Determination of metformin transfer across the human placenta using a dually perfused \textit{ex vivo} placental cotyledon model

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Abstract

Objective: The aim of the study was to quantify and characterize metformin transfer across the human placenta using an \textit{ex vivo} placental perfusion model.

Study design: Placentas were obtained from vaginal deliveries or caesarean sections and selected cotyledons were cannulated and dually perfused. Metformin (1 $\mu$g/ml) and a permeability reference marker, antipyrine (50 $\mu$g/ml), were added to the maternal circulation. Each perfusion experiment was conducted for 180 min while samples were taken from the maternal and fetal compartments. The integrity and viability of the placenta were determined by measuring the flow rates, fetal artery inflow pressure, and hCG production during the experiments.

Results: Six complete experimental set-ups were completed. The maternal–fetal transport rates for metformin and antipyrine were 10.61 $\pm$ 2.85% and 30.98 $\pm$ 5.62%, respectively. The clearance index, calculated as the ratio between the permeabilities of metformin and antipyrine, was 0.34 $\pm$ 0.05.

Conclusion: The results indicate that metformin is able to cross the mature human placenta; thus, fetal exposure must be considered when treating pregnant women with metformin.

Keywords: Placenta; Metformin; \textit{Ex vivo} placental cotyledon model

1. Introduction

The oral hypoglycemic agent, metformin, is indicated for the treatment of diabetes mellitus type 2. Reports on the safety and efficacy of this treatment modality in diabetic pregnant women are inconsistent [1–3] although a meta-analysis failed to show increased teratogenic risk among women treated with oral hypoglycemic agents (OHAs) during the first trimester [4]. Recently, metformin was compared with insulin treatment in gestational diabetes mellitus (GDM) patients, and found to be similarly as effective as insulin for maternal blood glucose control and neonatal outcome [5]. In addition to its role in the treatment of diabetes mellitus, its value in treating women with polycystic ovarian syndrome (PCOS) to induce ovulation [6], reducing insulin resistance [7] and improving conception rates, has been well established [8]. Recently, several studies have indicated that the maintenance of metformin therapy throughout pregnancy reduces the risk of first trimester abortions [9,10], and enhances pregnancy outcomes [11,12]. Consequently, many patients with polycystic ovaries, who conceive while taking metformin, continue the treatment throughout pregnancy.

The placenta plays a pivotal role in the transfer of drugs from the mother to the fetus. OHAs exemplify a group of medications that has been excluded from use in obstetrics for
many years, mostly because of the little information about their transplacental passage available. The transplacental passage of the four sulfonylurea agents has been studied by Elliott et al. [13,14], using the *ex vivo* human placental model, finding that only insignificant transport of glyburide (glibenclamide) occurs across the human placenta. This finding has led to clinical studies [15,16] and to an alteration in the current management algorithms of gestational diabetes.

Even though metformin treatment during pregnancy has become an accepted clinical approach, there is still a paucity of information on the trans-placental passage of this oral hypoglycemic agent, thus preventing many clinicians from advocating the treatment throughout pregnancy [17,18]. Only scarce data exist explaining the mechanism by which metformin crosses biological membranes [19–21] and the exact mechanism of the transplacental transfer of metformin is unknown. Several organic cation transporters (OCTs) have been shown to be involved in the intestinal and hepatic uptake of metformin, as well as the paracellular transport of this small and polar molecule. The purpose of this study was to investigate the transplacental transfer of this biguanide across the human placenta using an *ex vivo* placental cotyledon model.

2. Materials and methods

2.1. Perfusion system

Placental transport was studied using an isolated perfused single cotyledon model in a normal-term human placenta [22]. Approval for the study was obtained from the local ethics committee. In brief, this system contains independent maternal and fetal perfusion circuits that individually maintain stable pH, temperature, flow rates, and perfusion pressure during the dual-perfusion process. Term placentas were obtained immediately after vaginal deliveries or cesarean sections and a suitable fetal artery and vein pair supplying a single placental cotyledon were cannulated and perfused with heparinized Krebs–Ringer solution. After having verified proper flow and absence of leakage via tissue tears, the placenta was mounted, maternal surface upwards, in a plexiglass perfusion cabinet. Perfusion of the maternal side was achieved through blunt cannulation of the intervillous space of the lobe corresponding to the perfused isolated cotyledon, by four needles. Both circulations were maintained by peristaltic pumps and were perfused in a closed recirculation fashion. Maternal perfusate that returned from the intervillous space was continuously drained by a venous catheter placed at the lowest level of the maternal decidual surface to avoid significant pooling of perfusate. The cotyledon was perfused for 30 min with heparinized Krebs–Ringer solution in order to stabilize the tissue and to exclude any leakage by determining equality between arterial inflow and venous outflow.

After 30 min, the perfusion medium was changed to tissue culture medium M199 (Sterile Earle’s salt base with L-glutamate; Biological Industries, Kibutz Beit Haemek, Israel), maintained at 37 °C, enriched by 3 g/l of bovine serum albumin (Sigma, Munich, Germany), 1 g/l of glucose, 10 IU/ml of heparin (Kamada, Rehovot, Israel), and 48 μg/ml of gentamycin (Teva, Netanya, Israel). Sodium bicarbonate was added to maintain pH of the perfusate within the physiologic range (7.35–7.45). The fetal perfusate (500 ml) was equilibrated with 95% N2/5% CO2 to mimic the low oxygen tensions observed *in utero*, whereas, the maternal perfusate (500 ml) was equilibrated with 95% O2/5% CO2. Perfusion rates were 4–6 and 10–12 ml/min in fetal and maternal circulation, respectively. During the whole perfusion period lateral pressure was measured by a pressure transducer (HP 8040A) in the fetal inflow line next to the point of cannulation.

When stable perfusion parameters were achieved for 30 min, the maternal perfusate was replaced with medium containing metformin (1.0 μg/ml) and the reference substance, antipyrine (50 μg/ml). The perfusion was maintained for 3 h, and samples were taken from both maternal and fetal systems (arterial and venous) at 0, 5, 10, 15, 20, 30, 60, 90, 120, 150, and 180 min and were stored at −20 °C until analysis of the drug concentrations. In addition, samples of perfusates were obtained from maternal and fetal circuits at 30 min intervals for measurements of human chorionic gonadotropin (hCG) concentration. At the end of the experiment, the perfused cotyledon was separated from the remainder of the placenta, weighed, cut into 1000 mg pieces, and frozen.

Control over the quality of each perfusion was maintained by several indicators, and the experiment was terminated and excluded from analysis if any indication existed that stable perfusion had not been achieved. Such indicators included:

1. >10% volume leakage from the maternal to the fetal perfusate or *vice versa*, as determined by comparison of final reservoir volumes.
2. Inability to achieve adequate circuit perfusion rates within fetal inflow pressure ranging between 40 and 70 mmHg.
3. Lack of satisfactory antipyrine transfer, to a predetermined extent of 20%, by the end of the perfusion experiment.

2.2. Sample analysis

Antipyrine concentrations were determined by the HPLC method applying a Kontron chromatograph (pump 420, auto-injector 465, detector 432, Data Acquisition System 450; Kontron, Zurich, Switzerland) and Xierra RP C18 (3.5 μm) column (Waters, Wexford, Ireland). The mobile phase consisted of methanol, acetonitrile, and 0.1% TFA solution (1:2.8:12.8, v/v). Flow rate was 1 ml/min and
Maternal clearance index. To diminish this bias, we used different perfusion experiments solely on the basis of the fact that the sizes of the perfused cotyledons are usually not equal, a potential bias exists in comparing the results of different perfusion experiments, and is used by various authors, is the clearance index. The clearance index is the ratio between the percentage of maternal–fetal transport rate of metformin across the human placenta using the ex vivo perfusion as evidenced by an antipyrine placental transfer rate of >20%. The mean (±S.D.) weight for the cotyledons used was 22.4 g ± 3.2 (Table 1).

Beta-hCG accumulation in the maternal compartment was observed throughout the experiments. The mean hCG production by the perfused cotyledon was 108.74 ± 70.64 mIU/min/g. The production of hCG by the perfused tissues and its appearance only in the maternal circulation, reflects the expected preferential secretion of the hormone and indicates physical integrity of the placental preparation (Table 1).

The mean (±S.D.) rate of antipyrine transfer was 30.98 ± 5.62%, and the mean (±S.D.) rate of metformin transfer was 10.61 ± 2.85% (Fig. 1). The mean clearance index was 0.34 ± 0.05 and the adjusted maternal–fetal transport rate (mean ± S.D.) for metformin was 0.585 ± 0.41 mg/ml/g of cotyledon (Table 2). Finally, two 1 g samples of each perfused cotyledon were tested for accumulation of metformin; however, metformin was not detected above the limit of quantification in any of the 12 samples tested by HPLC.

3. Results

A total of six experiments could be validated for integrity of the placental membrane and adequate conditions of perfusion as evidenced by an antipyrine placental transfer rate of >20%. The mean (±S.D.) weight for the cotyledons used was 22.4 g ± 3.2 (Table 1).

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4. Comment

In our study, we used placentae from women near or at term. Therefore, our data apply to mature placentae only. This study demonstrates the maternal–fetal transport of metformin across the human placenta using the ex vivo perfusion as evidenced by an antipyrine placental transfer rate of >20%. The mean (±S.D.) weight for the cotyledons used was 22.4 g ± 3.2 (Table 1).

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placental cotyledon model. We have shown that there is some maternal–fetal transport of this substance in a concentration that corresponds to steady-state therapeutic plasma levels. The clearance index of metformin was quite low, 0.34 ± 0.05. Furthermore, we did not observe any accumulation of the drug in the placental tissue. Our data show that the clearance index of metformin is only slightly higher than that of the second generation sulfonylurea glyburide (0.21 ± 0.09), comparable with that of the second generation sulfonylurea glipizide (0.32 ± 0.12), and is significantly lower than that of the first generation sulfonylureas (tolbutamide [1.05 ± 0.21] and chlorpropamide [0.5 ± 0.18]), as evaluated by Elliott et al. [14]. Since we could not detect metformin in the placental tissue we concluded that its concentration in the perfused cotyledon was lower than the quantification limit, although there is a possibility that the extraction techniques we used were not sensitive enough.

Metformin is a weak base, highly polar, positively charged hydrophilic compound, with a small molecular weight (166 g/mol), a low binding capacity to plasma proteins, and is not known to freely diffuse through cell membranes. The exact mechanism by which metformin crosses the biological membranes is not completely understood. Metformin has been shown to act as a substrate for three organic cation transporters OCT1, OCT2, and OCT3. There are no current data indicating the presence of OCT1 presence in human placenta. OCT2 is only moderately expressed and OCT3 has considerable expression in human placental tissue [27]. OCTs are the only transporters known to be involved in metformin transport to date; therefore, it can be speculated that OCT3, and to some extent OCT2, are responsible for the transport of metformin across the human placenta.

Considering the substantial volume of distribution of metformin, with its mean serum level around 1 μg/ml and its low clearance index, we could have assumed, based on our results, that fetal exposure to this medication is low, suggesting the safety of its use during pregnancy. However, this assumption contradicts recently published data that reveals that metformin concentrations in both umbilical artery and vein are roughly the same as in maternal serum [28]. Although the ex vivo human placental studies generally reflect maternal–fetal transfer in vivo, still this model lacks the effect of maternal and fetal metabolism. The available data of metformin metabolism in the fetus are insufficient. In

<table>
<thead>
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<th>Experiment no.</th>
<th>Antipyrine percentage maternal–fetal transport</th>
<th>Metformin percentage maternal–fetal transport</th>
<th>Clearance index</th>
<th>Adjusted transport rate (μg/h/g placenta)</th>
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<td>0.34</td>
<td>0.605</td>
</tr>
<tr>
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<td>38.24</td>
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</table>
adults metformin is excreted by the kidneys. It may be speculated that in the amniotic sac, after excretion of metformin to the amniotic fluid, there might be some re-absorption of metformin to the fetal circulation during swallowing, thus resulting in the high fetal serum metformin concentrations, despite its low clearance index.

In conclusion, the results from the present study indicate that metformin passes across the placenta; thus, a certain degree of fetal exposure must be considered when treating pregnant women with metformin. Additional studies are needed to assess the exact mechanism of metformin transport across the human placenta, and possible accumulation in the fetal compartment. Although no teratogenic effects were reported among women treated with metformin during the first trimester, the long-term outcome of exposed fetuses should be evaluated before advocating this treatment throughout pregnancy.

References