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Effect of human serum albumin on transplacental transfer of glyburide

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ABSTRACT

Glyburide is a second-generation sulfonylurea hypoglycemic drug used for the treatment of select women with pregestational and gestational diabetes mellitus (GDM). In vitro and in vivo investigations demonstrated its very low transplacental transfer to the fetal circulation. However, the factors influencing its low transfer across the human placenta remain unclear. Therefore, the goal of the current investigation was to determine the effect of human serum albumin (HSA) on the transfer and distribution of glyburide across the human placenta. To achieve this goal, the technique of dual perfusion of the placental lobule was utilized. The effect of HSA on the transfer of glyburide was determined at the range of glyburide to HSA molar ratios of 1:2–1:100. The transfer rate of free/unbound glyburide to the fetal circuit was 73 ± 10% of the freely diffusible marker compound antipyrine (AP). Data obtained indicates the dependence of glyburide transfer and its retention by the placental tissue on the concentration of HSA.

1. Introduction

Glyburide (Glibenclamide) is a second-generation sulfonylurea used in the treatment of non-insulin-dependent diabetes mellitus. Clinical trials in adult patients with type 2 diabetes demonstrated that glyburide is an effective and safe hypoglycemic drug [1]. Currently, the use of glyburide in the treatment of gestational diabetes mellitus (GDM) is under investigation by the NIH Obstetric-Fetal Pharmacology Research Units Network.

The rationale for the use of glyburide during pregnancy is based on the similarities of the pathophysiology of GDM and type 2 diabetes. It is assumed that the effect of glyburide in decreasing glucose levels in patients with type 2 diabetes should be as effective in women with GDM who have the mildest form of glucose intolerance [2].

A major concern in the pharmacotherapy of pregnant women is the potential of a drug's teratogenic or toxic effects to the developing fetus. Earlier experience with first-generation sulfonylureas revealed their association with fetal macrosomia, increased rate of congenital abnormalities, and increased rate of fetal hypoglycemia due to hyperinsulinemia [3]. However, in contradistinction to first-generation sulfonylurea drugs, the transfer of glyburide across the dually perfused placental lobule was insignificant [4,5]. Moreover, glyburide was not detected in the cord blood of infants born to mothers that were treated with the drug [6]. Therefore, data obtained from in vitro and in vivo investigations suggest that glyburide use during pregnancy should not affect fetal growth and or development.

The transfer of a drug, with a molecular weight less than 1000, from the maternal to fetal circulation across the human placenta is mainly dictated by its physicochemical properties, but other factors are also involved. The molecular weight of glyburide is 494, but the presence of large nonpolar groups

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renders it less water soluble and more lipophilic [1]. Therefore, according to the physicochemical properties of glyburide alone, the drug should diffuse readily across the syncytiotrophoblast to the fetal circulation. However, this is not the case as is apparent from the data obtained by Elliott et al. They concluded that the extremely low transfer of glyburide across the human placenta is not comparable to other drugs with the same physicochemical properties such as tolbutamide, chlorpropamide, and glipizide [5]. Other properties of the drug were offered as explanations – namely, the effect of glyburide binding to proteins (99.8%), its short elimination half-life (6 h), and the role of placental efflux transporters [7–9]. Accordingly, the factors influencing glyburide transfer across the human placenta remain unclear.

Therefore, the goal of this investigation was to determine the effect of human serum albumin (HSA) on the transfer and distribution of glyburide across the human placenta. To achieve this goal the technique of dual perfusion of the placental lobule was utilized.

2. Materials and methods

2.1. Materials

Radioactive [^{3}H] glyburide at specific activity of 44.6 Ci/mmol was purchased from Perkin-Elmer (Boston, Mass). All other chemicals were purchased from Sigma–Aldrich (Dallas, Tex) including radioactive [^{14}C] antipyrine (AP) at specific activity of 4.7 mCi/mmol.

2.2. Placentas

Placentas were obtained immediately after vaginal or abdominal deliveries from the Labor & Delivery unit of the University of Texas Medical Branch in Galveston, according to an approved protocol by the Institutional Review Board. Placentas from uncomplicated term pregnancies and from women with GDM were utilized in this study.

2.3. Placental perfusion

The technique of dual perfusion of a placental lobule was used according to the method of Miller et al. [10] and as described in detail in earlier reports from our laboratory [11]. Briefly, each placenta was examined for tears, and vessels supplying a single intact peripheral cotyledon were cannulated with umbilical catheters. The selected lobule was placed maternal side up in the chamber and perfused within 20 min of delivery by inserting two catheters into the intervillous space on the maternal side. Both reservoirs and peristaltic pumps were at a lower level than that of the perfusion chamber. The flow rate of the medium in the fetal and maternal circuits was 2.8 and 12 mL/min, respectively, i.e., a proportional reduction of in vivo conditions. The perfusion medium was made of tissue culture medium M199 (Sigma, St. Louis, Mo) supplemented with: Dextran 40 (7.5 g/L in the maternal and 30 g/L in the fetal reservoir), 25 IU/mL heparin, 40 mg/L gentamicin sulfate, 80 mg/L sulfamethoxazole, and 16 mg/L trimethoprim. The maternal perfusate was equilibrated with a gas mixture made of 95% O_2, 5% CO_2, and the fetal perfusate with a mixture of 95% N_2, 5% CO_2. Sodium bicarbonate was added to the maternal and fetal circuits to maintain the pH at 7.4 and 7.35, respectively. All experiments were carried out at a temperature of 37 °C.

2.4. Control period

Each placenta was perfused for an initial control period of 2 h. The control period allowed the tissue to stabilize to its new environment and perfusion was terminated if one of the following occurred: fetal arterial pressure exceeding 50 mm Hg, a volume loss in fetal circuit in excess of 2 mL/h, or a pO_2 difference between fetal vein and artery dropping less than 60 mm Hg.

2.5. Experimental period

The experimental period was initiated by replacing the medium in the maternal and fetal reservoirs with a fresh medium and adding the drugs. Glyburide was added at its final concentration of 150 ng/mL (the average peak serum levels following administration of a single oral dose of 2.5–5 mg) [2] together with the inert, lipophilic, and highly diffusible marker compound AP (20 μg/mL). Glyburide and AP were added to either the maternal or fetal reservoir according to the transfer direction investigated, maternal to fetal or fetal to maternal, respectively. The radioactive isotopes of the drugs [^{3}H] glyburide and [^{14}C] AP (1.5–6 μCi) were cotransfused with nonlabeled drugs to enhance their detection limits. Human serum albumin was added to both circuits at concentrations between 42 μg/mL and 10 mg/mL regardless of whether the model system was utilized in its open–open or closed–closed configuration as stated in Section 3.

The perfusion system was used in two of its configurations, either closed–closed, when the perfusates are recirculated, or the open–open, without recirculation. The closed–closed configuration was used to investigate the distribution of glyburide between the placental tissue and the maternal and fetal circuits. The open–open system was used to determine the transfer of glyburide across the placenta under steady-state conditions.

The concentrations of glyburide and AP were determined in 0.5 mL aliquots taken from the fetal and maternal veins and arteries every 10–30 min during the experimental period. Scintillating cocktail (4 mL) was added to each aliquot, and the radioactivity of the two compounds determined simultaneously using a liquid scintillation analyzer (1900TR, Packard Instruments Inc., Shelton, Conn).

2.6. Retention of glyburide by placental tissue

At the end of each experiment, the perfused region was dissected from adjoining tissue, weighed, and homogenized in a volume of saline equal to four times its weight. To an equal volume of the homogenate, 1 mL of 1 M NaOH was added, and the samples were incubated for 12 h at 60 °C in the darkness to allow for luminescence decay. Scintillation cocktail (8 mL) was added to each sample and the concentration of each drug determined.

2.7. Data and statistical analysis

Transfer rate, clearance, and clearance index (ratio of glyburide transfer rate to antipyrine transfer rate) under steady-state conditions were calculated utilizing the reported equations [12] and as described earlier from our laboratory [11]. The difference between compared values was determined by two-tailed *t*-test and considered significant when $P < 0.05$.

3. Results

3.1. Transfer of glyburide under steady-state conditions

The transfer rate of glyburide under steady-state conditions in the absence and presence of HSA was determined utilizing the dual perfusion model system in its open–open configuration.

3.1.1. Transfer of glyburide across the human placenta in the absence of HSA

The marker compound AP was cotransfused with glyburide to normalize its transfer rate and account for interplacental variations. The transfer of AP under identical experimental conditions was described previously in detail [11].

The concentration of glyburide in the maternal artery was 150 ng/mL, i.e., its reported level in maternal circulation following administration of a single oral dose of 2.5–5 mg. Glyburide crossed the placenta and appeared in the fetal circulation with a lag time of 11.3 ± 0.59 min. This lag time is significantly longer than for marker compound AP (2.1 ± 0.64 min). The fetal transfer rate of glyburide and its clearance were $29 \pm 6\%$ mL/min and 0.82 ± 0.21 mL/min, respectively. Upon normalization of these values with the transfer rate of AP in each placenta, the transfer rate of glyburide across the human placenta in the absence of HSA was $73 \pm 10\%$ of that for AP transfer.

Taken together these data indicate that in medium devoid of HSA, the rate of ''free'' glyburide transfer across the placenta is slow, but the extent of its transfer to the fetal circuit is high.

3.1.2. The effect of HSA on glyburide transfer across human placenta

The mean serum albumin concentration during 37–40 weeks of gestation is 29 mg/mL [13,14]. The effect of HSA added to both circuits at concentrations of 42, 210, 630 μg/mL, and 2.1 mg/mL on the transfer of glyburide (150 ng/mL) was determined. These concentrations correspond to glyburide:HSA molar ratios of 1:2, 1:10, 1:30, and 1:100, respectively. Adding HSA to the perfusion medium did not have an effect on AP transfer parameters. Therefore, the transfer of glyburide across placentas in the presence of HSA was normalized to that for the transfer of the marker compound AP.

In the presence of HSA in the perfusion medium, the lag time for glyburide was 9.1 ± 1.5 min. This indicates that HSA did not affect the lag time for glyburide appearance in the fetal circuit. Moreover, HSA, at its concentration of 42 μg/mL, i.e., 2:1 molar ratio to that of glyburide, did not affect glyburide transfer. However, the increase in the molar ratio of HSA to glyburide resulted in a decrease in the amount of glyburide transferred to the fetal circuit (Table 1). These data indicate that the fetal transfer rate of glyburide across the placenta is dependent on the concentration of HSA present in the maternal circuit. Therefore, transplacental transfer of glyburide to the fetal circuit is inversely dependent on HSA concentration up to 10% of its levels in vivo.

3.1.3. Effect of HSA on the amount of glyburide retained by placental tissue

The effect of 42, 210, 630 μg/mL, and 2.1 mg/mL of HSA on the retention of glyburide by the tissue was investigated. Glyburide was added to the maternal reservoir and transfused under steady-state conditions for a period of 2 h. Trophoblast tissue retained the highest amount of glyburide when the perfusion medium was devoid of HSA. The addition of increasing amounts of HSA to the maternal circulation resulted in decreasing the amount of glyburide retained by the tissue. At a concentration of 630 μg/mL HSA, the amount of glyburide in the placental tissue was decreased by 80% (Fig. 1). These data confirmed that the amount of glyburide retained by trophoblast tissue is dependent on the circulating levels of HSA in the maternal circuit when the molar ratio of glyburide to HSA is less than 1:30. This was substantiated by the addition of 2.1 mg/mL of HSA to the perfusion medium, which did not decrease the amount of glyburide retained by placental tissue. These data suggest that, in the range of HSA concentrations between 630 μg/mL and 2.1 mg/mL, most of glyburide is bound to HSA and the amount of free/unbound glyburide available is at a minimum.

3.1.4. Transfer of glyburide across placentas obtained from women with GDM

Transfer of glyburide under steady-state conditions across placentas obtained from women with GDM was compared with its transfer across placentas obtained from women with uncomplicated pregnancies. In both placental groups, the perfusion medium was devoid of HSA, i.e., the transfer of the

Table 1 – Effect of HSA on glyburide transfer across human placenta in open–open system

Parameters	Placentas from uncomplicated pregnancies				
	Absence of HSA ($n = 6$)	42 μg/mL HSA ($n = 2$)	210 μg/mL HSA ($n = 2$)	630 μg/mL HSA ($n = 2$)	2.1 mg/mL HSA ($n = 2$)
TRf (%)	26 ± 6	29 ± 1.3	13 ± 1.3	10.7 ± 0.7	$2.5 \pm 0.5^{*}$
CL (mL/min)	0.82 ± 0.21	0.88 ± 0.04	0.38 ± 0.04	$0.30 \pm 0.04^{*}$	$0.08 \pm 0.02^{*}$
CL index	0.73 ± 0.09	0.66 ± 0.08	$0.35 \pm 0.04^{*}$	$0.32 \pm 0.04^{*}$	$0.08 \pm 0.02^{**}$

TRf: fetal transfer rate; Cl: clearance; Each value represents the mean ± S.D.; $^{*}P < 0.05$; $^{**}P < 0.01$.

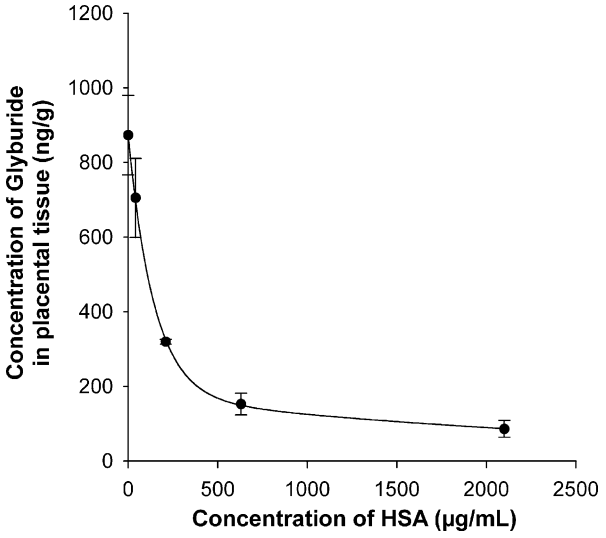

Fig. 1 – Effect of HSA on the retention of glyburide by placental tissue. Glyburide was added in the maternal reservoir at a final concentration of 150 ng/mL. HSA was added to both circulations. The retention of glyburide by the placental tissue decreased with the increase in the concentration of HSA in the perfusion medium.

free/unbound drug was compared. The transfer parameters of glyburide and its retention by placental tissue obtained from women with GDM was not different from that determined in placentas obtained from uncomplicated pregnancies (Table 2), indicating that GDM does not affect glyburide transfer across the human placenta.

3.2. *Distribution of glyburide between the tissue and the maternal and fetal circuits*

The effect of HSA on the distribution of glyburide between the tissue and the maternal (250 mL) and fetal (150 mL) circuits was investigated. The model system was utilized in its closed–closed (recirculating) mode. Glyburide was perfused in the maternal to fetal and fetal to maternal directions.

3.2.1. *Release of proteins from placental tissue during perfusion*

In preliminary experiments, the amounts of proteins released into the maternal and fetal circuit were determined following perfusion of a lobule for 4 h in a closed–closed system. The concentration of proteins in the maternal reservoir was approximately 630 μg/mL and below the detection limit of the Bio-Rad protein assay in the fetal reservoir.

These data indicate that the perfused tissue releases its proteins into the maternal reservoir only.

3.2.2. *Distribution of glyburide when perfused in the maternal to fetal direction*

In the absence of HSA, the transfer of glyburide from the maternal to fetal circuit was biphasic, as exhibited by an initial rapid decline in its concentration in the maternal circuit during the first 30 min, followed by no change during the 210 min of experimental time (Fig. 2A). This can be explained by the binding of the drug to proteins released from the perfused tissue into the maternal circulation. Under these conditions, the amount of glyburide transferred to the fetal (recipient) circuit was very low and the concentration ratio for recipient (fetal) to donor (maternal) circuit at the end of 4 h of perfusion was 0.09 ± 0.02. Accordingly, the initial rapid decline in the concentration of glyburide in the maternal circuit is most likely due to its uptake and retention by the tissue and not due to its transfer to the fetal circuit (Fig. 3A).

3.2.3. *Distribution of glyburide when perfused in the fetal to maternal direction*

Glyburide was added to the fetal reservoir (donor) and perfused to the maternal (recipient) circuit in the absence of HSA. The concentration of free glyburide, unbound to proteins, declined steadily in the donor circuit and increased in the recipient circuit (Fig. 2B). At the end of the experimental period, the glyburide concentration ratio in the recipient (maternal) to donor (fetal) circuit was 1.1 ± 0.42. The observed rapid decline in the concentration of glyburide in the fetal circuit could be explained by the absence of any proteins released by the placenta. On the other hand, the proteins released from placental tissue into the maternal circuit bound glyburide, resulting in its accumulation in the recipient circuit and in a decrease of the amount of the drug retained by the tissue (Fig. 3B).

Therefore, data from the above experiments indicate that the presence of proteins in either the donor or recipient circuits is a major factor in the distribution of glyburide between the placental tissue and the maternal and fetal circuits.

Table 2 – Transfer of glyburide across placentas from uncomplicated pregnancies and placentas obtained from women with GDM

Parameters	Placentas from uncomplicated pregnancies ($n = 4$)		Placentas from women with GDM ($n = 6$)	
	AP	Glyburide	AP	Glyburide
TRf (%)	41.3 ± 7	29.8 ± 7	38.5 ± 7.6	28.2 ± 6.2
CL (mL/min)	1.2 ± 0.25	0.86 ± 0.2	1.01 ± 0.35	0.76 ± 0.26
CL index	0.72 ± 0.1		0.72 ± 0.09	
Tissue retention (ng/g)		881 ± 85		944 ± 105

TRf: fetal transfer rate; CL: clearance. Each value represents the mean ± S.D.

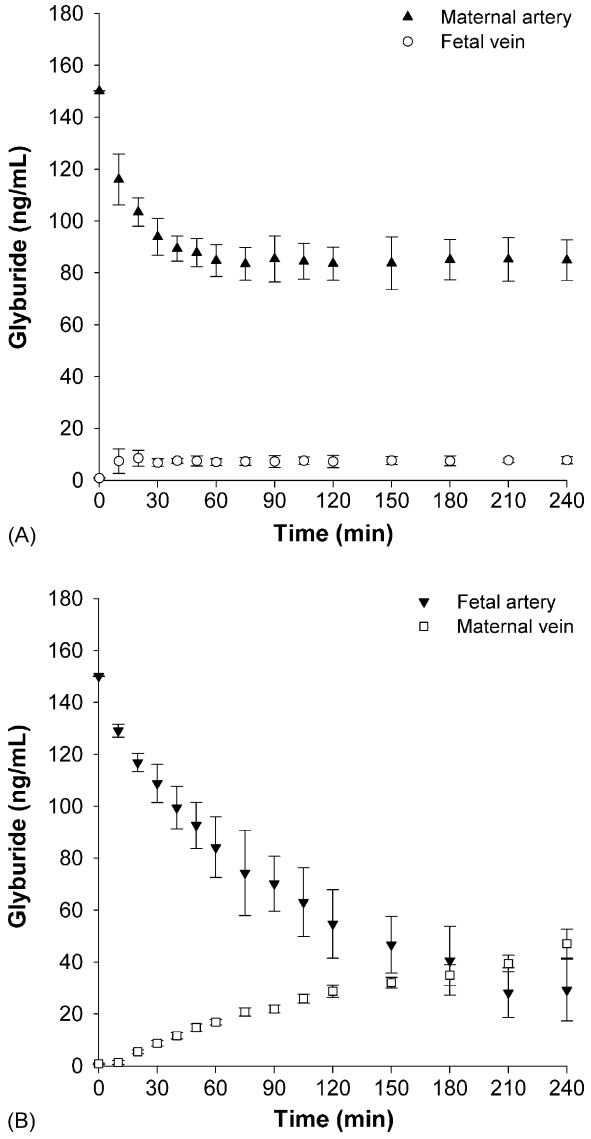

Fig. 2 – (A) Transfer of glyburide from the maternal to fetal circuit in the absence of added HSA ($n = 4$). Glyburide was added to the maternal reservoir and transfused in a closed–closed system. HSA was not added but the perfused tissue proteins were released into the maternal circuit only. The decline in the concentration of glyburide in the maternal artery during the initial 30 min is attributed to uptake of the drug by placental tissue. (B) Transfer of glyburide from the fetal to maternal circuit in the absence of HSA ($n = 5$). HSA was not added and tissue proteins are not released into the fetal circuit. Glyburide was readily transferred from the fetal circuit to the tissue and consequently into the maternal circuit. The proteins released from the tissue into the maternal circuit retained the drug and caused the observed gradual increase in its concentration.

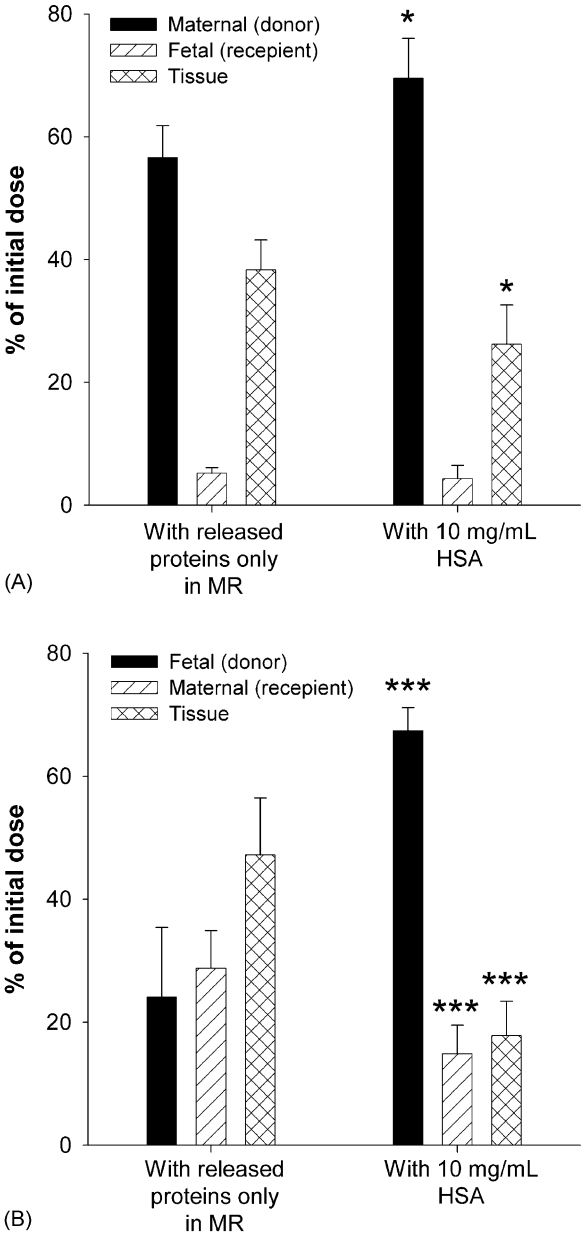

Fig. 3 – (A) The effect of HSA on the distribution of glyburide when transfused from the maternal to fetal circuit ($n = 4$). Glyburide was added to the maternal reservoir at a concentration of 150 ng/mL followed by the addition of 10 mg/mL HSA to both circuits. Adding HSA resulted in more of the drug being retained in the maternal (donor) circuit ($P < 0.05$) and less being transferred to the tissue ($P < 0.05$). (B) The effect of HSA on the distribution of glyburide when transfused from the fetal to maternal circuit ($n = 4$). The addition of 10 mg/mL HSA to both circulation resulted in a significant increase of the amount of glyburide remaining in the fetal (donor) circuit and a decrease in the amount of glyburide retained by the tissue and that transferred to the maternal (recipient) circuit. The effect of added HSA on the distribution of glyburide after its perfusion in fetal to maternal direction was more pronounced than after its perfusion in the opposite direction ($P < 0.001$).

3.2.4. The effect of HSA on glyburide distribution between the placental tissue and the maternal and fetal circuits

The effect of adding HSA (at a concentration of 10 mg/mL) to the maternal and fetal reservoirs on the distribution of

glyburide was investigated. This concentration of HSA is approximately 35–40% of that in vivo, and under these conditions, the binding of glyburide to albumin should be maximal. The presence of 10 mg/mL HSA in the maternal circuit (Fig. 3A) resulted in a decrease in the rate of decline in the concentration of glyburide as compared with that in the absence of the protein. Consequently, the transfer of glyburide to the placental tissue and fetal circuit was reduced.

3.2.5. *Distribution of glyburide in presence of HSA following its perfusion from the fetal to maternal circuits*

The effect of adding HSA to the fetal circuit on the transfer of glyburide is shown in Fig. 3B. The addition of HSA significantly increased the amount of glyburide retained in the donor (fetal) circuit and consequently, decreased its transfer to the placental tissue and the recipient (maternal) circuit. Moreover, it is apparent that the effect of 10 mg/mL HSA was more pronounced on the transfer of glyburide from the fetal to the maternal circuit than from the reverse direction, i.e., maternal to fetal.

Taken together, these data demonstrate that the binding of glyburide to HSA is the main factor affecting transfer of the drug across the human placenta, as well as its distribution between the tissue and two circuits.

4. Discussion

Pregnancy is associated with a decrease in circulating proteins, which includes an albumin concentration drop from a mean of 43 mg/mL in nonpregnant women to 29 mg/mL in pregnant women [13,14]. Undoubtedly, this drop in albumin concentration should affect the biodisposition of drugs and more so those that are extensively bound to it, such as glyburide (99.8%). Therefore, the goal of this investigation was to determine the effect of albumin and placental tissue proteins on the transfer of glyburide across the term human placenta and its distribution between the tissue, maternal, and fetal circuits of the dually perfused placental lobule.

The closed–closed (recirculating) type of perfusion system was used by Elliott to investigate the bidirectional transfer of glyburide across the human placenta [4]. These authors reported that insignificant amounts of glyburide were transferred across term placental tissue irrespective of the direction of its transfusion being from the maternal to fetal or fetal to maternal circuits. Subsequently, the authors reported on a comparison between the transplacental transfer of glyburide with the hypoglycemic drugs tolbutamide, chlorpropamide, and glipizide [5]. Although all four drugs had similar physicochemical properties (molecular weight, partition coefficient, and dissociation constant), the rate for transplacental transfer of glyburide was approximately 50% that of glipizide, 20% that of chlorpropamide, and 10% that of tolbutamide.

It should be noted that in the above investigations, the concentration of HSA added to the perfusion medium ranged between 2 and 20 mg/mL. In our investigation discussed here, glyburide was transfused in the absence of HSA to determine the transfer and distribution of the ''free'' drug. Under these experimental conditions, the transfer rate of unbound glyburide to the fetal circuit was $73 \pm 10\%$ of the readily diffusible marker compound AP. These data indicate that glyburide transfer, in the absence of HSA, is in agreement with its physicochemical properties. Moreover, in the absence of HSA from the perfusion medium, high amounts of glyburide were retained and accumulated by the perfused tissue due to the nonspecific binding of the drug to tissue proteins. Similar high retention of other lipophilic drugs, such as the opiate buprenorphine, by perfused tissue was reported earlier [11].

Glyburide binding to HSA is 99.8% and, even in the presence of 100 times its peak plasma levels and HSA concentration of 2 mg/mL, the drug remained in the bound state [4]. Accordingly, it can be assumed that glyburide was in the bound state at all the concentrations of HSA investigated by Elliott et al. (2–20 mg/mL). At HSA concentration of 2 mg/mL and the circulating therapeutic concentration of glyburide of 150 ng/mL, the molar ratio of glyburide:albumin is approximately 1:100. Therefore, we investigated the effect of a range of HSA concentrations on the transfer of glyburide corresponding to a drug:albumin molar ratio between 1:2 and 1:100.

The first set of experiments was designed to determine the effect of HSA on glyburide transfer under steady-state concentration and hence, the use of the open–open mode of the perfusion system. Increasing concentrations of HSA were added to the maternal reservoir and the fetal transfer rate of glyburide and its amounts retained by the tissue were determined. The data obtained clearly indicate that the effect of HSA on glyburide transfer (Table 1) and its retention by the tissue (Fig. 1) was concentration dependent and biphasic. The most pronounced effect of HSA on placental transfer and retention of glyburide was observed at its concentration range between 42–630 μg/mL (molar ratio for glyburide:HSA of 1:2–1:30). The concentration of 2.1 mg/mL HSA (glyburide:HSA molar ratio of 1:100) did not significantly affect the amount of drug retained by the tissue. These data suggest that the binding of glyburide to HSA, in the maternal circuit, was near its maximum and consequently, the amount of free drug available for transfer was significantly decreased. Moreover, the increase in HSA concentration in the perfusion medium resulted in a significant decrease in glyburide binding to placental tissue proteins, but an amount of glyburide still remained nonspecifically bound.

The second set of experiments was designed to investigate the effect of HSA on glyburide distribution between placental tissue, maternal circuits, and fetal circuits. In these experiments, the closed–closed system was used to simulate the in vivo conditions and the bidirectional transfer of glyburide, i.e., from the maternal to fetal and fetal to maternal circuits, was investigated. It should be emphasized here that the perfused placental lobule releases significant amounts of protein into the maternal circuit, but not into the fetal circuit, during the experimental period irrespective of any added HSA. The concentration of these released proteins into the maternal circuit accumulated in the closed–closed system, and reached approximately 630 μg/mL at the end of the 4 h experimental period. On the other hand, no protein was detected in the fetal circuit. Under these conditions, glyburide was added to the maternal reservoir, transfused and $56 \pm 5.2\%$ of it remained in the maternal circuit, $38 \pm 4.7\%$ was retained by the tissue and only $2.5 \pm 0.4\%$ of its initial dose was transferred to the fetal circuit. On the other hand, under the same conditions, when

glyburide was added to the fetal circuit and transfused, only $27 \pm 9.8\%$ of it remained in the fetal circuit, $48 \pm 8\%$ was retained by the tissue, and $25 \pm 1.9\%$ was transferred to the maternal circuit. Therefore, the proteins that were released from the tissue (630 μg/mL) into the maternal circuit, and not the fetal, had a significant effect on the distribution of glyburide between the tissue and the two circuits.

The above discussed effect of released proteins became more apparent when 10 mg/mL HSA was added to both the maternal and fetal circuits. Under these conditions, the following effects were observed irrespective of the addition of glyburide to either the maternal or fetal reservoir: increase in the amount of glyburide remaining in the donor circuit, decrease in the amount of glyburide retained in the tissue, and decrease in the amount of glyburide transferred to the recipient circuit (Fig. 3A and B). Moreover, the transfer of glyburide from the fetal (donor) to maternal (recipient) circuit was higher (recipient/donor concentration ratio of 0.17 ± 0.05) than in the reverse direction, i.e., maternal (donor) to fetal (recipient) (recipient/donor ratio of 0.05 ± 0.03, $P < 0.01$). A similar higher transfer of a drug from the fetal to maternal circuit than that in the opposite direction was reported earlier for the transfer of the opiate methadone, including a discussion of a role for the efflux transporter P-glycoprotein (P-gp) [15]. This explanation was confirmed by data utilizing the same technique of dually perfused placental lobule — namely, the addition of the P-gp inhibitor GF120918 resulted in a higher transfer of methadone to the fetal circuit [16].

Recently, Kraemer et al. demonstrated that glyburide is transferred from the fetal to maternal circuit against its concentration gradient and suggested the involvement of transporter proteins [17]. However, in their reported experimental design, HSA was excluded from the perfusion medium and the authors did not account for the binding of glyburide to proteins released from the placental lobule into the maternal but not the fetal circuits of their closed–closed perfusion system. Moreover, the authors did not provide a graph of their data on the rate of glyburide transfer; thus, it is impossible to compare the data with those reported here or earlier by Elliot et al., who reported a slightly higher transfer rate of glyburide in the fetal to maternal direction (0.89% versus 0.62%) than in the maternal to fetal direction but the difference did not reach statistical significance [4]. Therefore, it appears that the difference in the transfer of glyburide from the maternal to fetal circuit and vice versa in the model system of the dually perfused placental lobule could be explained by the influence of proteins released from the tissue and the added albumin to the perfusion medium as well as by the involvement of efflux proteins. Recently, Gedeon et al., utilizing a cell line overexpressing BCRP and MRP3, demonstrated a role for these efflux transporters in the transfer of glyburide [9]. However, it is unclear whether the role of one or more efflux transporters could account for the difference in the higher net transfer of glyburide across the human placenta and further investigations are needed.

In an earlier report from our laboratory [11], we demonstrated that the transfer of lipophilic drugs across human placenta is a two-step process as proposed by Sastry [18]. The first step is uptake and retention of the drug by trophoblast tissue and the second step is release of the drug to the recipient circuit. Therefore, it appears that glyburide binding to HSA in the maternal circuit is the main factor that limits its uptake by the tissue and consequently, its transfer to the fetal circuit.

In summary, data cited here, utilizing the technique of dual perfusion of the placental lobule in both of its configurations, the open–open and closed–closed, indicate that the presence of added HSA and released tissue proteins, that may also include albumin, in the maternal circuit are the major factors limiting the transfer of glyburide across the human placenta to the fetal circuit. Moreover, the binding of glyburide to human serum albumin reaches its maximum at a concentration of albumin significantly below that observed in pregnant women.

The above conclusions and speculations are based on data from an ex vivo model system, though they are in part substantiated by data obtained in vivo indicating very low transplacental transfer of glyburide. Therefore, it can be concluded that the decrease in albumin concentration associated with pregnancy is unlikely to affect the disposition of glyburide.

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