Poly(ADP-Ribose) Glycohydrolase in Bovine Retained and Not Retained Placenta

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Contents
Poly(ADP-ribose) glycohydrolase (PARG) is the enzyme which degrades poly(ADP-ribose) polymers synthesized by poly(ADP-ribose) polymerase (PARP). Both enzymes are activated in response to different stimuli like oxidative stress and are involved in DNA repair processes. The retention of bovine foetal membranes (RFM) is supposed to be connected with oxidative stress conditions. The aim of the study was to detect the presence of PARG protein in bovine placenta in order to find the relationship between the process of releasing/retaining placenta and DNA repair. Placentomes, collected after spontaneous delivery or caesarian section were divided into maternal as well as fetal part of placenta, homogenized and subjected to electrophoresis. Animals were divided into six groups as follows: A – caesarian section before term with RFM; B – caesarian section before term without RFM; C – spontaneous delivery at term with RFM; D – spontaneous delivery at term without RFM; E – caesarian section at term with RFM; F – caesarian section at term without RFM. PARG protein was detected in nitrocellulose membranes using commercially available bovine anti-PARG antibody and Western blotting technique. Single bands referred to bovine PARG standard were observed in all examined tissues as well as in human placenta used as the control of procedure. In addition, the intensity of staining was stronger in retained than properly released term placenta and in foetal than in maternal part of the placenta. These results may suggest the differences in enzyme protein content and careful conclusions can be drawn that the activities of PARG may be altered between compared groups of animals. It may confirm the presence of oxidative stress conditions and their consequences on metabolic pathways, the content of biologically active substances and processes of proper releasing placenta. Further experiments on PARG activity in bovine foetal membranes with respect to proper and improper placentonal release are necessary.

Introduction
Although the activity of poly(ADP-ribose) glycohydrolase (PARG) was described in the early 1970s (Ueda et al. 1972), its characteristics still require investigation. PARG, together with poly(ADP-ribose) polymerase (PARP), is involved in poly(ADP-ribose) metabolism. Upon binding to DNA breaks, activated PARG cleaves NAD$^+$ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors, and PARP itself (Virag and Szabo 2002). Poly(ADP-ribose) polymers synthesized by PARP in response to different stimuli are degraded later on by PARG. The activity of both enzymes is supposed to be associated with DNA repair processes (Sato et al. 1993; Davidovic et al. 2001), programmed cell death (Lazebnik et al. 1994) and ischemic cell damage (Eliasson et al. 1997). Oxidative stress-induced overactivation of PARP consumes NAD$^+$ and consequently ATP, culminating in cell dysfunction or necrosis (Virag and Szabo 2002).

The retention of bovine foetal membranes, which is one of the most important post-partum disorders, warrants the investigation of its biochemical background as well. It is supposed to be connected with oxidative stress conditions. These conditions not only induce the imbalance between production and neutralization of reactive oxygen species (ROS), but also increase the peroxidation processes of proteins and lipids (Kankofer 2001a,b). Oxidative damage to DNA molecules may be a consequence (Kankofer and Schmerold 2002) and may lead to stimulation of DNA repair processes that require PARP and PARG activity (Pieper et al. 1999; Shall and de Murcia 2000). ROS-induced alterations in protein, lipid and DNA pathways result in changes in hormone level which may disturb the proper separation of foetal membranes.

To check this hypothesis, the study on PARG activity in bovine placenta was undertaken. The aim of this study was to detect the presence of PARG protein in bovine retained and properly released placenta by means of the Western blotting technique and bovine anti-PARG antibody.

Materials and Methods
All pregnant cows included into this study were clinically healthy and of Holstein-Friesian breed, 2–6 years of age. They were fed a standard diet, i.e. sugar beet pulp, barley straw and grass silage. Days of gestation were calculated using dates of insemination. Preterm Caesarean sections were indicated for teaching purposes, term sections because of fetus size.

Placentomes were collected from pregnant horn (one per cow) immediately after normal delivery of a calf at term (282–288 days of pregnancy), after extraction of a calf during Caesarean section before term (272–277 days of pregnancy), and during Caesarean section at term. The remainder of foetal membranes were left in situ until they were released spontaneously within 8 h after parturition or removed by a veterinarian after 8 h and defined as retained placenta (Grunert 1983). Cows were divided into six groups according to the time of expulsion and the mode of delivery as follows: A – Caesarean section before term with retained placenta; B – Caesarean section before term without retained placenta; C – normal delivery at term with retained placenta; D – spontaneous delivery at term without retained placenta; E – Caesarean section at term with
Electrophoresis and Western blotting

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was carried out in 12% polyacrylamide gels using the method of Laemmli (1970) at 120 V. Samples were resuspended in a reducing loading buffer (62.5 mM/l Tris, 192 mM/l glycine, 20% methanol, pH 8.3. Electrophoresis was performed at 150 V for 1 h.

The PVDF membranes were washed with PBSTM solution (PBS 1x, pH 7.4; 3% non-fat powdered milk; 0.1% Tween 20) for 2 h. Anti-PARG polyclonal antibody (cat. no 210-773-R050; Alexis Biochemicals, Nottingham, UK) was diluted in PBSTM and the membranes were incubated for 2 h. The blot was washed in PBSTM 3x for 10 min and in substrate buffer (0.1 M/l Tris pH 9.5; 0.1 M/l NaCl; 50 mM/l MgCl2) for 10 min. Finally, the blot was developed in a substrate solution which was freshly prepared by adding 44 µl of nitroblue tetrazolium chloride solution [NBT; 75 mg/ml in 70% (v/v) dimethylformamide] and 33 µl of 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt [BCIP; 50 mg/ml in 100% (v/v) dimethylformamide, Life Technologies GmbH, Karlsruhe, Germany] to 10 ml of substrate buffer. Positive reactions revealed purple bands. The staining was stopped by washing the membrane in distilled water. The specificity of the primary antibody had previously been verified by comparing the reactivity of first and second antibody to sample preparations on Western blotting. Bovine PARG standard was purchased from Alexis Biochemicals.

Densitometric analysis was performed by use of Gel Doc 1000 Gel Documentation System (Bio-Rad, Hercules, CA, USA) and computer program Sigma Gel (Sigma, St Louis, MO, USA). Peak areas were expressed in arbitrary units.

Results

The presence of PARG protein is showed in Fig 1.

The enzyme was defined with respect to PARG bovine standard. Bovine placental samples, as well as human placenta, showed one band of molecular weight compared with enzyme standard (110 kDa).

Western blotting technique allows to confirm the presence of examined protein by the use of a specific antibody. To visualize the results, by colour, enzymatic reaction was implemented. Obtained intensity of staining only suggests the differences in amount of enzyme protein. The intensity of staining differed between examined samples what might, however, only semiquantitatively and indirectly, also suggest the alterations in enzyme activity.
In addition, densitometric analysis showed stronger staining in foetal than in maternal part of the placenta. In placental samples collected at term, a stronger intensity of staining was observed in retained than properly released foetal membranes as well as in Caesarean section than normal delivery groups. In preterm samples the intensity tended to be stronger in control cows than in RFM animals. For example the following peak areas were determined in membrane presented in Fig. 1: lane 2 – 111; lane 3 – 448; lane 4 – 176; lane 5 – 556; lane 6 – 253; lane 7 – 199; lane 8 – 1; lane 9 – 86; lane 10 – 441; lane 11 – 452; lane 12 – 55; lane 13 – 57.

Such densitometric analysis is valuable only together with the results of direct determinations of enzyme activity. In the present study this is the only suggestion for further experiments.

Discussion

Well evidenced changes in the levels of steroid hormones (Grunert 1983) and prostaglandins (Leidl et al. 1980; Slama et al. 1993), which are observed during the retention of foetal membranes in cows, are connected with the disturbances in their pathways. These disturbances reflect the alterations and/or inhibition of activities of enzymes which are involved in hormone metabolism. Enzyme activities may be changed because of ROS excess, causing protein peroxidation and proteolysis. Disturbed NAD/NADH ratio may also influence the proper activity of dehydrogenases responsible for steroid metabolism (Takayanagi et al. 1986).

Local interaction of ROS with prostaglandin F$_{2a}$ (PGF$_{2a}$) on progesterone release in ewes was described. The results demonstrated that PGF$_{2a}$ may induce ROS increase during ovine luteolysis (Hayashi et al. 2003).

The evidence for ROS imbalance during RFM is from experiments describing not only the alterations of antioxidative enzymes activity, but also the changes in the level of non-enzymatic antioxidants (Kankofer 2001c,d). The concentration of protein (Kankofer 2001a) and lipid peroxidation products (Kankofer 2001b) increase in RFM-affected cows in comparison with control animals. The determinations of 8-OH-dG, which is a marker of oxidative DNA damage, suggest the presence of this damage during improper placental release (Kankofer and Schmerold 2002). These determinations facilitated finding the relationship between RFM, ROS imbalance, and PARG activity.

PARG was purified from different tissues and partly characterized (Tanuma and Endo 1990; Maruta et al. 1991; Braun et al. 1994). Bovine cDNA encoding this enzyme was also isolated recently (Lin et al. 1997). It is a cytoplasmic and nuclear 110 kDa protein responsible for poly(ADP-ribose) polymers catabolism (Desnoyers et al. 1995; Winstall et al. 1999a). The known inhibitors of human placental PARG are three classes of tannins (gallotannins, ellagitannins, condensed tannins) (Aoki et al. 1993) as well as ADP dihydroxyxypyrrolidine (Slama et al. 1992). There are suggestions that PARG shows different affinity for long and short poly(ADP-ribose) molecules resulting in the hypothesis that polymer size and numbers are rate-determining factors in poly(ADP-ribose) metabolism (Hatakeyama et al. 1986; Winstall et al. 1999b). There is evidence for a 59-kDa protein with PARG activity which is supposed to be the product of proteolysis or the result of purification procedure (Winstall et al. 1999a,b).

PARG acts through endo- and exoglycosidic mode to remove poly(ADP-ribose) polymers (Braun et al. 1994). The synthesis of poly(ADP-ribose) polymers, catalysed by PARP, appears in response to different stimuli such as oxidant injury. This is the reason why the activity of both enzymes is closely connected. The presence of PARP protein was previously detected in bovine placenta by use of Western blotting technique (Kankofer and Guz 2003). The intensity of staining of enzyme bands differed between examined groups of animals with and without retained placenta.

Western blotting technique and anti-bovine PARG antibodies allowed to describe also PARG protein presence in bovine released and retained placenta. Although it is only a semiquantitative method, it was possible to see the differences in intensity of staining of obtained bands. It might indirectly suggest the differences in enzyme activity between cows affected with RFM and control animals. It may also provide evidence for oxidative/antioxidative imbalance present in examined animals. Human placental tissue which was used as the control showed the same electrophoretic pattern and also only one band of enzyme protein. The intensity of staining, however, was weaker in bovine tissue.

There are reports indicating that poly(ADP-ribose) catabolism may be involved in local increase of ATP, which could serve as an energy store for DNA repair enzymes as well as for the ligation step (Maruta et al. 1997; Oei and Ziegler 2000).

PARG, like other proteins, is cleaved during apoptosis. Responsible for this cleavage is caspase-3 which releases two enzymatically active fragments of 85 and 74 kDa in human cells (Affar et al. 2001). Partial examination of bovine placental proteins by means of SDS-PAGE and zymography of metalloproteinases showed differences in the number of fractions as well as differences in metalloproteinases activities in cases of retained and released placenta (Maj and Kankofer 1997, 1998). This may suggest the alterations in proteolytic processes in bovine placenta during improper placental release (Gross et al. 1985).

The present study brings additional evidence that bovine placenta is sensitive to ROS imbalance, leading to DNA damage. This damage is repaired by PARP and PARG action which are both present in bovine released and retained placenta. However, the control and efficiency of repair processes in bovine retained placenta requires further elucidation.

References


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