain solutions with concentrations 0.5, 5 and 25 mg ml⁻¹ for groups 2, 3 and 4, respectively.

Gastric submucosal microdialysis technique

Modified technique originally described by Kitano *et al.*¹⁵ was used. Briefly, following 3–4 cm long midline laparotomy, stomach was exposed. Firstly, respecting the course of blood vessels, a 21 G needle was introduced into the submucosa of gastric fundus with care neither to penetrate through the mucosa into the lumen nor to make an additional opening in the serosa. Secondly, a microdi-

alysis probe (MAB 11.8.10 with 6 kDa cut-off polyethylene sulphone membrane, active length 10 mm; outer diameter 0.5 mm; Microbiotech/se AB, Stockholm, Sweden) was cautiously inserted into the preformed tunnel (Fig. 1a). Finally, the probe was fixed to the serosa with a suture. Continuous microdialysis was effectuated by perfusing the catheters with ethanol solution by means of CMA 102 microdialysis pump (CMA Microdialysis AB, Solna, Sweden) at a perfusion rate of 2 µl min⁻¹. For tissue equilibration, an initial 60 min period (without specimen collection) was allowed which was succeeded by a 30 min period to yield the baseline sample. Thereafter, sampling ensued for the next 2.5 h in 30 min intervals into sterile 200 µl polyethylene microvials. The specimens were aliquoted and stored at -70 °C (for nitric oxide) or -20 °C (for ethanol) until analysis.

Experimental design

The animals were randomly allocated to four groups (6 in each). After the collection of baseline microdialysis sample, the first (sham-operated) group received by intraperitoneal injection an adequate volume of normal saline, whereas the second, third and fourth groups were intraperitoneally administered caffeine solutions with concentrations 0.5, 5 and 25 mg ml⁻¹ (caffeine dose of 1, 10 and 50 mg kg⁻¹ b. wt.) respectively. At the conclusion of experiments, the animals were sacrificed by blood withdrawal from abdominal aorta. The obtained plasma was stored at -70 °C for ensuing biochemical determination of caffeine and MDA. After the removal of stomach from the body, the mucosal surface of the glandular part was gently cleaned in cold tap water and closely inspected in search for macroscopic lesions. The tissues surrounding the probe (0.5 × 1.5 cm) with the probe itself *in situ* were then dissected and the biopsy immersed in 10% formaline for histological examination.

Probe performance stability and in vivo recovery determination for nitrate

The measurement of NO using microdialysis technique was validated in two consecutive steps employing other two groups (A and B) of pentobarbital-anesthetized rats. First, probe performance stability for nitrate was tested continuously for 7 h (measurements during equilibration period inclusive, group A, n = 5) in one experiment based on an assumption of stable NO production throughout the study period. Microdialysis sampling in gastric submucosa was realized in 30 min intervals at a perfusion rate of 2 µl min⁻¹. As perfusate, normal saline was utilized. Second, *in vivo* recovery of the same probe type was estimated (group B, n = 3) using zero-net flux method originally proposed by Lönnroth *et al.*¹⁶. Four perfusion media of increasing concentrations of sodium nitrate in sterile saline were consecutively applied as follows. After the initial tissue equilibration (1 h) with 10 µmol l⁻¹ NaNO₃, a 30 min sample was collected. The perfusion medium was changed for 15 µmol l⁻¹ nitrate and following 30 min equilibration, another sample harvest (30 min) ensued respecting the probe's lag time (3 min). The experiment

Fig. 1. Morphological studies. Panel “a” confirms the position of the microdialysis probe within the submucosal layer of gastric wall (arrow). The catheter is surrounded by noticeable edema and hyperemia with diffuse inflammatory infiltrate of mild degree penetrating at some places into the mucosa. The probe’s tougher inner membrane was slightly damaged during tissue slicing (mu = mucosa, sm = submucosa, mp = muscularis propria). Panel “b” depicts the detail of the probe’s outer membrane permeated by polymorphonuclear leukocytes (arrow). Pictures “c-e” show normal gastric mucosa, i. e. grade 0, and typical lesions of grade I and II – detached cells and necrosis of pits (arrows), respectively. Standard hematoxylin-eosine stain, original magnification “a” 125×, “b” 500×, “c-e” 250×.

was completed with 50 and finally 120 $\mu\text{mol l}^{-1}$ nitrate solutions. The results were plotted on a graph and probe recovery was read from the regression equation (slope gradient). Besides microdialysis, these two groups of animals underwent no further experimental treatment. All general steps (anesthesia, surgery, gastric submucosal microdialysis technique including probes but excluding perfusion media, sacrifice and analytical techniques) were equal to the experimental groups of the present study.

Nutritive blood flow measurement

Using flow marker (e.g. ethanol)-enriched perfusion medium, microdialysis offers opportunity to indirectly assess tissue microcirculation (nutritive blood flow) in

various organs. The principle of the so called “ethanol dilution technique” is based on the negative correlation between blood perfusion and ethanol efflux from the probe represented by out/in ratio of ethanol concentrations^{14, 17, 18}.

Biochemical analyses

Total NO was quantitatively determined as the sum of its stable metabolites (nitrite plus nitrate) in an enzymatic colorimetric assay, which involves an enzymatic conversion of nitrate to nitrite by nitrate reductase. Resulting nitrite is detected colorimetrically as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm. In the experiments commercially available

ELISA kits (Assay Designs, Ann Arbor, MI, USA) were utilized only. All procedure steps were performed according to the manufacturer's instructions.

The analyses of ethanol were carried out using GC-MS apparatus consisting of Varian 3300 Gas Chromatograph (GC) coupled to Finnigan MAT Magnum Mass Spectrometer (MS; Thermo Fisher Scientific, formerly Finnigan). SupelcowaxTM-10 (30 m x 0.25 mm x 0.25 µm film thickness) GC Capillary column with helium as a carrier gas was employed. Injector and transferline temperatures were set to 230 °C for both. GC oven was programmed as follows: 55 °C, 2 min, 25 °C min⁻¹ to 150 °C, hold for 1 min. The specified MS parameters were 70eV for electron ionization mode (EI) and 209 °C for ion trap temperature. The resulted MS spectra were scanned in 15–70 a.m.u. mass range under 4 microscans s⁻¹ of detection speed regimen.

Plasma caffeine concentrations were determined using a modified HPLC method by Biederbick *et al.*¹⁹. Briefly, caffeine and internal standard hydroxyethyltheophylline (aqueous solution 40 mg l⁻¹) from plasma samples (100 µl + 50 µl) were extracted using 2.5 ml of dichloromethane. The organic phase was evaporated under nitrogen at 40 °C and the samples were reconstituted in 150 µl of mobile phase, and 30 µl were injected into the HPLC column. Analysis was performed on a 2695 Waters Separations Module equipped with 996 photodiode array detector and Peltier column-thermostat Jet-Stream (Thermotechnic Products). The mobile phase was made up of an aqueous solution of sodiumdihydrogenphosphate (0.5 mmol l⁻¹, pH 5) to acetonitrile (ratio 88:12) and was pumped isocratically at a flow rate of 0.9 ml min⁻¹. The temperature of the column was set at 40 °C. The quantification of caffeine was performed at 270 nm.

Plasma MDA was determined as a secondary product of lipid peroxidation in an attempt to evaluate the level of oxidative stress produced by caffeine. The analysis was based on the reaction of MDA with thiobarbituric acid (TBA) producing a red MDA-TBA complex measured photometrically at three distinct wavelengths (485, 532 and 560 nm) and the absorbance corrected according to Allen's formula $A_{\text{corr}} = A_{532} - [(A_{560} - A_{485}) \times 0.63 + A_{485}]$ for enhanced specificity²⁰.

Histological analyses

Stomach biopsies were taken to evaluate possible effects of caffeine on tissue morphology at microscopic level and for probe position verification. The samples (0.5 × 1.5 cm) were taken from glandular segment surrounding the probe. The tissues were fixed in 10 % neutral buffered formalin and further treated according to standard procedures for hematoxylin-eosin (HE) stain. The sections were evaluated by a blinded professional observer and photographed at 125, 250 and 500-fold magnification. The grading criteria were adopted from Natale *et al.* and were as follows: grade 0 for normal mucosa, grade I for lysis and segregation of cells on the luminal surface (with intact pit cells), grade II for damage confined to gastric pits with detachment of the surface epithelium and grade III

Fig. 2. The graph depicts total gastric submucosal nitric oxide (NO) production (presented as a sum of nitrite and nitrate), as monitored in anaesthetized rats by *in vivo* microdialysis and confirms its stability throughout the experiment. The arrows specify the time frame of *in vivo* recovery determination (measured in another study, see Fig. 3). Data are expressed as means ± SEM of 5 measurements.

Fig. 3. *In vivo* probe calibration ("zero net-flux" method). The Δ microdialysate (dialysate – perfusate) total NO plotted against the perfusate total NO gives a gradient representing the probe's recovery (38 %). The x-intercept corresponds to the concentration of NO in the surrounding extracellular medium (~23 µmol l⁻¹). The y-intercept approximates the mean concentration obtained in due course from stability experiment (11 µmol l⁻¹, Fig. 2). Single results of three animals are displayed as dots.

which involves injured gastric glands (whole-thickness mucosal necrosis with swelling and possible disconnection of mucosal layers)²¹.

Statistics

Data are expressed as means $\pm$ standard error of mean (SEM), unless otherwise noted. For statistical evaluation, normality tests and repeated measures ANOVA were used. The data were processed by the program NCSS 2004. The chosen level of significance was $\alpha = 0.05$.

RESULTS

Histology

Microdialysis probes were positioned correctly within the submucosal layer of gastric wall without penetration into the organ's lumen. The histological picture was comparable to previous reports^{15, 22} as indicated in Fig. 1a, b. Lack of macroscopically measurable whole organ mucosal alterations due to caffeine treatment was mirrored in standard microscopy, which depicted similar grades of tissue damage ranging within grades 0-II around the probe in slides from all experimental groups including sham-operated animals (Fig. 1c-e). The severity of mucosal injury was unrelated to the administered caffeine dose. No grade III lesions were observed.

Microdialysis data

The results of probe performance stability are displayed in Fig. 2. In this experiment, the calculated average outflow concentration of total NO (within 5 h-time frame corresponding with the probe calibration study) was 11 $\mu\text{mol l}^{-1}$. This is in agreement with the expected value ($\sim 9 \mu\text{mol l}^{-1}$) calculated using extrapolation of data obtained from the probe calibration study, i.e. in case the concentration of total NO in the perfusate was close to zero (Fig. 3). The outcomes of these experiments were consistent with one another indicating stable function of the microdialysis probe and NO production over time. The *in vivo* recovery of the used probe type under given experimental conditions was determined by the gradient of the regression line and found to be 38 %. The x-intercept corresponds to the concentration of nitrite + nitrate in the surrounding extracellular medium ($\sim 23 \mu\text{mol l}^{-1}$, Fig. 3). This level of extracellular NO was not affected by the i.p. administration of caffeine ($p = 0.9$, ANOVA, Fig. 4a). The microcirculation as represented by the ethanol out/in ratio showed a tendency for dose-related alterations after caffeine but the difference was not statistically significant ($p = 0.1$, ANOVA, Fig. 4b).

Plasma analytes

Along with caffeine, plasma MDA was measured as general oxidative stress-related marker at the conclusion of the experiments. Even here, no statistically significant dose-related trend was observed ($p = 0.8$, ANOVA, Tab. 1).

Fig. 4. Panel “a” presents the total NO production in response to increasing caffeine load. No difference was registered within 2.5 h of monitoring. Panel “b” illustrates the time course of microdialysate ethanol out/in ratio (negatively correlates with nutritive blood flow) indicating a slight tendency of microcirculation to deteriorate in a dose-dependent manner (particularly at intervals 3 and 5) following the injection of caffeine. However, this trend lacks statistical significance. Results are means $\pm$ SEM of 6 measurements.

DISCUSSION

Effect of caffeine on (sub)mucosal morphology

Even though macroscopical observations (e.g. a computerized planimetry) are a “golden standard” in the evaluation of gross gastric mucosal injury for their ability to assess the whole organ, they are inconvenient for detection and scoring of minor (visibly hardly discernible) changes. Since, in accordance with literature²³, the latter was also the case in our experiment, gastric mucosal impairment was studied microscopically using standard

histology. However, owing to the exactingness of the suggested method of whole organ histological evaluation²¹, we decided for the present time to grade the most prominent cellular/tissue changes found in comparable parts of glandular stomachs (in the vicinity of probe implantation site) of experimental groups. Since this time we did not quantify the lesions, the statistical approach was not feasible. Hence, the present study provides only qualitative data on the effects of microdialysis and caffeine on rat gastric tissue morphology.

NO measurement using microdialysis

There is very limited data on gastric NO measurement with *in vivo* microdialysis. Iversen *et al.* measured nerve-induced release of NO in the wall of rabbit stomach. They utilized different probe types (CMA/10, CMA Medical AB, Stockholm, Sweden, 3 x 0.7 mm dialysis membrane with 20 kDa cut-off) and perfusion rate (1 $\mu\text{l min}^{-1}$) and estimated interstitial concentrations of nitrite and nitrate to approximate 10 and 70 $\mu\text{mol l}^{-1}$, respectively. The calculated *in vitro* recovery of the probes (for given perfusion rate) equaled 31 - 33 % for nitrate and nitrite, respectively. The authors did not determine the recovery *in vivo*, but assumed that it would be in the range 10–40 %²⁴. Suzuki *et al.* recently employed similar probes to our catheters (MAB 7.8.10 with 15 kDa cut-off dialysis membrane, active length 10 mm; outer diameter 0.5 mm; Microbiotech/se AB, Stockholm, Sweden) and validated them for studying nitrosative chemistry in the lumen of human stomach. The assessed *in vitro* recovery for nitrite at pH 1.5 and perfusion rate 0.15 ml h^{-1} was 71 %²⁵. Our results of *in vivo* recovery may be comparable to these studies since the dialysis function of membranes *in vivo* generally diminishes¹⁶. The interstitial concentration of total NO found in our study ($\sim 23 \mu\text{mol l}^{-1}$) was lower than reported by Iversen *et al.* in rabbits. It is a matter of debate to what extent this difference may be attributable to species and/or technique used.

Effect of caffeine on NO production

Besides vascular tone regulation, nitric oxide released by Ca^{2+} -dependent endothelial (e)NOS as well as Ca^{2+} -independent inducible (i)NOS plays a large number of (patho)physiological roles many of which may be related to mucosal defence and injury¹³. It is therefore of interest

whether caffeine interferes with the release of this reactive pluripotent radical. Bruce *et al.* report of a significant decrease in exhaled NO levels in humans one hour after 100–200 mg caffeine intake²⁶. The latest findings indicate that caffeine (16 mg kg^{-1} i.v.) may decrease nitric oxide synthase (NOS) expression in rat skeletal muscles²⁷ and attenuate glutamate-induced NO synthesis in murine spinal cord *in vitro*²⁸. Moreover, caffeine negates the protective effect of ischemic preconditioning, i.e. eliminates reactive hyperemia due to the hypoperfusion-induced accumulation of adenosine and enhanced NO production²⁹. In contrast, endothelium of isolated rat aorta responds to caffeine by promotion of NO synthesis³⁰. In the present study, we failed to detect significant shifts in NO production up to 2.5 h after the application of increasing doses of caffeine. However, eNOS releases NO in nanomolar quantities, changes we may have been unable to detect with the analytical procedure we used while micromolar amounts of NO are produced by activated iNOS as soon as 2 h after application of inducing substance³¹. Therefore, our results indicate no effect of caffeine on Ca^{2+} -independent NO production in resting gastric submucosa of anesthetized rats.

Effect of caffeine on blood flow

Despite the generally recognized constricting role of caffeine in the brain³², heart³³, limb³⁴ or gut³⁵ vasculature, the literature is inconsistent as far as gastric (sub)mucosal perfusion is concerned. Understandably, the discrepancy with respect to blood flow is reflected in the contrary effects of caffeine on acute induced gastric mucosal injury^{36, 23, 12}. Ozturkcan *et al.* showed that a single i.p. injection of 7.5 – 30 mg kg^{-1} caffeine leads to elevations in rat gastric mucosal blood flow 90 min after drug application¹⁰. Moreover, Koyama *et al.* found that *ex vivo* intraluminal administration of caffeine doses as high as 50–100 mg kg^{-1} result in a dose-dependent increase in mucosal blood flow lasting up to 90 min¹². Although in the present study some tendencies to decreased nutritive blood flow could be observed 30–90 min after caffeine administration, the results show a lack of statistically significant change in this variable throughout the experiment (Fig. 4b). This would be consistent with other studies showing no direct effect of caffeine on resting blood flow^{37–39} and/or induced vascular contractility⁴⁰. Along these lines, there is conflict-

Table 1. The table summarizes the effect of increasing doses of caffeine on plasma caffeine and malondialdehyde (MDA) levels at the conclusion of experiments. In spite of a negative trend, no statistically significant difference or association between administered caffeine and plasma MDA was found. Data are displayed as means $\pm$ SEM of 6 measurements.

i. p. caffeine dose (mg kg^{-1} b.wt.)	0	1	10	50
plasma caffeine (mg l^{-1})	0 ± 0.00	1.43 ± 0.07	11.80 ± 0.42	56.51 ± 1.68
plasma MDA ($\mu\text{mol l}^{-1}$)	0.94 ± 0.05	0.91 ± 0.12	0.84 ± 0.03	0.80 ± 0.15

ing data on the effect of caffeine on endothelial function. Papamichael *et al.* describe acute detrimental effects of caffeine up to 90 min after oral intake of 80 mg of caffeine on endothelium-dependent flow-mediated dilatation of the brachial artery⁴¹ whereas Umemura *et al.* report its favorable role on (acetylcholine-induced) forearm vasodilation one hour after oral administration of 300 mg of caffeine, whereas baseline blood flow remained unaltered³⁷. Interestingly, the latter authors ascribe these findings to enhanced endothelial NO synthesis caused by released Ca^{2+} from endoplasmic reticulum through activation of ryanodine-sensitive Ca^{2+} channels and the suppression of cGMP degradation³⁰, while the former investigators account for their observation as the inhibitory effect of caffeine on soluble GC with eventual suppression of cGMP formation. Unfortunately, neither group measured real *in situ* NO production. Nevertheless, these explanatory discrepancies may have some rationale as the differential pharmacologic actions of caffeine depend on the plasma caffeine levels⁴². Hence, the final vasoactive action of caffeine may result from a balance between its vasoconstrictive (adenosine-antagonizing) and possibly vasodilating (NO-releasing) ability.

Effect of caffeine on oxidative stress

There are data suggesting multifarious mechanisms whereby caffeine might play a role in augmenting oxidative stress⁴¹. However, the acute unfavorable consequence of caffeine could also follow from its antagonism with adenosine – a substance producing NO and thus preventing mitochondrial oxidant damage in rat cardiomyocytes⁴³. In rats that underwent concussive head injury, Al Moutaery *et al.* demonstrated an increase in the level of inflammation and oxidative stress (significant increase in neutrophil infiltration and brain MDA) associated with a dose-dependent increase in mortality of caffeine-pretreated animals⁴⁴. In the present experiment, the plasma concentrations of caffeine were consonant with previous findings⁴⁵ and indicate good bioavailability of caffeine after i.p. application. However, 2.5 h after drug administration, only a slight dose-dependent decreasing trend of plasma MDA levels lacking statistical significance was noted. This indicates no acute detrimental effect of caffeine on whole body's oxidative stress as far as systemically manifested effects are concerned (Tab. 1).

CONCLUSIONS

The results of the present study indicate that i.p. administration of caffeine in given dosages does not produce acute macroscopic changes to gastric mucosa and is unlikely to alter gastric submucosal nutritive blood flow and nitric oxide production or aggravate systemic oxidative stress level. Additional histomorphometric studies are needed to describe the effect of caffeine on gastric mucosa and further attempts need to be made to explore possible mechanisms whereby caffeine might participate in irritant effects of coffee.

ABBREVIATIONS

cGMP – cyclic guanosine monophosphate
GC – guanylate cyclase
MDA – malondialdehyde
NO – nitric oxide
e/iNOS – endothelial/inducible nitric oxide synthase

ACKNOWLEDGEMENTS

The authors acknowledge skillful technical assistance of Mrs. Ježková in the vivarium, Mrs. Menclová and Mrs. Lošonská in the biochemical and histopathological laboratories, respectively. This work was supported by Research Project from the Czech Ministry of Foreign Commerce MZO 00179906.

REFERENCES

1. Marotta RB, Floch MH. Diet and nutrition in ulcer disease. *Med Clin North Am* 1991; 75(4):967-79.
2. Terry P, Lagergren J, Wolk A, Nyren O. Reflux-inducing dietary factors and risk of adenocarcinoma of the esophagus and gastric cardia. *Nutr Cancer* 2000; 38:186-91.
3. Cibickova L, Cibicek N, Zdansky P, Kohout P. The impairment of gastroduodenal mucosal barrier by coffee. *Acta Medica (Hradec Králové)* 2004; 47(4):275-8.
4. Ehrlich A, Basse H, Henkel-Ernst J, Hey B, Menthe J, Lückner PW. Effect of differently processed coffee on the gastric potential difference and intragastric pH in healthy volunteers. *Methods Find Exp Clin Pharmacol* 1998; 20(2):155-61.
5. Fiebich BL, Valente P, Ferrer-Montiel A, Candelario-Jalil E, Menthe J, Luecker P. Effects of coffees before and after special treatment procedure on cell membrane potentials in stomach cells. *Methods Find Exp Clin Pharmacol* 2006; 28(6):369-72.
6. Roth JA, Ivy AC. The pathogenesis of caffeine-induced ulcers. *Surgery* 1945; 17:644-9.
7. Pfeiffer CJ, Roth JLA. Studies on the secretory and cytotoxic actions of caffeine on the ferret gastric mucosa. *Exp Mol Pathol* 1970; 13:66-78.
8. Hamada E, Nakajima T, Hata Y, Hazama H, Iwasawa K, Takahashi M *et al.* Effect of caffeine on mucus secretion and agonist-dependent Ca^{2+} mobilization in human gastric mucus secreting cells. *Biochim Biophys Acta* 1997; 1356(2):198-206.
9. Dziaduś-Sokołowska A, Orlef A, Bilski R, Mroczka J. The effect of ethanol-caffeine interaction on the gastric mucosal barrier. *Pol J Pharmacol Pharm* 1989; 41(3):253-8.
10. Ozturkcan O, de Saint Blanquat G, Derache R. Effet de la caféine sur le flux sanguin de la muqueuse gastrique chez le Rat. *Thérapie* 1974; 29:941-4.
11. Wittmers LE, Alich A, Quirk DR. Effect of caffeine on the Gastric Potential Difference (GPD). *FASEB Journal* 1998; 12(5):A737.
12. Koyama R, Kataoka H, Tanaka Y, Nakatsugi S, Furukawa M. Effect of caffeine on ibuprofen-induced gastric mucosal damage in rats. *J Pharm Pharmacol* 1999; 51(7):817-24.
13. Elliott S, Wallace JL. Nitric oxide: a regulator of mucosal defence and injury. *J Gastroenterol* 1998; 33:792-803.
14. Arner P, Hellmér J, Hagström-Toft E, Bolinder J. Effect of phosphodiesterase inhibition with amrinone or theophylline on lipolysis and blood flow in human adipose tissue in vivo as measured with microdialysis. *J Lipid Res* 1993; 34:1737-43.
15. Kitano M, Norlén P, Håkanson R. Gastric submucosal microdialysis: a method to study gastrin- and food-evoked mobilization of ECL-cell histamine in conscious rats. *Regul Pept* 2000; 86:113-23.

16. Lönnroth P, Jansson PA, Smith U. A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol* 1987; 253(2):228-31.
17. Hickner RC, Ekelund U, Mellander S, Ungerstedt U, Henriksson J. Muscle blood flow in cats: comparison of microdialysis ethanol technique with direct measurement. *J Appl Physiol* 1995; 79(2):638-47.
18. Korth U, Krieter H, Denz Ch, Janke Ch, Ellinger K, Bertsch T et al. Intestinal ischaemia during cardiac arrest and resuscitation: comparative analysis of extracellular metabolites by microdialysis. *Resuscitation* 2003; 58:209-17.
19. Biederbick W, Joseph G, Rump A, Theisohn M, Klaus W. Caffeine in Saliva After Peroral Intake: Early Sample Collection as a Possible Source of Error. *Therapeutic Drug Monitoring* 1997; 19:521-4.
20. Hendrix T, Assman R. Spectrophotometric correction for bile pigments in the thiobarbituric test for malondialdehyde-like substances in plasma. *Med Lab Sci* 1990; 47:10-6.
21. Natale G, Lazzeri G, Blandizzi C, Gherardi G, Lenzi P, Pellegrini A et al. Seriate histomorphometry of whole rat stomach: an accurate and reliable method for quantitative analysis of mucosal damage. *Toxicol Appl Pharmacol* 2001; 174:17-26.
22. Cibicek N, Micuda S, Chládek J, Živný P, Zadák Z, Čermáková E et al. Lithium microdialysis and its use for monitoring of stomach and colon submucosal blood perfusion – a pilot study using ischemic preconditioning in rats. *Acta Medica (Hradec Králové)* 2006; 49(4):227-31.
23. Parmar NS, Tariq M, Ageel AM. Effect of nicotine, alcohol and caffeine pretreatment on the gastric mucosal damage induced by aspirin, phenylbutazone and reserpine in rats. *Jpn J Pharmacol* 1985; 39(1):1-6.
24. Iversen HH, Celsing F, Leone AM, Gustafsson LE, Wiklund NP. Nerve-induced release of nitric oxide in the rabbit gastrointestinal tract as measured by *in vivo* microdialysis. *Br J Pharmacol* 1007; 120:702-6.
25. Suzuki H, Moriya A, Iijima K, McElroy K, Fyfe VE, McColl KEL. Validation of microdialysis probes for studying nitrosative chemistry within localized regions of the human upper gastrointestinal tract. *Scand J Gastroenterol* 2003; 38:856-63.
26. Bruce C, Yates DH, Thomas PS. Caffeine decreases exhaled nitric oxide. *Thorax* 2002; 57:361-3.
27. Corsetti G, Pasini E, Assanelli D, Saligari E, Adobati M, Bianchi R. Acute caffeine administration decreased NOS and Bcl2 expression in rat skeletal muscles. *Pharmacol Res* 2007; 55(2):96-103.
28. Godfrey L, Bailey I, Toms NJ, Clarke GD, Kitchen I, Hourani SM. Paracetamol inhibits nitric oxide synthesis in murine spinal cord slices. *Eur J Pharmacol* 2007; 562(1-2):68-71.
29. Riksen NP, Zhou Z, Oyen WJG, Jaspers R, Ramakers BP, Brouwer RMHJ et al. Caffeine prevents protection in two human models of ischemic preconditioning. *J Am Coll Cardiol* 2006; 48:700-7.
30. Hatano Y, Mizumoto K, Yoshiyama T, Yamamoto M, Iranai H. Endothelial-dependent and -independent vasodilatation of isolated rat aorta induced by caffeine. *Am J Physiol* 1995; 269:H1679-84.
31. Huang CJ, Tsai PS, Pan WHT, Skimming JW. Microdialysis for measurement of hepatic and systemic nitric oxide biosynthesis in septic rats. *Acta Anaesthesiol Scand* 2005; 49:28-34.
32. Couturier EG, Laman DM, van Duijn MA, van Duijn H. Influence of caffeine and caffeine withdrawal on headache and cerebral blood flow velocities. *Cephalalgia* 1997; 17(3):188-90.
33. Bottcher M, Czernin J, Sun KT, Phelps ME, Schelbert HR. Effect of caffeine on myocardial blood flow at rest and during pharmacological vasodilation. *J Nucl Med* 1995; 36(11):2016-21.
34. Casiglia E, Bongiovi S, Paleari CD, Petuccio S, Boni M, Colangeli G et al. Haemodynamic effects of coffee and caffeine in normal volunteers: a placebo-controlled clinical study. *J Intern Med* 1991; 229(6):501-4.
35. Hoecker Ch, Nelle M, Poeschl J, Beedgen B, Linderkamp O. Caffeine impairs cerebral and intestinal blood flow velocity in pre-term infants. *Pediatrics* 2002; 109(5):784-7.
36. Yano S, Isobe Y, Harada M. The etiology of caffeine-induced aggravation of gastric lesions in rats exposed to restraint plus water-immersion stress. *J Pharmacobiodyn* 1982; 5(7):485-94.
37. Umemura T, Ueda K, Nishioka K, Hidaka T, Takemoto H, Nakamura S et al. Effects of acute administration of caffeine on vascular function. *Am J Cardiol* 2006; 98:1538-41.
38. Wierema TK, Houben AJ, Kroon AA, Postma CT, Koster D, van Engelsehoven JM et al. Mechanisms of adenosine-induced renal vasodilatation in hypertensive patients. *J Hypertens* 2005; 23(9):1731-6.
39. Daniels JW, Mole PA, Shaffrath JD, Stebbins CL. Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise. *J Appl Physiol* 1998; 85(1):154-9.
40. Barton B, Kleinert JM. The effect of caffeine on digital haemodynamics. *J Hand Surg [Br]* 1994; 19(3):301-2.
41. Papamichael CM, Aznaouridis KA, Karatzis EN, Karatzi KN, Stamatiopoulos KS, Vamvakou G et al. Effect of coffee on endothelial function in healthy subjects: the role of caffeine. *Clin Sci (Lond)* 2005; 109(1):55-60.
42. Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999; 51:83-133.
43. Xu Z, Park SS, Mueller RA, Bagnell RC, Patterson C, Boysen PG. Adenosine produces nitric oxide and prevents mitochondrial oxidant damage in rat cardiomyocytes. *Cardiovasc Res* 2005; 65(4):803-12.
44. Al Moutaery K, Al Deeb S, Khan HA, Tariq M. Caffeine impairs short-term neurological outcome after concussive head injury in rats. *Neurosurgery* 2003; 53(3):704-12.
45. Wang Y, Lau ChE. Caffeine has similar pharmacokinetics and behavioral effects via the IP and PO routes of administration. *Pharmacol Biochem Behav* 1998; 60(1):271-8.