Blood–Brain Barrier Breakdown in Reduced Uterine Perfusion Pressure: A Possible Model of Posterior Reversible Encephalopathy Syndrome

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**Background:** Posterior reversible encephalopathy syndrome (PRES) is a clinical entity characterized by headaches, altered mental status, seizures, and visual disturbances and is associated with white matter vasogenic edema. There are no experimental models to study PRES brain changes. **Methods:** Twenty-eight pregnant Wistar rats were divided into 4 groups of 7: (1) pregnant-control; (2) reduced uterine perfusion pressure (RUPP); (3) invasive blood pressure (IBP); and (4) reduced uterine perfusion pressure plus invasive blood pressure (RUPP-IBP). The RUPP and RUPP-IBP groups were submitted to a reduction of uterine perfusion pressure at pregnancy days 13 to 15. The invasive mean arterial pressure of the IBP and RUPP-IBP groups was measured on day 20. The blood–brain barriers (BBBs) of all groups were analyzed using 2% Evans Blue dye on day 21. **Results:** RUPP rats had higher blood pressures and increased BBB permeability to Evans Blue dye compared with the control animals. Brain staining occurred in 11 of 14 RUPP rats and in none of the control groups (P < .0001). **Conclusions:** The physiopathology of PRES remains unclear. Here, we described the use of RUPP rats as a potential model to better comprehend this syndrome. **Key Words:** Posterior reversible encephalopathy syndrome—blood–brain barrier—experimental model—reduction of uterine blood pressure—hypertension.

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**Posterior reversible encephalopathy syndrome (PRES)** is a clinical entity characterized by headaches, altered mental status, seizures, and visual loss. It associated with white matter vasogenic edema on radiologic imaging, predominantly affecting the occipital and parietal lobes of the brain. Numerous factors can trigger the syndrome, most commonly an acute elevation of blood pressure, renal dysfunction, and/or immunosuppressive therapy. Pre-eclampsia (PE) is one of the most common syndromes that is associated with PRES. Other clinical conditions that are associated with PRES are transplantation, cancer and chemotherapy treatment, systemic infections, and acute or chronic renal diseases.
The most characteristic imaging pattern in PRES is the presence of edema involving the white matter of the posterior portions of both cerebral hemispheres, especially the parieto-occipital regions, in a relatively symmetric pattern that spares the calcarine and paramedian parts of the occipital lobes. However, other structures (such as the brain stem, cerebellum, and frontal and temporal lobes) may also be involved. The abnormality primarily affects the subcortical white matter, but the cortex and the basal ganglia may also be involved.7

The cause of PRES is unknown. Autoregulatory failure with resultant vasodilation, as observed in hypertensive encephalopathy, is often suggested as the underlying mechanism. On the other hand, vasospasm with ischemic changes is also observed in some patients.8,9 To our knowledge, a PRES animal model evaluating the underlying mechanisms of this serious syndrome has not yet been established. A well-established model of PE is the reduced uterine perfusion pressure (RUPP), which is associated with arterial hypertension, increased urinary protein excretion, reduced glomerular filtration rate and renal plasma flow, and decreased litter size and pup weight.10,11

The blood–brain barrier (BBB) of RUPP animals has not been previously examined. We hypothesize that altered permeability is present and that this model may be proposed to study PRES.

Methods

All the studies were performed in age-matched, timed pregnant Wistar rats. The animals were housed in a temperature-controlled room (23°C) with a 12:12-hour light:dark cycle. All the experimental procedures and protocols executed in this study were approved by the Institutional Animal Care and Ethics Committee from protocols executed in this study were approved by the Pontifícia Universidade Católica do Rio Grande do Sul.

Twenty-eight pregnant rats were divided in 4 groups: (1) pregnant-control group (n = 7); (2) RUPP group (n = 7); (3) invasive blood pressure (IBP) group (n = 7); and (4) reduced uterine perfusion pressure plus invasive blood pressure (RUPP-IBP) group (n = 7).

Protocol for RUPP

The animals of the 2 groups (RUPP and RUPP-IBP) were submitted to the intervention to reduce the uterine perfusion pressure.

From day 13 to day 15 of pregnancy, the pregnant rats were anesthetized with 5% of ketamine and 2% of xylazine by intraperitoneal injection. The abdominal cavity was approached via a midline incision. The lower abdominal aorta was exposed, and a silver clip (2 mm interdiameter) was placed around the aorta above the iliac bifurcation and below the renal arteries as previously described.10,12,13

This procedure has been shown to reduce the uterine perfusion pressure in the gravid rat by 40%.14 The compensation of blood flow to the placenta occurs in pregnant rats by an adaptive increase in the ovarian blood flow. Consequently, a silver clip (2 mm interdiameter) was also placed on the main uterine branches of both the right and left ovarian arteries.15

The RUPP rats in which the clipping procedure resulted in maternal death (n = 1) or total reabsorption (n = 3) of the fetuses were excluded from the study. All the other animals had at least 8 pups.

Protocol for Measurement of Invasive Blood Pressure

The animals of the 2 groups (mean invasive blood pressure and RUPP-IBP) were submitted to the measurement of mean arterial pressure (MAP).

On day 20, an arterial catheter was placed in the carotid artery under anesthesia. On day 21, the measurement of MAP in the conscious rat was performed using a pressure transducer. The arterial pressure was monitored with a pressure transducer connected to the Kananda arterial pressure recording device (Dr. Marcio Flavio Dutra Moraes, Belo Horizonte, Brazil). Kananda is a device that transforms blood pressure measurements via sphygmomanometer into records on a microcomputer in real time.16

The measured blood pressure values were transferred to the Excel 2007 software to calculate the MAP of these groups.

Evaluation of the BBB by Evans Blue and Brain Tissue Processing

The BBB permeability was evaluated in all the animals using Evans Blue at day 21. Evans Blue dye (2% wt/vol in 9% NaCl) was intravenously administered (3 mL/kg) via the tail vein at the start of a 3-hour perfusion. At the end of perfusion, the rats were transcardially perfused with 250 mL cold phosphate buffered saline to remove the intravascular Evans Blue dye. The brains were then removed and rapidly frozen in a −20°C freezer.

Brain Evaluation

All the rat brains were macroscopically evaluated by a pathologist who was unaware of the groups. After this analysis, the brains were prepared for a microscopic evaluation using an Olympus CH-30 electronic microscope (Olympus, Tokyo, Japan). The brains were cut into 30 μm coronal sections with a cryostat for microscopic evaluation.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Science version 16.0 (SPSS/IBM–Chicago, IL). The results are presented as the mean and standard deviation. The comparisons between the groups were performed using Student t test or the chi-square test.
The MAP in the RUPP group was significantly higher than that of the controls (102.5 ± 8.3 mm Hg vs 85.4 ± 2.2 mm Hg, P < .002; Fig 1).

The BBB permeability was examined in the 28 rats from the 4 groups. Evans Blue dye was detected in brain parenchyma in the 2 groups that were submitted to reduced uterine perfusion pressure (RUPP and RUPP-IBP). Evans Blue was present in the parenchyma of 6 of the 7 animals in the RUPP group and 5 of the 7 in the RUPP-IBP group. Only 1 hemispheric brain staining was observed in 2 animals from each of the RUPP groups.

In the control groups (pregnant-control and IBP groups), there was no staining of the brain. Brain staining occurred in 11 of the 14 RUPP or RUPP-IBP brains (Fisher exact test, P < .0001). Macroscopic examination revealed an intense diffused uptake, especially of the blue pigment in the cortex of the RUPP brains (Fig 2).

On microscopic examination, histologic differences were observed between the RUPP and the controls. RUPP animals had an increased perivascular space, which was not present in the pregnant-controls or IBP (Fig 2). An interesting observation on microscopic examination was that the cell density was reduced in the group submitted to RUPP when compared with the groups that were not subjected to RUPP (7.9 ± 1.1 cells per field × 10.6 ± 1.5 cells per field, P < .0001).

Figure 1. Reduced uterine perfusion pressure (RUPP) group mean arterial pressure (MAP) increased significantly compared with the control group (102.5 ± 8.3 mm Hg vs 85.4 ± 2.2 mm Hg, P < .002).

Figure 2. Macroscopic and microscopic samples. Brain slices from animals subjected to reduced uterine perfusion pressure (RUPP) after staining with Evans Blue in the RUPP group (A) and the control group (B); histologic sections stained with hematoxylin and eosin (400×) RUPP animal (C) vs control animal (D). Lower cell density and increased perivascular space (arrowhead) in RUPP animal (C). (Color version of figure is available online.)
Discussion

For the first time, we have demonstrated the altered BBB permeability in the RUPP model of PE. We suggest that this model may be used to further investigate the pathophysiologic mechanisms involved in PRES.

The search for an experimental model that simulated PRES made us consider using a PE animal model. In our experience, PE is the most frequent cause of PRES at São Lucas Hospital (Porto Alegre, Brazil). The RUPP model is an established model for the study of the physiopathologic features of PE and is an excellent opportunity to examine the brain. The changes found in the brains of the RUPP animals, arising from the breakdown of the BBB, were confirmed with the macroscopic and microscopic identification of Evans Blue dye in the cerebral cortex.

An acute increase in blood pressure is a frequent finding in PRES and a typical aspect in the RUPP model. The mechanism involved in the BBB breakdown is not clear. According to Brewer et al., PRES is the primary central nervous system injury in patients with eclampsia. Some of the changes in normal pregnancy include substantially increased levels of vasoangiogenic growth factors and cytokines in the maternal circulation. However, during normal pregnancy, the BBB adapts to prevent these circulating permeability factors from entering the brain and thus leading to vasogenic brain edema. Interestingly, plasma from pre-eclamptic women has been found to increase the BBB permeability. This suggests that an increase in BBB permeability could permit the passage of damaging antivasogenic and antiendothelial proteins into the brain to cause the neurologic complications of eclampsia.

PRES is commonly observed in the setting of hypertension, most likely because of a breakdown of cerebral blood flow autoregulation. The cerebral blood flow autoregulation is an intrinsic function of the brain, which is designed to maintain a stable blood flow independent of the variation of blood pressure. In animal models, an arteriolar dilation, injury to the capillary bed, vasogenic edema, and vessel injury with altered artery morphology would occur when a severe increase in blood pressure beyond the upper limit of autoregulation was caused.18

The histologic evaluation of the brains allowed us to verify that the Evans Blue dye was only found in animals that were subjected to the RUPP model. Some changes may be evidenced in the RUPP samples in which there is a persistently expanded perivascular space that appears to be empty and without any dye stains; this may correspond to perivascular edema. The lower cell density may also indicate that these cells are more spaced by interstitial edema in the RUPP groups. In humans, the abnormal findings are mainly in the posterior areas of the brain for unknown reasons. The anterior cerebral circulation, via carotids and arteries, has more autonomic receptors than the posterior circulation via the vertebrobasilar system.19,20 This fact can be a potential difference that leads to the predominance of PRES in the posterior lobes. If the cerebral autoregulation is more sensible in the posterior circulation, this predisposes patients to an autoregulation breakdown in this vascular zone. In this model, all the encephalic parenchyma showed an increased permeability of the BBB because this vascular difference is not present in Wistar rats.27 In addition, a difference in the brain territories is not described in rats. We speculate that this may explain the lack of preference for alteration in the posterior regions of the rat brain.

The reversibility of the increased permeability of the BBB is observed in a few clinical situations. When tissue damage occurs as a result of brain tumors, infection, and vascular disorders, the permeability of the BBB remains persistently increased. In these cases, there is a change in the anatomic substrate of the BBB that includes a functional synergism between the various tissue elements.21 The reversibility of transient BBB permeability was observed in previous experimental studies that induced seizures in rats, suggesting functional changes in the permeability of the BBB. Thus, the electrophysiologic changes determined by the seizures could explain the changes in the selectivity of the BBB to dyes.22 The major limitation of this model is that we were unable to verify the reversibility of cerebral edema. The reduced uterine perfusion was obtained by a mechanical mechanism; the clip was permanent. We believe that the evaluation of the reversibility of cerebral edema development and evolution could be monitored with the help of neuroimaging evaluations in the future.

In summary, the physiopathology of PRES remains enigmatic. In this article, we suggested for the first time the use of the RUPP model as a PRES model, which we believe will be crucial for a better comprehension of this syndrome.

References