J Vect Borne Dis 44, June 2007, pp. 116–121

Oxidoreductases in early gestational monkey placenta during maternal malarial infection : histochemical localisation

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Abstract

Background & objectives: Early gestational malaria is more deleterious than late gestational infection. Still the pathophysiology of maternofoetal organ—the placenta in malaria remains almost unexplored during early gestation. Present study dealing with oxidoreductases in early gestational placenta during maternal malarial infection of *Plasmodium cynomolgi bastianellii* in rhesus monkeys was anticipated to provide a better insight into the functional impairment of this organ leading to foetal abnormalities.

Methods: Three control and four experimental monkeys (*Macaca mulatta*) were quarantined for one month prior to experimentation. Experimental monkeys at 2–2½ months of gestation were inoculated with *P. cynomolgi bastianellii*. On attaining first peak of parasitaemia the placentae were collected from anesthetised animals. The snap-frozen, cryostat sections were subjected to histochemical localisation for 3 (or 17) β-hydroxysteroid dehydrogenase (β-HSD) [3 (or 17) β-hydroxysteroid: NAD (P^+) oxidoreductase, EC 1.1.1.51 hydroxysteroid dehydrogenases] and NADPH-tetrazolium reductase [NADPH : (acceptor) oxidoreductase, EC 1.6.99.1 NADPH-TR]. Comparative microscopy of control and malaria infected placental sections was performed and analysed.

Results: A localised decrease in both the enzymes was observed in syncytiotrophoblast layer of malaria infected monkey placenta. The areas showing morphological damage of syncytiotrophoblast were also depicting gross reduction in NADPH-TR activity.

Interpretation & conclusion: The altered enzymatic activities [3 (or 17) β-HSD and NADPH-TR] in malaria infected early gestational monkey placenta have been discussed in the light of placental function. It could be concluded by present studies that these alterations would affect the cellular metabolism especially steroidogenesis and detoxification process which in turn would affect the normal development of the foetus as well as maintenance of gestation.

Key words 3 (or 17) β-HSD – malaria – NADPH-TR – placenta

Introduction

Placenta, the most vital organ of mammalian pregnancy poses barriers of several cells between maternal and foetal circulation through which the transportation of endogenous or exogenous materials takes place[1,2]. Any pathological alteration in maternal blood as imposed by malaria parasite may have direct bearing on placental structure and function as is evident by the incidences of abortions, reduced mean birth weight of foetus, pre-term deliveries and maternofoetal deaths[3–8] during maternal malarial infection.

Incidences of neonatal and congenital malaria[9] indicate involvement of placenta in transmission of malaria. Information on malaria is limited to human term placenta[10–12] or term animal placenta[13], however, early gestational malaria has been found to be more deleterious[14–18], still the pathophysiology of early gestational placenta in malaria remains unexplored. It is, therefore, considered worthwhile to investigate the structural and functional alterations in the placenta of a non human primate model—*Macaca mulatta* infected with *P. cynomolgi bastianellii*. A part of these investigations dealing with the malfunctioning of infected placenta at histological, scanning electron microscope, biochemical and histochemical localisation of hydrolases has already been published[15–18] and the present communication deals with the histochemical investigations on some metabolic marker oxidoreductases, the steroidogenic enzymes of malaria infected early gestational (2–2½ months) monkey placenta.

Material & Methods

Adult pregnant rhesus monkeys (*Macaca mulatta*) of 5–6 kg body weight were procured from commercial suppliers and quarantined for one month before experimentation in temperature (range 23 ± 2°C) relative humidity (range 50–55%) and photoperiod (12 h of alternating light and dark periods) regulated room. Animals were fed on monkey pellet diet (Hindustan Lever Ltd., Mumbai, India) and fresh fruits while drinking water was allowed *ad libitum*. Experimental protocol had prior approval from Institutional Animal Ethics Committee and animals were handled/used accordingly. The gestation period of monkeys was determined by palpation and X-ray diagnosis. Only those monkeys which were free from tuberculosis (determined by tuberculin test and X-ray diagnosis) were included in the study.

Four animals at 2–2½ months of gestation were inoculated intravenously with 1×10^6 RBCs infected with *P. cynomolgi bastianellii* (B-strain; kind courtesy of Microbiology Division, Central Drug Research Institute, Lucknow, India) while three monkeys were taken as control. Peripheral parasitaemia in monkeys was monitored by daily examination of ear vein blood samples following the protocol of Puri & Dutta[19]. After 24 h of ascertaining first peak of parasitaemia that appeared on Day 11 post-inoculum ranging from 5.1–7.1[14]. The experimental and control animals were anaesthetised with nembutol and placentae were collected in chilled physiological saline by the procedure published by our group[15–18].

After extraction of placental tissue all the animals were treated with antimalarials and rehabilitated till they tested negative for parasitaemia following the rehabilitation procedure of institutional primate house. Two to three millimetre thick slice of placental tissue were briefly soaked in 5% polyvinyl alcohol at 0–4°C for 5 min; snap-frozen in Freon-22 cooled by liquid nitrogen and cryostat sections of 8 μm thickness were cut at –25°C. A minimum of five section sets each consisting of eight sections were prepared from each monkey placenta. The section sets were obtained by random sampling of almost the entire thickness of the tissue slice. The sections mounted on clean glass slides were air-dried and used for different histochemical reactions immediately.

The oxidoreductase 3 (or 17) β-hydroxysteroid dehydrogenase [3 (or 17) β-hydroxysteroid : NAD (P^+) oxidoreductase, E.C. 1.1.1.51. HSD] was demonstrated using dehydroepiandrosterone ($\triangle^5$ – androsten-3 β-ol-17-one) as substrate and nitroblue tetrazolium as hydrogen acceptor as described by Lojda[20]. The oxidoreductase NADPH-tetrazolium reductase (NADPH : (acceptor) oxidoreductase, E.C. 1.6.99.1; NADPH-TR) was localised by using NADPH as substrate and nitroblue tetrazolium as hydrogen acceptor[20].

Appropriate controls for all the above enzymes were also employed by omitting the substrates from the respective incubation media.

Results

After attaining peak parasitaemia several changes were observed in histological scanning electron microscopic, biochemical and histochemical analysis of hydrolases in malaria infected monkey placenta[15–18] while some of the oxidoreductases were localised as follows:

3 (or17) β-HSD activity: In normal placenta the syncytial cytoplasm of chorionic villi showed intense HSD activity whereas the cytotrophoblasts, villous mesenchyme and cells in the intervillous spaces were devoid of the oxidoreductase activity (Fig. 1).

The placenta of malaria infected animals showed a patchy distribution of the enzyme activity (Fig. 2) in the syncytial cells. All other cellular elements of the placenta were without enzyme activity.

NADPH-TR activity: In normal placenta, NADPH-TR activity was localised mostly in the syncytiotrophoblasts while the cytotrophoblasts were found devoid of the enzyme activity, some of the mesenchymal cells showed positive reaction. No enzyme activity could be demonstrated in the intervillous cells (Fig. 3).

In contrast to normal placenta the malaria infected placenta had decreased enzymatic activity in the syncytiotrophoblast and mesenchymal cells (Fig. 4). However, as in normal placenta, the cytotrophoblast cells and intervillous cells were devoid of the enzyme activity. In a few foci of cells in the syncytiotrophoblasts in which morphologically detectable damage was observed, there was a gross reduction in the enzyme activity (Fig. 5).

Discussion

Structural and functional similarity of human and rhesus monkey placenta establishes the relevant importance of present study as both of them are of

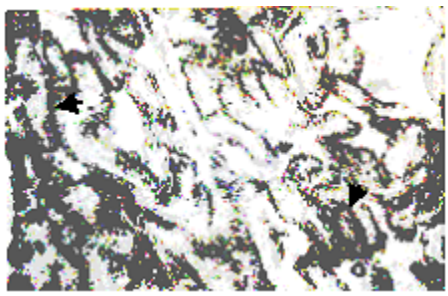

Fig. 1: Cryostat section of normal placenta showing 17 β-HSD activity localised to the syncytiotrophoblast layer (arrow). X 220

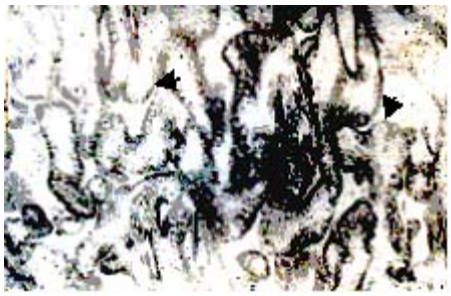

Fig. 2: Malaria infected placenta exhibiting patchy distribution of 17 β-HSD activity in trophoblastic syncytium (arrow). X 220

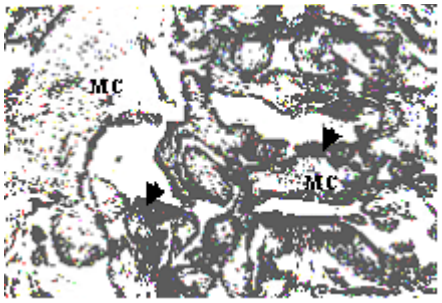

Fig. 3: Cryostat section of normal placenta showing NADPH-TR activity in synctiotrophoblastic cytoplasm (arrow) and mesenchymal cells (MC). X 220

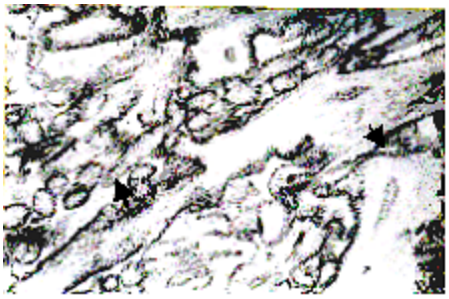

Fig. 4: Fresh frozen cryostat section of infected placenta showing decreased tetrazolium reductase activity in the syncytiotrophoblast (arrow) and mesenchymal cells (MC). X 220

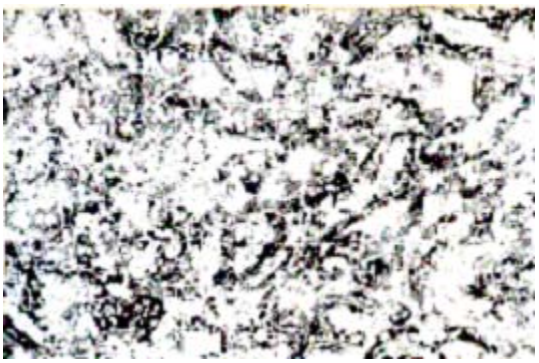

Fig. 5: Cryostat section of infected monkey placenta showing loss of NADPH-TR activity in apparently damaged foci of syncytiotrophoblast cells. X 220

haemochorial type[1,2]. Present investigations prove altered activity of few oxidoreductases leading to functional impairment of placental tissue following early gestational maternal malarial infection.

The oxidoreductase, 17β-HSD, is known to catalyse oxidoreduction of estradiol 17β and estrone, thus participating in the biosynthesis of steroid hormones[21,22]. In the present study the enzyme was chiefly localised in syncytiotrophoblast of normal placental villi. These findings are in accordance with that of Dupont *et al*[23], who immunocytochemically demonstrated the presence of 17β-HSD in syncytiotrophoblastic cytoplasm in early gestational human placenta. It thus appears that this enzyme is involved in the synthesis of pregnenolone and progesterone in villous syncytiotrophoblast which seems to be the major site of steroid hormone synthesis in rhesus monkey. A reduction in 17β-HSD activity in malaria infected monkey placenta, thus denotes a decreased steroid hormone biosynthetic activity. As the two steroid hormones, progesterone and estrogen are essential for the maintenance of pregnancy and normal growth and development of the foetus, the observed decrease in 17β-HSD activity in infected monkey placenta may be expected to adversely affect the maintenance of pregnancy. This gains support from the findings of Breuer *et al*[24] who correlated threatened abortions as well as foetal growth retardations with low levels of the oxidoreductases.

The existence of a 'diaphorase' activity capable of transferring hydrogen from $NAD(P)H_2$ to atmospheric oxygen via cytoplasmic (microsomal) cytochromes (*e.g.* cytochrome P-450) has attracted much attention. This 'microsomal respiratory pathway' has been shown to be involved in detoxification of several drugs, hydroxylation of many steroid hormones, ω-oxidation of fatty acids etc[25,26]. The clear localisation of NADPH-TR in the syncytiotrophoblast and some of the mesenchymal cells in the villi of normal placenta suggest the existence of a similar oxidation-reduction system in the monkey placenta also. The visible decrease in the activity of this enzyme in malaria infected placenta may indicate several possible functional changes in the tissue, of which an impaired detoxification of the 'putative malaria toxins' might result in, or could be the result of damage to the endoplasmic reticulum, which is the site of NADPH-TR activity[27]. Indeed, our preliminary electron microscopic studies[28] did reveal extensively damaged endoplasmic reticulum in malaria infected monkey placenta.

Conclusion

In conclusion, changes in distribution pattern of oxidoreductases in placenta following early gestational malaria have posed severe threat to maternal-foetal well-being which is thought to be responsible for foetal deaths and abortions[15–18] in infected monkeys.

Acknowledgement

Authors are grateful to Microbiology Division, Central Drug Research Institute, Lucknow for providing parasites and to Council of Scientific and Industrial Research, New Delhi for technical and financial help.

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Received: 4 May 2006 *Accepted in revised form:* 30 December 2006