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MDR1 P-gp Expression and Activity in Intact Human Placental Tissue; Upregulation by Retroviral Transduction

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This study investigates P-gp activity in placental villous fragments and the possibility of upregulating its expression and function by retroviral transduction.

In fresh fragments, cyclosporin A caused a significant increase in ^{3}H-vinblastine accumulation (187 ± 48% at 180 min $n = 4$), consistent with multi-drug resistance activity.

After 7 days in culture, villous fragments showed a similar increase in ^{3}H-vinblastine accumulation (143 ± 10% at 180 min $n = 4$), which was not significantly different from that in fresh tissue. Following transduction, immunohistochemistry revealed increased P-gp expression. However, the distribution of the protein differed from that in controls, with P-gp being located throughout the tissue as opposed to the normal specific location on the maternal facing plasma membrane. Transduced explants showed a significantly larger increase in ^{3}H-vinblastine accumulation in the presence of cyclosporin A than control explants (245 ± 15.5% at 180 min, $n = 4$), suggesting reduced capacity to efflux vinblastine. This study demonstrates P-gp activity in intact placental tissue which is maintained in explant culture. Retroviral transduction of P-gp to such tissue leads to increased but undirected expression of the protein. The consequent increased activity at sites such as the basal, fetal facing, plasma membrane probably explains the increased substrate accumulation within the tissue.

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INTRODUCTION

The multi-drug resistance gene MDR1 encodes P-glycoprotein (P-gp), an active drug efflux pump that has been shown to extrude a wide range of natural product drugs and xenobiotics from cells in which it is expressed [1]. In concert with drug metabolizing enzymes, such as the cytochrome P450s, this ATP-dependent efflux pump influences bioavailability and disposition of its substrates by limiting their uptake from the gastrointestinal tract and facilitating excretion from the kidneys and liver. Moreover, its expression in the blood brain barrier has been shown to protect the brain from the toxic effects of xenobiotics in the circulation [2]. Initially identified as the gene that confers multi-drug resistance on cancer cells [3], MDR1 has been shown to transport not only the cytotoxics used in cancer chemotherapy but also other therapeutics such as HIV-protease inhibitors, anti-convulsants, immunosupressants, Ca^{2+} channel blockers, cardiac glycosides and antibiotics [4]. This huge range of structurally unrelated compounds transported by P-gp, together with its known vectorial transport capacity and its expression in excretory tissues and classic pharmacological barriers, has led to the hypothesis that the protein has evolved to protect an organism from drugs and environmental xenobiotics.

Several reports have shown that MDR1 P-gp is expressed in the human placenta throughout gestation [5–9] and that the protein is localized predominantly on the microvillous, maternal facing, plasma membrane of the syncytiotrophoblast at term [5,9]. This layer forms the transporting epithelium of the placenta and is in direct contact with the maternal blood. Therefore, localization of P-gp to the microvillous membrane is the ideal situation for it to reduce transfer of xenobiotics from maternal to fetal circulations. This protective role of placental P-gp has been clearly demonstrated in animal studies, where absence of P-gp in mouse placenta results in a 100% susceptibility to avermectin induced cleft palate [10] and a sevenfold increase in transfer of substrate drugs such as digoxin, saquinavir and taxol to the fetus [11].

P-gp has been shown to be important in the capacity of cultured human trophoblast cells to efflux xenobiotics. Studies

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in choriocarcinoma cell lines, primary cytotrophoblast cells in culture and isolated membrane vesicles [5,8,12–14] have all demonstrated P-gp activity. However, little information is available on P-gp activity in intact human placental tissue. The first aim of this study was therefore to measure P-gp activity in fresh placental villous fragments. Such fragments have their overlying syncytiotrophoblast intact, as well as retaining other elements of the exchange barrier such as the extracellular matrix in the villous core and the fetal capillary endothelium.

Administration of drugs in pregnancy to treat maternal conditions such as epilepsy, hypertension and diabetes carries the inherent risk of transplacental transfer to the fetus, with the potential to cause undesirable side effects [15–17]. Upregulation of P-gp activity in the placenta in such situations could therefore be advantageous in protecting the fetus. In previous studies we showed that over-expression of P-gp in BeWo cells, by retroviral transduction of MDR1, increases the capacity of these cells to efflux xenobiotics [5]. The second aim of this study was therefore to investigate the possibility of upregulating P-gp expression and function in placental tissue by retroviral gene transfer. In order to achieve retroviral transduction, the target cells have to be actively dividing. Placental villous fragments in explant culture have been shown to undergo a process of degeneration over the first 24 h of culture, with the syncytiotrophoblast becoming vacuolated and eventually sloughing off. This is then followed, over the next 3 days, by a process of regeneration, during which a new syncytiotrophoblast develops. By day 7 of culture this is indistinguishable from that of fresh term placenta [18]. The new syncytiotrophoblast appears to be produced by active division and fusion of the underlying stem cytotrophoblast cells. We hypothesized that this would provide a suitable system in which to investigate retroviral transduction of placental tissue with P-gp.

MATERIALS AND METHODS

Functional studies in fresh placental fragments

Accumulation of ^{3}H-vinblastine (a substrate of P-gp) by placental villous fragments was measured in the presence and absence of cyclosporin A (an inhibitor of multi-drug resistance proteins), using a method adapted from that described by Jansson et al. [19] to measure amino acid transport.

Briefly, placentas were collected at term after uncomplicated pregnancies, from the delivery unit at St Mary's Hospital Manchester, following local ethical guidelines. Small fragments of villous tissue were dissected within 30 min of delivery. These were washed in Tyrodes solution [135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM Mg Cl_2, ($6H_2O$), 10 mM HEPES, 5.6 mM glucose, pH 7.4, 290 mosmol/ kgH_2O] and were then tied, with thread, to hooks supported by perspex rods. Uptake of ^{3}H-vinblastine was measured at 37 °C at six time points (5, 15, 30, 60, 120 and 180 min). Each timed uptake was made in duplicate i.e. two fragments were attached to each rod. Incubations were carried out in Tyrodes solution containing 0.2 μCi/ml, 17.7 μM ^{3}H-vinblastine (Km = 20 μM) (Amersham Pharmacia Biotech), either with or without 20 μM cyclosporin A (Sigma). This concentration of inhibitor had been previously established to give maximal inhibition of P-gp in trophoblast cells [5].

After each timed incubation, fragments were washed (2×15 s) in ice cold Tyrode's to remove extracellular isotope and to reduce leak of isotope from the tissue. They were then placed in distilled water to lyse the tissue and release accumulated isotope into the water. The lysates were counted using liquid scintillation to assay for ^{3}H activity. The fragments were then dissolved in 0.3 M NaOH and aliquots assayed for protein using a Bio-Rad protein assay (BioRad, Richmond, CA). Accumulation of vinblastine was calculated as pmol/mg protein.

Placental explant culture

The method described previously by Siman et al. [18] was used to culture placental villous fragments. Briefly, several 1 cm cubes of villous tissue were dissected within 10 min of delivery. These were rinsed in sterile Dulbecco's phosphate buffered saline (PBS) with calcium and magnesium (Sigma, Poole Dorset) and then transferred to CMRL-1066 medium (Invitrogen, Paisley) for transport to The Paterson Institute of Cancer Research, where culture was to be carried out.

On arrival, small fragments of villous tissue (0.46 ± 0.1 mg protein) were dissected out and carefully rinsed twice in sterile PBS and once in culture medium (CMRL-1066 Sigma, 5% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1 μg/ml insulin, 0.1 μg/ml hydrocortisone and 0.1 μg/ml retinol acetate; Sigma). The fragments were then transferred to Costar Netwell supports (15 mm diameter, 74 μm mesh; Corning, Corning N.Y.) in 1.5 ml of culture medium. Three fragments were placed in each Netwell and cultured at 37 °C in a humidified mixture of 5% CO_2 and 95% air for 7 days. The medium was changed every 24 h and the supernatant from three designated wells on each culture plate was retained and frozen at −20 °C for later analysis of human chorionic gonadotrophin (hCG), used as a marker of trophoblast differentiation [18]. In all cases, the time elapsed from collection of the placenta to culture was <2 h.

Retroviral transduction of placental explants

The SF MDR retroviral vector [20] was used to achieve a stable transduction of the regenerating syncytiotrophoblast of the placental explants with MDR1. The packaging cell line for the MDR1 vector, GPAM-MDR, was seeded into the lower compartment of the Netwell plates 24 h before setting up an explant culture. The placental tissue was then prepared exactly as described above and co-cultured with the packaging cell line for 7 days. The medium was again changed every 24 h but in the case of the transduced explants it was replaced with conditioned medium i.e. medium that had been used to culture the packaging cell line for 24 h. Daily collections of medium were made from three designated wells for hCG analysis as for control explants.