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Acid phosphatase and cathepsin D are active expressed enzymes in the placenta of the cat

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Abstract

Enzymes are crucial for the metabolism of macromolecular substrates. In the great majority of cells, most enzymes are constitutive. Nevertheless, inducible enzymes can predominate, determining specialized cell functions. Within this context, histochemistry/immunohistochemistry and biochemistry were used to investigate expression of peroxidase and reduced nicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase, as well as the expression and activity of cathepsin D and acid phosphatase, in trophoblast cells within the endotheliochorial labyrinth and marginal hematoma of the term cat placenta. In the marginal hematoma, elevated Cathepsin D expression and activity was accompanied by erythrophagocytosis. In contrast, acid phosphatase activity was much more intense in the labyrinth, where metabolic exchanges occur. Peroxidase and NAD(P)H-oxidase were predominantly active in trophoblast cells within endosomal vesicles of different placental compartments, indicating that, although reactive oxygen species might participate in endosomal/lysosomal processes, they are not territorially specific or functional markers. These findings highlight differential characteristics of cathepsin D and acid phosphatase activity within each placental compartment, thereby contributing to the comprehension of the territorial role played by the placenta and facilitating future metabolic studies.

Keywords: Placenta; Chorionic villi; Phagocytosis; Lysosomes; Trophoblasts; Cats

1. Introduction

The placenta of the domestic cat is zonary, lamellar, endotheliochorial and deciduate. It is subdivided into two distinct areas designated as the main placenta and the paraplacenta (Mossman, 1987; Leiser and Koob, 1993; Leiser and Kaufmann, 1994). The main placenta is responsible for pronounced metabolic and molecular exchanges, whereas the paraplacenta contains auxiliary areas such as the marginal hematoma, which is a specialized hematophagous region characterized by intense erythrophagocytosis. Iron uptake for fetal hemopoiesis has been considered one of the main roles of this placental region, in which the columnar phagocytic cytotrophoblasts are bathed in extravasated maternal blood (Malassiné, 1977, 1982, 2001; Leiser and Enders, 1980a,b; Mossman, 1987; Leiser and Koob, 1993).

Although the carnivore placenta, and especially the cat placenta, has been extensively described, the cellular physiology of placental compartments has not been completely

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explored (Malassiné, 1977; Malassiné, 1982; Mossman, 1987; Leiser and Koob, 1993; Leiser and Kaufmann, 1994). Within this context, enzymes that are crucial for the metabolism might provide valuable information on cell specializations in topographically distinct tissue regions. Various studies have shown that acid phosphatase, which is an important marker of phagocytic activity in professional phagocytes (activated macrophages and neutrophils, Rodman et al., 1990) and other cell types (Araki et al., 1995), is widely distributed among the different compartments of the maternal–fetal interface of the cat placenta (Christie, 1968; Malassiné, 1976; Miyoshi and Sawamukai, 2004). The enzyme cathepsin D is another marker of phagocytic activity in macrophages and neutrophils. It is the most significant aspartic-protease, and has also been found in uterine macrophages and stromal cells in the placentas of macaques (Blankenship and Enders, 1997) and horses (Green et al., 1998). Recently, cathepsin D has been reported to be correlated with invasion and phagocytic activity in the rodent trophoblast (Elangovan and Moulton, 1980; Afonso et al., 1999). Peroxidase is a hemoprotein present in all aerobic organisms, containing an iron porphyrin complex that takes part in substrate-specific oxidative process, as well as in the formation and degradation of oxygen peroxide (Nelson and Kulkarni, 1986). Finally, reduced nicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase is a plasma membrane-bound enzyme complex present in a wide variety of cells of mesodermal origin. Acting as an electron transporter, NADPH-oxidase is intrinsically associated with the generation of reactive oxygen species (ROS) in neutrophils and macrophages (Gagioti et al., 1996; Segal and Shatwell, 1997; Babior, 1999; Cui et al., 2006). According to Matsubara and Tamada (1991), NADPH-oxidase might also play an important role in the molecular transport between mother and fetus.

Thus, in the present study, we investigated the expression and activity of the lysosomal enzymes cathepsin D and acid phosphatase, and the activity of peroxidase and nicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase in an attempt to better comprehend the biology of the cat placenta, correlating our findings with specific trophoblast functions.

2. Materials and methods

The cat placentas were kindly provided by the Veterinary Hospital of the University Santo Amaro, obtained from females undergoing elective cesareans performed for ovariohysterectomy procedures on a routine basis for animal population control. The entire procedure was performed in an operating room under aseptic conditions. All experimental procedures were performed in accordance with the guidelines established by the Brazilian School of Animal Experimentation and were approved by the Ethics Committee for Animal Experimentation of the Institute of Biomedical Sciences of the University of São Paulo.

Ten adult females of domestic cat (*Felis catus*) of undefined race, from 1.5 to 3 years of age were selected for this study. On average, 3 placentas were obtained from each female. After delivery the females were clinically evaluated. Only were experimentally processed the placental fragments from females that did not exhibit infections or systemic diseases and, were in term gestation phase (50–60 days post-coitus). For the biochemical assays, 12 placentas were used, and 18 were used for the remaining assays.

2.1. Collection of samples

2.1.1. For morphological analysis

The placentas were immediately washed in 0.1 M phosphate-buffered saline (PBS) (pH 7.4), and immersed in a fixative solution of 4% paraformaldehyde in PBS. The paraplacenta (marginal hematoma) and the main placenta (labyrinth) were cut into small fragments. The samples were stored in the fixative solution for an additional 4–24 h. Due to the bilateral localization of the paraplacenta in relation to the main placenta, both regions were dissected and processed as individual units: the proximal and distal, having the ovary as the anatomical reference.

After 1 h of fixation, samples for histochemical identification of acid phosphatase and peroxidase were incubated in a cryoprotective solution (1% Arabic gum and 0.88 M sucrose in 0.1 M PBS) and rapidly frozen in chilled isopentane in a dry-ice/acetone bath. Five-micrometer sections were then obtained on a cryostat (IEC Minotome, Needham Heights, MA, USA) and mounted onto slides pretreated with 1% gelatin and 1% formaldehyde in 0.1 M PBS.

2.1.2. For hydrogen peroxide-formation sites (NAD(P)H-oxidase activity)

To evaluate areas with NAD(P)H-oxidase activity, fragments of the paraplacenta and the main placenta were sequentially washed in 0.1 M PBS/0.1 M Tris–maleate buffer (pH 7.5), and incubated in 0.1 M PBS at 4 °C to be taken to the laboratory.

2.1.3. For biochemical analysis

The placentas were first washed in PBS, and then the main placenta, as well as the distal and proximal paraplacenta (marginal hematoma), were identified and kept for at least 1 h on ice until starting the biochemical procedures. To that, samples were cut into tiny pieces.

2.2. Immunohistochemistry

Immunohistochemical reactions were performed to identify the enzyme cathepsin D, as well as the mesenchymal and trophoblastic components of the placenta. To identify the mesenchymal and trophoblastic components, the intermediate filaments vimentin and cytokeratin, respectively, were used as markers.