Reproductive Toxicology 65 (2016) 359–364

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

# Evaluation of the protective effect of thiamine pyrophosphate based on the biochemical analysis of rabbit foetuses at 30 days of gestation

M.A. Jiménez-Bravo[a,b], D. Mota-Rojas[c,*], H. Orozco-Gregorio[d], B. Pérez-Guille[b], R. Soriano-Rosales[b], P. Roldan-Santiago[c], M. Alonso-Spilsbury[c], E. Arch-Tirado[e], P. Mora-Medina[f], J. Martínez-Burnes[g]

[a] Ph. D. Program in Biological Sciences and Health, Universidad Autónoma Metropolitana (UAM), Mexico City 04960, Mexico
[b] Experimental Surgery Department, Instituto Nacional de Pediatría (INP), Mexico City 04530, Mexico
[c] Stress Physiology and Farm Animal Welfare, Department of Animal Production and Agriculture, Universidad Autónoma Metropolitana (UAM), Mexico City 04960, Mexico
[d] Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luis Potosí (UASLP), San Luis Potosí 78321, Mexico
[e] Bioacoustics Laboratory, Instituto Nacional de Rehabilitación (INR), Mexico City 14389, Mexico
[f] Department of Livestock Sciences, FESC., Universidad Nacional Autónoma de México (UNAM), State of Mexico 54714, Mexico
[g] Postgraduate Division and Research, Veterinary Medicine Faculty, Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico

ARTICLE INFO

*Article history:*
Received 4 November 2014
Received in revised form 3 September 2016
Accepted 9 September 2016
Available online 11 September 2016

*Keywords:*
Foetuses
Hypoxic-encephalopathy
Protective effect
Thiamine pyrophosphate
Foetal welfare
Cocarboxylase

ABSTRACT

This study evaluated the effects of thiamine pyrophosphate (PPT) on the biochemical profiles of full-term rabbit foetuses that were subjected to experimental ischemia followed by 24 h reperfusion. A total of 16 gestating rabbit dams were divided into two groups, one of which was treated by administering PPT and subjected to a process ischemia. During this interval, fetal blood samples were drawn from each dam (in the ischemia group) at 0, 15 and 45 min. Ischemia for 15 and 45 min was not associated with changes in lactate levels of the Ischemia group foetuses. However, in the foetuses in the reperfusion groups without PPT lactate levels were significantly higher after 15 and 45 min of arterial occlusion compared to time zero. These results demonstrate that PTT alters some acute and some longer-term biochemical outcomes of uterine ischemia perhaps important in preserving energy metabolism under hypoxic conditions.


## 1. Introduction

Disorders that damage the developing brain are a significant cause of death or permanent disability, such as cerebral palsy. Hypoxic-ischemia encephalopathy (HIE) occurs at a rate of about three per thousand live-born, full-term infants [1]. While mild forms of encephalopathy have good prognosis, in moderate and severe cases the risk of death [2] or neurodevelopmental sequal in the surviving offspring increases greatly [3]. Asphyxia events have been evaluated in several animal models, including pigs [4,5], guinea pigs [6], and rats [7]. In these animals, asphyxia triggers a cascade of cellular biochemical events that lead to temporary alterations in cellular function and/or cell death. Tissue hypoxia and ischemia, meanwhile, can result in the depolarization of neuronal membranes, alterations of cellular ion homeostasis, and changes in energy metabolism [8]. Various alternative therapies have been utilised to treat the secondary lesions that result from hypoxic events, including hypothermia and the administration of free radical scavengers or calcium channel blockers [9]. Some of these measures have been incorporated into clinical practice with relative success. However, considering that damage is progressive and largely-dependant on the energy supplies that reach the cell, an additional option could consist in administering molecules that act on the enzymatic pathways associated with energy production in order to reduce the extent of damage. Thiamine exerts its metabolic action primarily through thiamine pyrophosphate, or cocarboxylase (PPT), which acts through two different pathways; first, by donating phosphate groups to form ATP and other energy molecules, such as TATP (adenosine-thiamine-triphosphate); and, second, as an indispensable co-factor in the activation of enzymes involved in energy-generating processes through the Krebs Cycle or the pentose cycle [10]. Although Valenzuela [7] has demonstrated

* Corresponding author at: Calzada del Hueso 1100, Col. Villa Quietud, 04960 Coyoacán, Mexico, D.F., Mexico.
*E-mail addresses:* dmota100@yahoo.com.mx, dmota@correo.xoc.uam.mx (D. Mota-Rojas).

http://dx.doi.org/10.1016/j.reprotox.2016.09.003
0890-6238/

that administering PPT reduces the damage induced by neonatal hypoxia in 11-day-old rats, it has not yet been possible to develop a treatment designed to prevent the cellular damage caused by a pre-birth hypoxic event. Therefore, the objective of this study was to evaluate the protective effect of PPT on the physiological profiles of full-term rabbit foetuses that suffer a controlled ischemic insult.

## 2. Materials and methods

### 2.1. Ethical note

This protocol for animal research was approved by the Institutional Animal Care and Use Committee of the Universidad Autónoma Metropolitana (Mexico City), in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study was conducted in accordance with the guidelines for the ethical use of animals in experimental studies [11]. Upon completing the ischemia or reperfusion period, all the surviving foetuses and dams were sacrificed by administering an overdose of sodium pentobarbital, following the Mexican Official Norm NOM-062-ZOO for the humanitarian euthanasia of animals.

### 2.2. Animals and treatments

The study was performed at the Laboratory of Experimental Surgery at the National Paediatric Institute in Mexico City. It involved 96 foetuses from 16 New Zealand rabbit dams at 30 days of gestation. The dams were housed in individual cages under strictly-controlled environmental conditions, including 15–20 air exchanges per hour, incoming air forced through 0.3-μm particle filters, light intensity fixed at 150 lx, and room temperature maintained at 22 °C ± 2 °C.

Feed was provided in the form of a commercial brand of pellets that contained 18% proteins, 3.5% ethereal extract and 13% fibre. It was provided *ad libitum* every day at 8:00 AM in stainless steel containers. Potable water was also provided *ad libitum* in glass bottles equipped with pipettes.

Day one of gestation was established upon observing direct mounting, and was confirmed by palpation at 20 days after mounting.

### 2.3. Experimental groups

Of the 16 gestating rabbit dams, 8 were treated with 40 mg/kg of PPT (Laboratorios Manuell®, Mexico) via I.M, and assigned to the following groups: Ischemia with PPT (ICP n = 4), and reperfusion with PPT (RSP n = 4). The other 8 dams received a saline solution (placebo) administered in the same way and were assigned to the following sub-groups: Ischemia without PPT (ISP n = 4) and reperfusion without PPT (RSP n = 4).

#### 2.3.1. Procedure followed with the ischemia groups

Prior to uterine artery occlusion (UAO) time zero fetal blood samples were taken at 15 min and at 45 min further samples from different ischemic foetuses (one sample per foetus) were taken. Immediately upon concluding sample collection from each rabbit, the foetuses and dams were sacrificed.

#### 2.3.2. Procedure followed with the reperfusion groups

Regarding the groups of rabbits subjected to the reperfusion process (RSP and RCP), they were first exposed to an ischemic process similar to the one performed with the ISP and ICP groups, but without drawing foetal blood samples at any moment of the ischemic period. Samples considered at time zero were also obtained prior to inducing ischemia. Later, the uterine arteries of the rabbits in these groups were freed after periods of ischemia of 15 or 45 min to allow blood reperfusion. Once the arteries were released, the foetuses and uterine horns were re-introduced into the abdominal cavity and the incision was closed to evaluate the effect of foetal reperfusion 24 h after induction of ischemia by means of a second laparotomy. At the moment of exposing the uterus and the foetuses during the second surgical procedure, we observed that all the foetuses previously exposed to ischamia were still alive. The next step in the procedure consisted in obtaining foetal blood samples. Due to the experimental design, and, in contrast to the ISP and ICP groups, each dam in the RSP and RCP groups was assigned to a control group (0 min) and to one of two subgroups that were exposed to periods of ischemia (15 or 45 min), in such a way that during the evaluation of reperfusion all the foetuses obtained from one dam (n = 8) belonged to only one of the three previous periods of ischemia. Immediately upon concluding sample collection from each rabbit, the foetuses and dams were euthanized.

All blood samples (0.3-mL) were obtained by cardiac puncture. The time required to draw each sample was 15–20 s. Samples were transferred individually to a 150-μl microcapillary tube treated with lithium heparin and analysed by a gasometer (IL GEM Premier 3000; Instrumentation Laboratory Diagnostics, Milano, Italy/Lexington, USA). The blood gas measurements were then used to evaluate pH, partial carbon dioxide pressure and partial oxygen pressure (mmHg), as well as glucose (mg/dL), calcium and lactate (mmol/L) levels.

### 2.4. Caesarean sections

The dams were anaesthetized by simultaneous intramuscular administration of ketamine (50 mg/kg) and xylazine (3 mg/kg), and maintained in a sterilized polypropylene cage until loss of consciousness was observed. Afterwards, the ventilation of the animals was carried out by endotracheal intubation using an endotracheal tube of 3.5 mm (Kendall Health Care Group® USA). At that point, the lower abdominal surface was cleaned with an iodinated solution, the dam was positioned on a surgical table for small animal species, and its 4 extremities were gently immobilized. The lower abdominal area was then shaved. A 6–7 cm abdominal incision was made to gently expose the bicornuate uterus, and an incision was made on the antimesenteric edge of each horn for each foetus. The uterine arteries were occluded using bulldog-type clamps (Integra Miltex®, USA) placed simultaneously and bilaterally on the edge of the uterus. During this surgical procedure the rabbits were continuously monitored by blood perfusion to ensure that during the ischemic procedure blood flow was inhibited completely; whereas during the reperfusion procedure complete re-establishment of perfusion was verified. During exposure, the temperature and humidity of the uterine horns were maintained stable by applying moist compresses at 37 °C. The verification of the normality in the maternal physiological constants as well as the uterine blood perfusion during the processes of ischemia and reperfusion, as well as the monitoring of temperature were conducted with BIOPC MP100 (Biopac® Systems Inc, USA) equipment.

At the end of the surgical procedure, the incision was covered with an argentic ointment and the rabbits were housed in their cages under the conditions mentioned above. No rabbits showed signs of infection or dehiscence in the wound at 24 h. At the end of this period, foetal survival was seen to be 100%. No medication was administered during the post-operatory period so as not to interfere with the experimental procedure.

### 2.5. Statistical analyses

Results are presented as means and standard errors. Normality was tested for all the variables examined to ensure: (1) that