Small Ruminant Research 141 (2016) 77–83

Contents lists available at ScienceDirect

Small Ruminant Research

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

The conceptus induces a switch in protein expression and activities of superoxide dismutase 1 and 2 in the sheep endometrium during early pregnancy

K.H. Al-Gubory[a,*], C. Garrel[b], N. Sugino[c], P.A. Fowler[d]

[a] UMR BDR, INRA, ENVA, Université Paris Saclay, 78350, Jouy en Josas, France
[b] Unité de Biochimie Hormonale et Nutritionnelle, Département de Biologie – Toxicologie – Pharmacologie, Centre Hospitalier Universitaire de Grenoble, 38043 Grenoble Cedex 9, France
[c] Yamaguchi University Graduate School of Medicine, Department of Obstetrics and Gynaecology, Minamikogushi 1-1-1, Ube 755-8505, Japan
[d] Institute of Medical Sciences, Division of Applied Medicine, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form 6 June 2016
Accepted 9 July 2016
Available online 15 July 2016

Keywords:
Sheep
Endometrium
Copper-zinc superoxide dismutase
Manganese superoxide dismutase
Early pregnancy

ABSTRACT

There has been a growing interest in the importance of superoxide dismutases (SODs) in the regulation of endometrial function. However, little is known about endometrial SOD1 and SOD2 protein expression and activity associated with early conceptus development. We aimed to investigate changes in protein levels and activities of SOD1 and SOD2 in the sheep caruncular (CAR) and intercaruncular (ICAR) endometrium during the transition from pre-implantation (day 14) to implantation (day 16) and post-implantation (day 18) periods of pregnancy. Lipid peroxidation was assessed by measuring CAR and ICAR malondialdehyde (MDA) content. SOD1 activity increased from day 14 to day 18 ($P < 0.05$) in CAR and from day 14 to day 18 ($P < 0.05$) and from day 16 to day 18 ($P < 0.01$) in ICAR. SOD1 protein level increased from day 16 to day 18 ($P < 0.05$) in CAR and from days 14 to days 16 and 18 ($P < 0.05$) in ICAR. SOD2 activity increased from day 16 to day 18 ($P < 0.05$) in CAR and from days 14 and 16 to day 18 ($P < 0.001$) in ICAR. SOD2 protein level increased from day 14 to day 18 ($P < 0.05$) in CAR and from days 14 and 16 to day 18 ($P < 0.05$) in ICAR. The content of MDA in CAR or ICAR did not alter significantly between stages of pregnancy. In conclusion, the early post-implanting conceptus co-ordinately up-regulates SOD1 and SOD2 protein expression and bioactivity within CAR and ICAR. By the maintenance of adequate endometrium SOD1 and SOD2 activity, the conceptus limits lipid peroxidation during the peri-implantation period of pregnancy.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The superoxide dismutase (SOD) family is a ubiquitously distributed group of metalloenzymes that catalyze the dismutation of superoxide radicals ($^{\bullet}O_2^{-}$) into hydrogen peroxide (H_2O_2). By scavenging $^{\bullet}O_2^{-}$, which is a precursor molecule for all other reactive oxygen species (ROS), SOD is the first line of defence against cellular oxidative damage and its subsequent effects on tissues of biological systems. Copper-zinc containing SOD (Cu, Zn-SOD or SOD1) is a dimeric protein, essentially located in the cytoplasm, whereas manganese-containing SOD (Mn-SOD or SOD2) is a homotetrameric protein, located in the mitochondria (McCord et al., 1971). The selenium glutathione peroxidases (seGPXs or GPXs), located within the mitochondrial matrix and the cytoplasm, are responsible for the conversion of H_2O_2 to water (Hayes and McLellan, 1993). Studies have indicated that SODs may have important roles in rodent (Laloraya et al., 1991; Jain et al., 2000), human (Sugino et al., 1996; Sugino et al., 2002a,b; Lucic and Milicevic, 2011) and sheep (Al-Gubory and Garrel, 2012; Al-Gubory et al., 2014) endometrial function early in pregnancy.

The enzyme activity is primarily determined by protein expression level. The corresponding changes in protein levels and enzyme activities of SOD1 and SOD2 in mammalian endometrium associated with early conceptus (embryo and associated extraembryonic placental membranes) development have not been fully described. The sheep is a useful animal model to explore the endometrial antioxidant machinery and its regulation during the oestrous cycle (Al-Gubory et al., 2008) and early pregnancy (Al-Gubory and Garrel, 2012). The ovine uterine endometrium consists of large numbers of well-delimited aglandular caruncules (CAR) and glandular

* Corresponding author at: Institut National de la Recherche Agronomique (INRA), Département de Physiologie Animale et Systèmes d'Elevage, UMR 1198, Biologie du Développement et de la Reproduction, 78352 Jouy-en-Josas Cedex, France.
E-mail address: kais.algubory@jouy.inra.fr (K.H. Al-Gubory).

http://dx.doi.org/10.1016/j.smallrumres.2016.07.006
0921-4488/© 2016 Elsevier B.V. All rights reserved.

intercaruncular (ICAR) areas. The CAR and ICAR endometrium are histoarchitecturally different and play different roles in the establishment of pregnancy (Cooke et al., 2013). The glands of ICAR areas produce a complex mixture of growth factors, cytokines, adhesion proteins and enzymes to support early conceptus development whereas the CAR areas allow conceptus attachment of and early placentation (Filant and Spencer, 2014). Our hypothesis is that the conceptus-derived factors influence the uterine environment favourably for conceptus attachment via the regulation of SOD1 and SOD2 protein expression in sheep endometrium. To test our hypothesis, CAR and ICAR tissues from pregnant ewes were used to characterise peri-implantation specific alterations of protein expression and activities of SOD1 and SOD2, GPX activity and the content of malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative stress.

2. Materials and methods

2.1. Animals and management

The French Ministry of Agriculture approved all procedures relating to care and use of animals according to the French regulation for animal experimentation (authorization no. 78-34). Ewes of the Préalpes-du-Sud breed (18 months of age) were used in this study. All the ewes were treated for 14 days with intravaginal sponges containing 40 mg fluorogestone acetate (Intervet, Angers, France) to synchronize oestrous. Immediately after removal of the sponge, each ewe received an intramuscular injection of 400 IU of equine chorionic gonadotropin (eCG, Intervet). Ewes were mated twice with fertile rams of the same breed, at an interval of 12 h during the synchronized oestrus.

2.2. Endometrial tissue collection

The ewes were slaughtered at a local abattoir in accordance with protocols approved by the local institutional animal use committee at the Institut National de la Recherche Agronomique (INRA, Jouy-en-Josas, France). Ewes ($n = 4$ ewes per group) were randomly allocated for slaughter at the pre-implantation period (day 14), initial conceptus implantation (day 16) and the early conceptus post-implantation period (day 18). Immediately after slaughter of the ewes, the reproductive tracts were collected, placed on crushed ice and transported to the laboratory. All subsequent manipulation of the tissue was performed at 4 °C. The uterine horns were opened and the CAR and ICAR endometrial tissues were separately dissected from the entire two uterine horns of each ewe, snap-frozen in liquid nitrogen and then stored at −80 °C until processed for activities of the O_2^- scavenging antioxidant enzymes, SOD1 and SOD2, the H_2O_2 scavenging antioxidant enzyme, GPX, and the content of MDA.

2.3. Malondialdehyde measurement

The content of MDA in CAR and ICAR endometrial tissues was determined by reversed-phase high performance liquid chromatography (HPLC) in which the MDA-thiobarbituric acid (TBA) adducts are separated from interfering substances (Londero and Lo Greco, 1996). The breakdown product of 1,1,3,3-tetraethoxypropane (TEP) was used as a standard. TEP undergoes hydrolysis to liberate stoichiometric amounts of MDA. Stock standard solution (480 μl of TEP in 100 ml ethanol) was prepared and this primary solution was diluted to the concentrations of 0, 1, 2, 3, 4, 5 and 6 μM. Tissue extract aliquots or standards (100 μl) were mixed with 750 μl of 0.8% TBA. The tubes were placed in a water bath (95 °C, 1 h), and then they were cooled. Samples were neutralized with methanol-NaOH mixture (pH 6). After centrifugation, 50 μl of protein-free supernatant were chromatographed in the HPLC system. The column used for the separation was Adsorbosphere C18 (5 μm particle diameter, 250 mm × 4.6 mm ID). The MDA-TBA adduct is eluted from the column with potassium dihydrogen phosphate buffer (10 mM, pH 6.0)-acetonitrile (17%). The quantification of MDA derivative was established by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalysed hydrolysis of TEP as μmoles per g tissue protein.

2.4. Antioxidant enzyme activity assays

The CAR and ICAR endometrial tissues were homogenized separately in cold phosphate buffer (50 mM, pH 7.4) and then the homogenates were centrifuged at 15,000 × g for 30 min, 4 °C. The resulting supernatant was used for determination of protein concentration (Lowry et al., 1951). Enzyme activities of SOD1 and SOD2 were determined as described previously (Al-Gubory and Garrel, 2012). Total SOD activity was measured using the pyrogallol assay based on the competition between pyrogallol oxidation by $^{\bullet}O_2^-$, and O_2^- dismutation by SOD. Enzymatic activity of SOD2 was determined by assaying for SOD activity in the presence of sodium cyanide, which selectively inhibits SOD1 but not SOD2 (Jin et al., 2005). SOD1 activity was calculated by subtracting SOD2 activity from total SOD activity. The rate of auto-oxidation is taken from the increase in the absorbance at 420 nm. GPX activity was measured using the glutathione reductase (GR)-NADPH method. Activity was determined by a coupled assay system (Nzengue et al., 2008) in which oxidation of glutathione (GSH) was coupled to NADPH oxidation catalysed by GR. The rate of GSH oxidized by tertiary butyl hydroperoxide was evaluated by the decrease of NADPH in the presence of ethylenediaminetetraacetic acid (EDTA), excess GSH and GR. The rate of decrease in concentration of NADPH was recorded at 340 nm.

2.5. Western blot

CAR and ICAR endometrial tissue lysates were loaded (30 μg protein/lane) onto 26-lane 1DE gels (NUPAGE Novex Midi gels, 4–12%, Invitrogen) under reducing conditions and then electroblotted onto immobilon-FL membrane (Millipore Ltd, Watford, UK) as described previously (Fowler et al., 2008). After blotting, membranes were incubated in blocking buffer, 1:1 Odyssey blocking buffer (LI-COR Biosciences UK Ltd, Cambridge, UK) and PBS, at 4 °C overnight. Primary antibodies were diluted in Odyssey blocking buffer 1:1 with 0.2 μm filtered PBST as follows: rabbit anti-Cu/Zn superoxide dismutase (SOD1, Abnova, Taipei City, Taiwan, PAB14492), 2 μg/ml; mouse anti-Mn superoxide dismutase (SOD2: AbCam Ltd, Cambridge, UK, ab16956), 1–10000; rabbit anti-Alpha Tubulin (AbCam Ltd, Cambridge, UK, ab4074), 1 μg/ml. The membranes were incubated with primary antibodies at 4 °C overnight and then incubated with secondary antibodies for 60 min at room temperature. Secondary antibodies including anti-mouse IgG IRDye^{TM}800 (all secondary antibodies were provided from LI-COR, Cambridge, UK, 610-732-124), 1-10,000 and anti-mouse IRDye^{TM}700DX (610-730-124) 1-5,000 were diluted in Odyssey blocking buffer 1:1 with 0.2 μm filtered PBST + 0.01% SDS. Following washing the membranes, the digital images were captured using Odyssey LI-COR Infrared Imager (LI-COR, Cambridge, UK). The band volumes and molecular weights (kDa) were then obtained following a background subtraction using Phoretix-1D Advanced software (Nonlinear Dynamics).

Download English Version:

https://daneshyari.com/en/article/5795390

Download Persian Version:

https://daneshyari.com/article/5795390

Daneshyari.com