Scientia Medica

e-ISSN: 1980-6108 | ISSN-L: 1806-5562

10.15448/1980-6108.2018.2.29631

ORIGINAL ARTICLE

Open Access

Using the Reduced Uterine Perfusion Pressure model of preeclampsia to study the blood brain barrier permeability

Uso do modelo de Redução da Pressão de Perfusão Uterina de pré-eclâmpsia para estudar a permeabilidade da barreira hematoencefálica

Daniele Cristóvão Escouto[1,2] ✉, Giovani Gadonski[2], Luiz Porcello-Marrone[3], Jaderson Costa da Costa[3], Nathália Paludo[2], Rayssa Ruszkowski do Amaral[2], Bartira Ercília Pinheiro da Costa[1,2], Carlos Eduardo Poli-de-Figueiredo[1,2]

[1] Programa de Pós-Graduação em Medicina e Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.
[2] Laboratório de Nefrologia, Escola de Medicina da PUCRS. Porto Alegre, RS, Brazil.
[3] Instituto do Cérebro do Rio Grande do Sul, União Brasileira de Educação e Assistência. Porto Alegre, RS, Brazil.

How to cite this article:

Escouto DC, Gadonski G, Porcello-Marrone L, da Costa JC, Paludo N, do Amaral RR, da Costa BEP, Poli-de-Figueiredo CE. Using the Reduced Uterine Perfusion Pressure model of preeclampsia to study the blood brain barrier permeability. Sci Med. 2018;28(2):ID29631. http://doi.org/10.15448/1980-6108.2018.2.29631

ABSTRACT

AIMS: To use the Reduced Uterine Perfusion Pressure (RUPP) model for preeclampsia to describe and evaluate the blood brain barrier permeability in pregnant rats.

METHODS: Forty-one pregnant Wistar rats were divided into different intervention groups between 13 to 15 days of gestation: Pregnant-Control (PC; n=12), Reduced Uterine Perfusion Pressure (RUPP; n=15), Invasive Blood Pressure-Control (IBP; n=7) and Reduced Uterine Perfusion Pressure and Invasive Blood Pressure (RUPP-IBP; n=7). The 14 rats of groups IBP and RUPP-IBP had their mean arterial pressure measured at day 21. All animals were then sacrificed, administered Evans Blue dye through the tail vein and perfused with paraformaldehyde 4%. Brains were removed and evaluated by a blinded pathologist. Results are presented as means and standard errors. Comparisons between the groups were performed using Student's t-test for continuous variables and Fisher's exact test for categorical variables. Statistical significance was set as a p value less than 0.05.

RESULTS: Mean arterial pressure averaged 85.4±2.2 mmHg in the IPB group and 102.5±8.3 mmHg in the RUPP-IPB group (p=0.002). Among all the RUPP rats (RUPP and RUPP-IBP groups), 82% had a positive staining with Evans Blue dye for at least one of the brain hemispheres, while none of the pregnant control rats (PC and IBP groups) had brain staining (p<0.001).

CONCLUSIONS: In this study, altered permeability of the blood brain barrier was successfully reproduced in pregnant rats exposed to the RUPP protocol. Therefore, we concluded that the RUPP model is a valid surrogate to study blood brain barrier abnormalities.

KEYWORDS: animal models; blood-brain barrier; preeclampsia; eclampsia; toxemia of pregnancy; hypertension; posterior leukoencephalopathy syndrome; pregnancy complications.

RESUMO

OBJETIVOS: Usar o modelo de Redução da Pressão de Perfusão Uterina / *Reduced Uterine Perfusion Pressure* (RUPP) de pré-eclâmpsia para descrever e avaliar a permeabilidade da barreira hematoencefálica.

MÉTODOS: Quarenta e uma ratas Wistar prenhes foram estratificadas em diferentes grupos de intervenção entre 13 a 15 dias de gestação: grupo controle (PC; n=12), grupo modelo de redução da pressão de perfusão uterina (RUPP; n=15), grupo monitorização invasiva da pressão arterial (IBP; n=7) e grupo redução da pressão de perfusão uterina e monitorização invasiva da pressão arterial (RUPP-IBP; n=7). As 14 ratas dos grupos IBP e RUPP-IBP tiveram sua pressão arterial média aferida no dia 21. Logo após todos os animais foram sacrificados e foi administrado o corante Azul de Evans pela veia da cauda, seguido de formaldeído 4%. Os cérebros foram removidos e avaliados por um patologista cegado para os grupos. Os resultados são apresentados em médias e erros padrão. As comparações entre os grupos foram realizadas utilizando o teste t de Student para variáveis contínuas e o teste exato de Fisher para variáveis categóricas. A significância estatística foi definida como um valor de p inferior a 0,05.

RESULTADOS: As médias e desvios padrões da pressão arterial média foram 85,4±2,2 mmHg no grupo IPB e 102,5±8,3 mmHg no grupo RUPP-IPB (p=0,002). Entre todas as ratas RUPP (grupos RUPP e RUPP-IBP), 82% tiveram marcação positiva pelo corante em pelo menos um dos hemisférios cerebrais, enquanto nenhuma das ratas controle (grupos PC e IBP) teve marcação cerebral positiva (p<0,001).

CONCLUSÕES: Neste estudo, a permeabilidade alterada da barreira hematoencefálica foi reproduzida com sucesso em ratas prenhes expostas ao protocolo RUPP. Portanto, concluímos que o modelo RUPP é um substituto válido para estudar anormalidades da barreira hematoencefálica.

DESCRITORES: modelos animais; pré-eclâmpsia; eclampsia; toxemia gravídica; hipertensão; síndrome da leuconencefalopatia posterior; complicações da gestação.

Received: *2018/01/09*
Accepted: *2018/05/02*
Published: *2018/06/12*

✉ ***Correspondence:*** daniele.escouto@acad.pucrs.br
ORCID: http://orcid.org/0000-0002-4470-9834
Hospital São Lucas da PUCRS, Laboratório de Nefrologia
Av. Ipiranga, 6690, 2º andar – CEP 90610-000, Porto Alegre, RS, Brazil

Abbreviations: BBB, blood brain barrier; IBP, invasive blood pressure control; ID, inner diameter; MAP, mean arterial pressure; PC, pregnant-control; PE, preeclampsia; PRES, posterior reversible encephalopathy syndrome; RUPP, reduced uterine perfusion pressure.

INTRODUCTION

Preeclampsia (PE) is a pregnancy disorder characterized by hypertension and pathologic proteinuria after the 20th gestational week [1]. During 2002-2010 its incidence was estimated in 2-8% of all pregnancies worldwide [2]. Understanding the pathophysiology of PE is still a challenge and the main focus of many researchers. The reduced uterine perfusion pressure (RUPP) model of PE, developed at the University of Mississippi, reassembles numerous features of human PE, and helps to study the underlying mechanisms of hypertension induced by placental ischemia in pregnancy. The model has demonstrated approximately 40% chronic reduction in uterine perfusion pressure and an increase of mean arterial pressure (MAP) in approximately 20-30 mmHg [3]. Other characteristics of PE have been demonstrated in the RUPP model: increased urinary protein excretion, decreased glomerular filtration rate and renal plasma flow, fetal intrauterine growth restriction, reduction in cardiac index, increased inflammatory markers (tumor necrosis factor [TNF] alpha, interleukin [IL]-6) and angiogenic factors (sFlt-1, soluble endoglin) [3-5]. Projects in collaboration with Professor Joey P. Granger, from the University of Mississippi, have brought the opportunity of learning and establishing the RUPP rat model in our research group [5].

Eclampsia is characterized by occurrence of seizures without other recognizable cause during pregnancy or at the early post-partum in women with PE features [1]. Despite of occurring in less than 0.3% of pregnancies, the presence of eclampsia elevates up to 40 folds the odds ratio for maternal mortality [6]. Posterior reversible encephalopathy syndrome (PRES) is a clinical disorder characterized by neurological symptoms like headache, visual loss, altered mental status and seizures. PRES is commonly associated with eclampsia [7]. The cause of PRES is unknown, but the syndrome is usually triggered by acute elevation of blood pressure, renal injury, systemic infections, and/or use of immunosuppressive therapy. Radiologic images demonstrate vasogenic edema of white matter predominantly at occipital and parietal lobes of brain, characteristically sparing other brain structures and reducing after weeks of evolution [8].

Nevertheless, the underlying mechanisms of these complications have not been established. Pathogenesis of the neurologic presentations of PE seems to be similar to hypertensive encephalopathy. Absence of elevated blood pressure in some cases suggests that hypertension is not the single component responsible for the neurologic manifestations [9]. We have previously proposed an animal model with altered blood brain barrier (BBB) permeability using the RUPP model for PE [10]. The aim of this study is to describe the BBB altered permeability in RUPP model. We believe this model may be of use for further pathophysiological investigations of PE neurologic manifestations.

METHODS

The study was performed in 41 age-matched, timed pregnant Wistar rats with 90 days life. Animals were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle and were given free access to water and standard rat chow. Rats intended to become pregnant were placed with a fertile male for one night, and day one of pregnancy was considered with a perineum smear with presence of semen. The pregnant rats were divided in four groups: Pregnant-Control (PC; n=12), Reduced Uterine Perfusion Pressure (RUPP; n=15), Invasive Blood Pressure (IBP; n=7) and Reduced Uterine Perfusion Pressure and Invasive Blood Pressure (RUPP-IBP; n=7). All rats had at least eight pups and were sacrificed at day 21 after procedures.

All protocol procedures were in accordance to the preconized standards for animal use in research. The study was approved by the Institutional Animal Care and Ethics University Committee, protocol 11/00222.

Reduced Uterine Perfusion Pressure protocol

Animals from RUPP group were subjected to the surgical procedure between days 13 to 15 of pregnancy. The animals underwent intraperitoneal anaesthesia, using insulin needles, with Ketamine 5% (0.21 mg/kg) and Xylazine 2% (0.42 mg/kg), allowing midline abdominal incision and exposure of the lower abdominal aorta. The RUPP was performed according to the University of Mississippi Medical Centre protocol [3]: a silver clip of 0.2 mm inner diameter (ID) was placed at the abdominal aorta below the renal arteries, and to avoid compensation of blood flow to

the placenta through an adaptive increase in ovarian blood flow [11], silver clips of 0.2 mm ID were placed around both the left and right uterine arcade, after the ovarian arteries and before the first uterine segmental artery. Silver clips were obtained by specific order to a goldsmith. After the procedure, suture by surgical layers was made with silk line 2.0 (deeper level) and *mononylon* 2.0 (superficial level). **Figure 1** shows the procedure schematically.

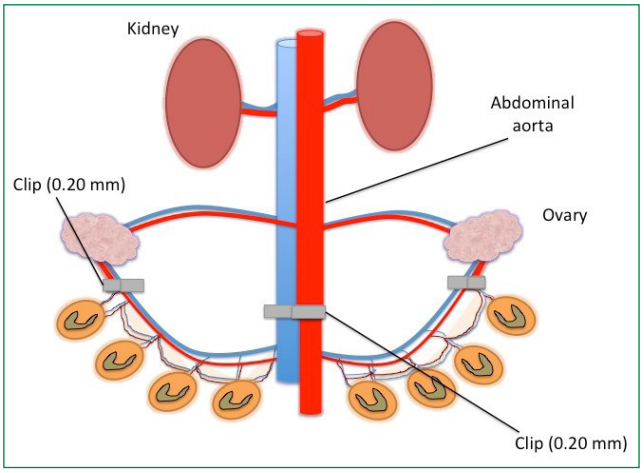


Figure 1. Modified from Li et al., 2012 (ref. 14). Representation of the Reduced Uterine Perfusion Pressure model procedure. Demonstrating placement of silver clips in the abdominal aorta below the renal arteries and around both the left and right uterine arcade, resulting in narrowing of the distal arterial flow.

Protocol for invasive measurement of blood pressure in conscious rats

At day 20 of gestation, animals of both IBP and RUPP-IBP groups had MAP measurement. Under the same anaesthesia protocol, an anterior cervical midline incision was made in order to expose the left carotid artery. A catheter (Micro Medical Tubing 85 Durometer Vinyl, BB31785-V/3, Scientific Commodities Inc., Lake Havasu City, AZ, USA) was then inserted into the left carotid artery with the opposite extremity exteriorized in the posterior cervical region. One day after, when full anaesthesia recovering was achieved, conscious rats had their MAP monitored by a transducer (PX 260, Edwards Lifesciences, Irvine, CA, USA) with saline solution connected to a sphygmomanometer and a water column to equalize with atmospheric pressure. The transducer was connected to the Kananda pressure transducer-recording device (Dr. Marcio Flavio Dutra Moraes, Belo Horizonte, Brazil). This device converts blood real time pressure measurements registered in digital records in a microcomputer and displayed in mmHg [12]. The animals were motorized for an average of two minutes. Measured blood pressure values were transferred to Excel 6.0 software to calculate MAP.

Evaluation of the blood brain barrier with Evans Blue

All animals were sacrificed at 21 days of gestation. Evans Blue dye (2% wt/vol in 0.9% NaCl) was then administered to the rat's tail vein with a 12.7 mm needle, dosage of 3 mL/kg. Thoracic incisions were made to enable transcardiacal cannulation of aortic artery. A solution of paraformaldehyde 4% was used for animal perfusion, through a perfusion pump until observed stiffness of the rat. After, 250 mL of cold phosphate buffered saline were perfused to remove intravascular Evans Blue dye [13]. Brains were removed and immediately frozen at -20°C.

Brain evaluation

Brains underwent evaluation by a blinded pathologist. After macroscopic evaluation they were sliced using a cryostat into 30 µm coronal sections. An Olympus CH-30 electronic microscope (Olympus, Tokyo, Japan) was used for microscopic evaluation.

Statistical Analysis

Data were analysed using the SPSS Statistics version 17.0 software (IBM, Chicago, IL, USA). Results are presented as means and standard errors. Comparisons between the groups were performed using Student's t-test for continuous variables and Fisher's exact test for categorical variables. Statistical significance was set as a p value less than 0.05.

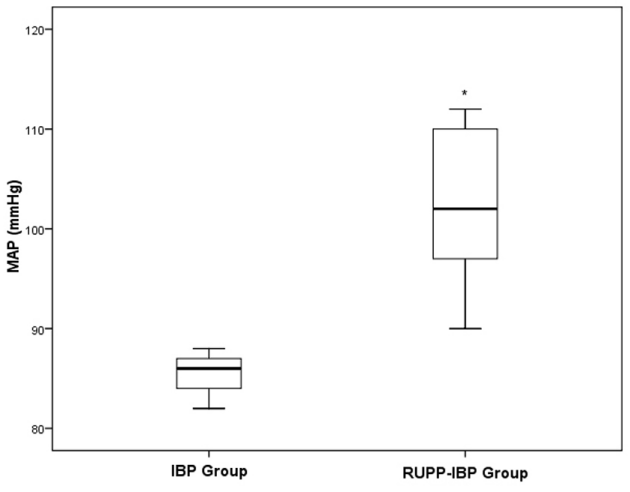
RESULTS

Rats that underwent blood pressure measurements (IBP and RUPP-IBP) had the MAP measured. MAP means and standard errors were 85.4±2.2 mmHg in the IBP group (n=7) and 102.5±8.3 mmHg in the RUPP-IBP group (n=7) (p=0.002). The values for each animal are shown in **Table 1**. There was a statistically significant increase in MAP in RUPP-IBP group (p=0.002) (**Figure 2**).

Table 1. Mean arterial blood pressure in the 14 rats from both control and RUPP groups submitted to invasive blood pressure measurement.

Group	Rats	Mean arterial pressure (mmHg)
IBP	1	84
	2	386
	3	84
	4	82
	5	88
	6	86
	7	88
Mean±SE		85.4±2.2*
RUPP-IBP	1	112
	2	110
	3	110
	4	90
	5	96
	6	102
	7	98
Mean±SE		102.5±8.3*

RUPP, reduced uterine perfusion pressure; IBP, invasive blood pressure measurement; RUPP-IBP, reduced uterine perfusion pressure rats submitted to invasive blood pressure measurement; SE, standard error.
* Student's t-test: p=0.002.



RUPP, reduced uterine perfusion pressure; IBP, invasive blood pressure measurement; RUPP-IBP, reduced uterine perfusion pressure rats submitted to invasive blood pressure measurement.
Student's t-test: p=0.002.

Figure 2. Boxplot for mean arterial blood pressure in both IBP and RUPP-IBP groups.

None of the pregnant control rats (PC, n=12; IBP, n=7) had brain staining with Evans Blue dye. Among all the RUPP rats (RUPP, n=15; RUPP-IBP, n=7), 82% had a positive staining for at least one of the brain hemispheres (p<0.001). One rat from RUPP group and one rat from RUPP-IBP group had only the right brain hemisphere stained. Two brains (RUPP n=1; PC=1) were stained with Hematoxylin and Eosin (H&E) and Periodic Acid–Schiff, to verify whether visualizing the dyed cerebral parenchyma was possible even after dehydration, parafinization and coloration.

Small histological differences were observed between submitted or not submitted to RUPP. Among these alterations, RUPP sample demonstrated a constant increase of the perivascular space, which does not occur in the brains of animals not submitted to RUPP. In these brains stained by H&E, the presence of Evans blue was not observed. Another microscopic observation was the lower cell density in the RUPP rats when compared with pregnant control groups.

DISCUSSION

Using an invasive methodology, the RUPP animals in this study had clearly a higher blood pressure, the main characteristic of PE, when compared to controls. We also demonstrated that rats submitted to the RUPP procedure had altered permeability of BBB, verified by cerebral staining with Evans Blue dye. No staining was detected in the pregnant control rats. The reproduction of this model allows us to develop research protocols to investigate the different pathways involved in the pathophysiology of PE and its cerebral manifestations.

Considering the ethical limitations of studies involving pregnant women, the RUPP model has been proposed as a useful tool to study the pathophysiology of PE [14]. The RUPP model in the rat has been the most extensively studied and is considered the experimental model that mostly reassembles severe features of PE, including proteinuria, impaired renal function, reduction in cardiac index and fetal intrauterine growth restriction, with decreased litter size and pup weight [14, 15]. The model has been reproduced in many experimental studies recently published, including studies evaluating the role of autoimmunity, angiogenic and anti-angiogenic factors in the physiopathology of PE [4, 16, 17].

In this study, we induced the reduction of uterine blood flow with the same specifications of the model proposed by Professor Granger, published by Alexander et al. [3]. Therefore, we decided to use the MAP elevation to demonstrate the success of the technique. We chose the RUPP model to evaluate the BBB altered permeability because this model is believed to be excellent for the study of many of the consequences of placental ischemia, including hypertension and vascular dysfunction.

This study successfully reproduced the altered permeability of the BBB in RUPP rats. However, instead of predominantly affecting posterior cerebral structures as in women affected by PRES [7], the entire cerebral parenchyma of RUPP rats was dyed in blue. A possible explanation would be that, differently of humans, rats do not present with specific pressure receptors that protect the anterior cerebral circulation from hypertensive lesions [10].

Understanding the mechanisms involved in cerebral microvasculature alterations of PE is one of the current research topics in our group. One of the possible mechanisms involved is similar to what happens in hypertensive encephalopathy. The acute blood pressure elevation would overcome the auto regulatory capacity of cerebral arteries to resist elevations of the cerebral blood perfusion pressure. That would cause endothelial cell damage, BBB breakdown and ultimately vasogenic edema [19]. However, in conditions like eclampsia and PRES, cerebral edema and neurologic manifestations can occur at normal blood pressures, suggesting that the auto regulatory breakthrough can be related to other factors, or even to a reduced auto regulatory capacity of cerebral arteries in pregnancy.

Alterations of the BBB permeability in preeclamptic women have also been proposed as a mechanism. Circulating factors increased in plasma of women with PE have been associated with increased BBB permeability. Especially the vascular endothelial growth factor that had increased activity of its tyrosine kinase receptor or s-Flit (a vascular endothelial growth factor receptor) associated to augmented BBB permeability [20].

Nevertheless, criticisms concerning this animal model have to be addressed. Etiology of PE cannot be inferred from this experimental model; the RUPP model only reproduces the placentary ischemia involved in PE; and induced vascular dysfunction occurs after the 14th gestational day, leaving only seven to eight days to evaluate gestational outcomes [14]. Pathophysiology investigation is also limited by the impossibility to evaluate reversibility of presentations, since the RUPP model is irreversible even after delivery. Furthermore, we have to be careful in addressing the translation of experimental studies to patient care.

There are several difficulties for the development of protocols for the study of PE in pregnant women, mostly concerning ethical limitations. In order to overcome some of those limitations, the establishment and validation of an animal model for research of the BBB is an important concern for our group. In this study, altered permeability of the BBB was successfully reproduced in pregnant rats exposed to the RUPP protocol. Therefore, we concluded that the RUPP model is a valid surrogate to study BBB abnormalities.

NOTES

Funding

This study received financial support from the Brazilian Coordination for Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES).

Conflicts of interest disclosure

The authors declare no competing interests relevant to the content of this study.

Authors' contributions

All the authors declare to have made substantial contributions to the conception, or design, or acquisition, or analysis, or interpretation of data; and drafting the work or revising it critically for important intellectual content; and to approve the version to be published.

Availability of data and responsibility for the results

All the authors declare to have had full access to the available data and they assume full responsibility for the integrity of these results.

REFERENCES

1. NHBPEPWG. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol. 2000;183(1):S1-S22. https://doi.org/10.1067/mob.2000.107928
2. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013;170(1):1-7. https://doi.org/10.1016/j.ejogrb.2013.05.005
3. Alexander BT, Kassab SE, Miller MT, Abram SR, Reckelhoff JF, Bennett WA, Granger JP. Reduced uterine perfusion pressure during pregnancy in the rat is associated with increases in arterial pressure and changes in renal nitric oxide. Hypertension. 2001;37(4):1191. https://doi.org/10.1161/01.HYP.37.4.1191
4. Spradley FT, Tan AY, Joo WS, Daniels G, Kussie P, Karumanchi SA, Granger JP. Placental Growth Factor Administration Abolishes Placental Ischemia-Induced Hypertension. Hypertension. 2016;67(4):740-7. https://doi.org/10.1161/HYPERTENSIONAHA.115.06783
5. Gadonski G, LaMarca BB, Sullivan E, Bennett W, Chandler D, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of interleukin 6. Hypertension. 2006;48(4):711-6. https://doi.org/10.1161/01.HYP.0000238442.33463.94

6. Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel J, Souza JP. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121 Suppl 1:14-24. https://doi.org/10.1111/1471-0528.12629
7. Brewer J, Owens MY, Wallace K, Reeves AA, Morris R, Khan M, LaMarca B, Martin JN Jr. Posterior reversible encephalopathy syndrome in 46 of 47 patients with eclampsia. Am J Obstet Gynecol. 2013;208(6):468.e1-6. https://doi.org/10.1016/j.ajog.2013.02.015
8. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, Pessin MS, Lamy C, Mas JL, Caplan LR. A reversible posterior leukoencephalopathy syndrome. N Engl J Med. 1996;334(8):494-500. https://doi.org/10.1056/NEJM199602223340803
9. Karumanchi SA, Lindheimer MD. Advances in the understanding of eclampsia. Curr Hypertens Rep. 2008;10(4):305-12. https://doi.org/10.1007/s11906-008-0057-3
10. Porcello Marrone LC, Gadonski G, de Oliveira Laguna G, Poli-de-Figueiredo CE, Pinheiro da Costa BE, Lopes MFT, Brunelli JFP, Diogo LP, Huf Marrone AC, Da Costa JC. Blood-brain barrier breakdown in reduced uterine perfusion pressure: a possible model of posterior reversible encephalopathy syndrome. J Stroke Cerebrovasc Dis. 2014;23(8):2075-9. https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.03.012
11. Nienartowicz A, Link S, Moll W. Adaptation of the uterine arcade in rats to pregnancy. J Dev Physiol. 1989;12(2):101-8.
12. Cardoso LM, Pedrosa ML, Silva ME, Moraes MF, Colombari E, Chianca DA. Baroreflex function in conscious rats submitted to iron overload. Braz J Med Biol Res. 2005;38(2):205-14. https://doi.org/10.1590/S0100-879X2005000200008
13. Gage GJ, Kipke DR, Shain W. Whole animal perfusion fixation for rodents. J Vis Exp. 2012;(65). pii: 3564. https://doi.org/10.3791/3564
14. Li J, LaMarca B, Reckelhoff JF. A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model. Am J Physiol Heart Circ Physiol. 2012;303(1):H1-8. https://doi.org/10.1152/ajpheart.00117.2012
15. McCarthy FP, Kingdom JC, Kenny LC, Walsh SK. Animal models of preeclampsia; uses and limitations. Placenta. 2011;32(6):413-9. https://doi.org/10.1016/j.placenta.2011.03.010
16. Brennan L, Morton JS, Quon A, Davidge ST. Postpartum Vascular Dysfunction in the Reduced Uteroplacental Perfusion Model of Preeclampsia. PLoS One. 2016;11(9):e0162487. http://doi.org/10.1371/journal.pone.0162487
17. Cornelius DC, Amaral LM, Wallace K, Campbell N, Thomas AJ, Scott J. Reduced uterine perfusion pressure T-helper 17 cells cause pathophysiology associated with preeclampsia during pregnancy. Am J Physiol Regul Integr Comp Physiol. 2016; 311(6):R1192-R1199. https://doi.org/10.1152/ajpregu.00117.2016
18. Cipolla MJ. Cerebrovascular function in pregnancy and eclampsia. Hypertension. 2007;50(1):14-24. https://doi.org/10.1161/HYPERTENSIONAHA.106.079442
19. Auer LM. The pathogenesis of hypertensive encephalopathy. Experimental data and their clinical relevance with special reference to neurosurgical patients. Acta Neurochir Suppl (Wien). 1978;27:1-111
20. Amburgey OA, Chapman AC, May V, Bernstein IM, Cipolla MJ. Plasma from preeclamptic women increases blood-brain barrier permeability: role of vascular endothelial growth factor signaling. Hypertension. 2010;56(5):1003-8. https://doi.org/10.1161/HYPERTENSIONAHA.110.158931