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Effects of oral caffeine administration to sows with induced parturition on hypoxia in piglets

Paola Superchi, Claudio Mazzoni, Paola Zanardelli, Chiara Piancastelli, Ernesto Mario Zambini, Valentino Beretti, Alberto Sabbioni *

Department of Veterinary Science, University of Parma, Via del Taglio 10, 43126 Parma, Italy

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ABSTRACT

To counteract the effects of perinatal hypoxia in piglets, the oral administration of caffeine to sows with induced parturition was evaluated. On day 113 of gestation 9 sows received 27 mg/kg body weight (BW) of caffeine mixed with 200 g of standard diet. The same amount of feed without the addition of caffeine was administered to 9 control sows. Additionally, on day 113 of gestation, all sows were treated by two injections in the perianal area of 1 mg of alfaprostol (at 8:00 am and 14:00 pm), and on the morning of farrowing by 14 IU of oxytocin in the perianal area. Caffeine did not affect BW of piglets and size of litters; however piglets from treated sows showed a higher capacity to adapt to extra-uterine life. Particularly, they showed a greater thermoregulatory ability ($P<0.001$) and a higher percentage of viability score ≥ 7 compared to piglets from control sows (92% *vs.* 75%, respectively; $P=0.030$). Caffeine furthermore reduced the frequency of high serum biopterin values (>80 nmol/L) in piglets born from treated sows ($P=0.001$). In conclusion, the caffeine orally administered to sows with induced parturition showed a protective effect on the consequences of neonatal hypoxia in tissue ischemia–reperfusion injury in piglets.

1. Introduction

Neonatal piglet mortality constitutes a continuing production problem and a significant welfare concern (Baxter et al., 2008). During the last decade, selection for improved prolificacy has been accompanied by an important increase of litter size at birth and at weaning. However, the improvement at birth is not completely recovered at weaning, as it is associated with a higher occurrence of peri- and postnatal mortality (Boulot et al., 2008; Cecchinato et al., 2007; Edwards, 2002; KilBride et al., 2012; Lucia et al., 2002; Sorensen et al., 2000). Actually, about 40% of all preweaning deaths occur at birth or during the first days after birth (Orozco-Gregorio et al., 2010). This high incidence is also related to litter size, because large litters are at greater risk of placental insufficiency. In this case, the flow of blood and oxygen to the unborn piglets can decrease or even interrupt and leads to fetal distress (Mota-Rojas et al., 2005; Svendsen et al., 1991). In piglet, oxygen availability reduction is characterized by metabolic acidosis, hypercapnia and depression of the respiratory center (Martínez-Rodríguez et al., 2011; van Dijk et al., 2008), alterations of thermoregulatory abilities (Herpin et al., 2002) and vascular responses, mediated mainly by the release of local vasodilator substances such as nitric oxide (NO) and adenosine (Alonso-Spilsbury et al., 2005; Fujioka et al., 2008).

In newborn infant and animals, the treatment of neonatal apnea related to asphyxia during birth has involved the use of caffeine (Orozco-Gregorio et al., 2010, 2011; Schmidt et al., 2006). Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid which plays a number of effects on various central

* Corresponding author. Tel.: +39 0521 032625; fax: +39 0521 032611.
E-mail addresses: alberto.sabbioni@unipr.it, sabbioni.alberto@gmail.com (A. Sabbioni).

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and peripheral tissues arising primarily from antagonism of adenosine's actions via blockade of adenosine receptors (Fredholm, 1995; Magkos and Kavouras, 2005). A marked increase in the levels of adenosine has been observed in chronic hypoxia-induced in mice (Back et al., 2006). By counteracting adenosine, which has generalized inhibitory functions, the effect of caffeine is broadly stimulatory. Caffeine also acts through the inhibition of cyclical nucleotides phosphodiesterase (PDE) leading to the accumulation of adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP). In this way caffeine may affect the functionality in the cell target for hormones and neurotransmitters (Magkos and Kavouras, 2005). In neonate piglets characterized by different vitality scale related to intrapartum asphyxia, a single oral administration of caffeine leads to improvements in the acid–base balance and in the metabolic status (Orozco-Gregorio et al., 2010). At higher and repeated subcutaneous administration doses, caffeine increases energy expenditure in low-birth weight piglets, reducing the weight gain at 8 days of life (Orozco-Gregorio et al., 2012). We observed that caffeine diffuses in biological fluids, as colostrum and milk; moreover its presence in the plasma of unsuckled piglets shows that caffeine is able to cross the placental barrier (Mazzoni et al., 2012).

The aim of this study was to verify whether caffeine administered orally to sows the day before the projected time of farrowing can affect the responses of piglets, by counteracting the effects of neonatal hypoxia.

2. Materials and methods

Experimental procedures for this study were approved by the Ethics on Animal Experimentation Committee of Parma University, in accordance with the EC Directive 2010/63/UE.

2.1. Animals, housing and treatment

Eighteen Large White × Landrace crossbred sows were homogeneously assigned to two groups ($n=9$), that were balanced for BW and parity. Sows in the control (C) group and treatment (T) group had a mean parity of 3.2 and 4.1, respectively, and a mean BW of 225 and 240 kg, respectively. Sows were introduced into the farrowing houses on day 109 of gestation. Until 113 days of gestation sows were fed a standard diet (14 MJ of DE/kg, 15% CP, and 0.6% lysine, as-fed basis) twice a day. Water was provided through nipple drinkers. On day 113 of gestation, T group sows received 27 mg/kg BW of caffeine (Sigma-Aldrich, St. Louis, MO) mixed with 200 g of feed, given once daily, before the morning meal (Li and Hacker, 1995; Mazzoni et al., 2012). The same amount of feed, without the addition of caffeine, was administered, at the same time, to C group sows. The day of farrowing sows were kept fasting. Parturition was induced by two injections in the perianal area of 1 mg of Alfaprostol (Gabbrostim®, Ceva Vetem, Agrate Brianza, Italy), on day 113 of gestation (at 8:00 am and 14:00 pm), as indicated by Kirkwood et al. (1996). To increase the effect of induction, on the morning of farrowing, 14 IU of oxytocin (Izossitocina®, IZO, Brescia, Italy), were injected in the perianal area (Raffi et al., 2011). The farrowing took place on day 114 of gestation for all sows. Farrowing was assisted and interventions were kept to a minimum. To standardize litter size, cross-fostering of piglets was allowed after 24 h within the group.

2.2. Piglets measurements

The number of piglets born alive, stillborn, and dead within the first 24 h and from 1 to 5 days of life were recorded. The piglets' viability was scored taking into account, with minimal modifications, the scale described by Mota-Rojas et al. (2005). Particularly, heart rates were classified as <110, 111–160, or >161 beats/min; time interval between birth and first breath as >1 min, 16 s – 1 min, or <16 s; muscle tone as flabby, scarce or good; muzzle skin color as pale, cyanotic or pink; the skin stained with meconium as severe (more than 40% of the body surface was stained), mild or absent. Each sign was rated from 0 (the worst) to 2 (the best) and the overall score was obtained for each piglet (maximum score = 10 points). Newborn piglets were individually weighed with an electronic dynamometer (Wunder 60, Trezzo sull'Adda, Milano, Italy) at birth, at 24 h and at 5 days post-partum. Temperature (°C) detection was obtained at birth and at 24 h post-partum by means of a tympanic membrane thermometer (ThermoScan, Braun GMBH, Kronberg, Germany). Weighing of piglets at birth was combined with ear tagging. Blood samples from piglets ($n=204$) were collected by venipuncture from the anterior vena cava within 30 min from the birth. Samples for biopterin determination, collected in heparin-coated tubes, were immediately centrifuged at 1327*g* for 10 min at room temperature and then stored at −20 °C until analysis.

2.3. Biopterin analysis

Measurement of biopterin was performed using HPLC with fluorometric detection (Carru et al., 2004). Samples and biopterin solutions were protected against light when handled. For protein removal, 250 μL of trichloroacetic acid (15%) were added to 500 μL of serum and vortexed for 10 s. After 15 min the sample was centrifuged at 1520*g* for 10 min, and 20 μL used for analysis. The chromatographic analysis was accomplished by means of a Perkin-Elmer Series 4 chromatographic system equipped with a quaternary pump and LS4 fluorescence spectrometer (Waltham, MA 02451, USA). The detection was used at the wavelengths selected as $\lambda_{ex}=353$ nm and $\lambda_{em}=438$ nm. Elution was performed on a 250 × 4.6 mm, 5 μm Waters Spherisorb ODS-2 column (Supelco Inc., Bellefonte PA, USA). The analysis was performed in isocratic mode, using water/acetonitrile (98:2v/v) as mobile phase, with a flow rate of 1 mL/min at room temperature. All solvents were HPLC/grade-purity. Biopterin analytical standard was purchased from Sigma-Aldrich (St. Louis, MO). Peak identification was achieved by comparison of retention time with biopterin standard. The linearity of fluorometric response, was measured in the concentration range of 50–2000 ng/mL ($y=7216.7x-226.46$; $r^2=0.9984$; C.V. = 3.19%). Detection limit was 15 ng/mL, determination limit 50 ng/mL, and recovery of biopterin from serum 98.5%.