TRANSPORT KINETICS OF VANADIUM IN PERFUSED HUMAN PLACENTAL LOBULE IN LATE GESTATION: IN VITRO STUDY

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ABSTRACT

Background: Reports relating to maternal-fetal transport kinetics of vanadium, an essential trace element in the human pregnancies are scanty. Hence we have attempted to investigate the transport kinetics of this trace element in the human placenta in late gestation in vitro Methods: Human placentae were collected immediately after delivery from normal uncomplicated pregnancies .Vanadium Perchlorate solution (GFS ChemInc, USA) at ten times the physiological concentrations and antipyrine (Sigma Chem co, USA) as internal reference marker were injected as a single bolus (100ul) into the maternal arterial circulation of perfused placental lobules and perfusate samples were collected from maternal and fetal circulations over a study period of 5 minutes. National Culture and Tissue Collection medium, diluted with Earle's buffered salt solution was used as the perfusate. Serial perfusate samples were collected from fetal venous perfusate for a period of 30 minutes. Vanadium concentration in perfusate samples was determined using atomic absorption spectrophotometry while the concentration of reference marker antipyrine was measured spectrophotometrically. Various transport parameters and transport kinetics of study and reference markers were computed using established permeation parameters. Results: Differential transport rates of Vanadium and antipyrine in 12 perfusions differed significantly for 25, 75, 90% efflux fractions (ANOVA test, $p<0.05$) while those of 10&50% efflux fractions were not significantly different between the study and reference substances. Transport Fraction (TF) of Vanadium averaged 54.9% of bolus dose in 12 perfusions while that of antipyrine averaged 89% of bolus dose, representing 61.80% of reference marker TF. The difference observed in TF values of Vanadium and antipyrine was statistically significant (Student's t-test, $p<0.05$).Pharmacokinetic parameters such as area under the curve, clearance, absorption rate, elimination rate of manganese compared to reference marker were significantly different (ANOVA Test, $p<0.05$) between the study and reference substances. Conclusions: Our studies report for the first time maternal-fetal transport kinetics of Vanadium in human placenta in vitro. Considering the restricted transfer of this essential trace element from maternal to fetal circulation despite its small molecular weight, we hypothesize an active transport of this trace across the human placental membrane. Further studies relating to placental transport kinetics of this trace element in diabetic pregnancies are in progress

Keywords: Vanadium; Transport Kinetics; Human placenta; In vitro

INTRODUCTION

Role of essential trace elements for maintenance of health has been widely established[1-4].Previous researches from our laboratory had explored the maternal-fetal transport and disposition of various essential trace elements in humans as well as experimental animals in control as well human diabetic pregnancies [5-8]. Though many research groups have emphasized importance of various trace elements in human metabolism including effects of anti-oxidant enzymes [9-12], no detailed study of transport of vanadium from mother to fetus has been reported even in experimental animals. However there have been no detailed reports to date, relating to maternal-fetal transport of Vanadium, an essential trace element in human pregnancies.

Though Vanadium has been reported by some research groups to be beneficial in treatment of Type 2 Diabetes in humans, studies by our research group in supplementation of vadium in experimentally induced diabetic rates showed no beneficial effect in reducing the blood sugar level. This study was aimed to investigate the transport kinetics of this trace element in the human placenta in order to establish its transport characteristics in normal and uncomplicated human pregnancies. Maternal-fetal transport kinetics of Vanadium was assessed in vitro, using perfusion of isolated human placental lobule. The in-vitro human placental perfusion model has been used previously by our research group to study maternal-fetal transport of a wide variety of nutrients and pharmaco-active agents [13-15]. The lobular perfusion method has the added advantage of exploring membrane transport behavior, independent of maternal and fetal hemodynamic and metabolic influences and under varying experimental conditions [15-16]. Antipyrine, widely used in placental permeation studies as the reference marker [17-18] was used as the internal control, to permit inter-experimental comparison and interpretation of data.

PERFUSION TECHNIQUE

Placentae were collected immediately after spontaneous delivery or caesarean section and fetal and maternal circulations of selected lobules perfused with National Culture and Tissue Collection (NCTC) medium diluted with Earle's buffered salt solution as perfusate[19, 20]. Perfusate flows in maternal and fetal circuits were monitored by "Brooks" flow meters and pressures assessed by mercury manometers. The flow rates were maintained within physiological range and pressure in both circuits in the perfusion pressure was monitored by mercury manometers. After initial wash-out period of 10 minutes, 1.5 mg/l Vanadium perchlorate c solution (GFS ChemInc, USA) corresponding to 10 times physiological Mn concentration and antipyrine (Sigma Chem co, USA) as reference marker (concentration : 100ug/L) were injected as 100 microliter bolus into the maternal circulation in separate experiments , at a site close to the insertion of the micro cannulas in the

maternal basal plate[25]. After a lag period of one minute, serial perfusate samples were collected from the fetal venous outflow for every 30 seconds for a study period of 5 minutes. The site, dose and duration of bolus injection were kept identical throughout the duration of the study.

The study period of 5 min was based on the time required for 90% of injected substance in the bolus to appear in the combined fetal and maternal venous efflux in control experiments. Fetal perfusion flow rate averaged 4.13 ± 0.29 ml/min while the maternal flow rate averaged 4.53 ± 0.38 ml/min in 12 successful perfusions.

Viability of perfusions was assessed by oxygen consumption of perfused tissue as well as absence of lactic dehydrogenase (LDH) enzyme in the perfusate samples before and after perfusion. Only those perfusions with minimum perfusion flow rate mis-match, minimum LDH leakage and within the established norms of oxygen consumption were considered as successful or valid experiments and data from unsuccessful perfusions were not considered for further analysis and evaluations.

Concentration of vanadium in perfusate and bolus samples was determined, using atomic absorption spectrophotometry [21]. Briefly the vanadium atoms in the samples are artificially excited and in the excited state, as the number of atoms in the light path increases , the amount of light absorbed also increases. By measuring the amount of light absorbed, the quantity of the trace element of the sample is determined. The spectrometer (Varian AA240 FS, Australia) uses the graphite furnace for combustion and exciting the manganese atoms present in the sample at a light source from hollow cathode lamps of wavelength 318.5nm and using high-intensity deuterium background corrector, covering wavelength range 185-425 nm. The graphite tube atomizer of the equipment requires dry argon gas at 140-200 kPa (20-29 psi) and gas pressure of 20 psi as an essential component for trace element detection and analysis [[25]].

Maternal-fetal transport parameters and kinetics were assessed using the following:

- Differential transport rates of test and reference substances was computed as described earlier [15.-16]. Transport of substance studied was expressed as transport rate index of different efflux fractions, as ratio of corresponding reference marker transport rates.

- Transport fraction (TF) of substances was calculated as per the formula [8, 19] A TF index of study substance was also computed by expressing the TF value as a ratio of that of the reference.
- Area under the curve (AUC) of substances was computed using trapezoid rule [21, 23] assuming a two-compartment model.

Parameters such as clearance, Kel (elimination constant), Tmax (time of maximum response), absorption rate and elimination rate were determined by

computer programmer based on PK SOLUTIONS software specially adapted for statistical applications.

RESULTS

Table 1 summarizes the clinical data of 12 pregnant women from whom placentae were collected after delivery for perfusion experiments. The women had no medical complications during the pregnancy period and had uncomplicated normal progression of pregnancy. Table 2 details the differential transport rates (TR) of Vanadium and antipyrine as well as transport rate index of manganese compared to reference marker in 12 successful perfusion experiments, ANOVA test showed that the differential transport rates of vanadium was significantly different ($p<0.05$) than that of antipyrine for TR25, 50, and 75 efflux fractions, but no statistical significance could be demonstrated (p.0.05) for the TR10&50 efflux fractions. Table 3 details the various pharmaco-kinetic parameters such as AUC, clearance, Tmax, absorption rate and elimination rate of manganese and antipyrine in 12 successful perfusions. ANOVA test showed the kinetic parameters of vanadium such as AUC, clearance, absorption Rate and elimination rate were significantly different ($p<0.05$) than the corresponding kinetic parameter of reference marker antipyrine. However, ANOVA test did not show any significant difference ($p>0.05$) between Tmax values of vanadium and antipyrine. Table 4 details the transport fractions (TF) of Vanadium and antipyrine in 12 perfusion experiments. Vanadium TF averaged 54.90 % of injected bolus dose while that of antipyrine averaged 89 % Student's t-test showed Mn TF to be significantly lower ($p<0.05$) than that of antipyrine TF. Vanadium TF averaged 61.8% of antipyrine TF in the 12 perfusions.. Vanadium TR50 averaged 2.48 min while that of antipyrine averaged 2.55 min in 12 perfusions. Student's t-test did not show any significant difference ($p>0.05$) of TR50 of Vanadium compared to that of antipyrine. Transport rates of vanadium and antipyrine from maternal circulation to fetal venous circuit in 12 perfusions are shown in (Fig.1). ANCOVA Test showed the Vanadium transport rate curve to be significantly lower ($p<0.05$) than that of antipyrine rate curve.

Table 1: Clinical Characteristics of pregnant women

Age (years)	32.14±7.1
Primiparous , m (%)	12.5
Previous Abortion, m (%)	12.5
Gestation Age (weeks)	39 ± 1.4
Cesarean Sections, m (%)	37.5
Spontaneous Delivery, m (%)	62.5
Newborn Weight (kg)	3.63±0.8
Weight of Placenta (g)	652.86±64.7
Apgar Score	8, 9

Data are Means±SEM of 12 pregnant women

Table 2: Differential Transport Rate parameter of Manganese &Antipyrine

	TR10 (min)	**TR25 (min)**	**TR50 (min)**	**TR75 (min)**	**TR90 (min)**	**QF/QM**
Vanadium	0.730 ± 0.16	1.643 ± 0.36*	2.48 ± 0.09	4.69 ± 0.05*	4.607 ±0.26*	0.45 ± 0.06
Antipyrine (A)	0.67± 0.04	1.37 ± 0.04	2.55 ± 0.03	3.73 ± 0.03	4.39 ± 0.033	0.45 ± 0.03

Means±SEM of 12 perfusions, QF= Fetal Perfusion Flow Rate (ml/Min); QM= Maternal perfusate Flow Rate (ml/min); Statistical Significance was assessed by ANOVA Test ; * p<

Table 3: Pharmacokinetic parameters of Vanadium and Antipyrine

	AUC (ug/hour)	CLEARANCE (ml/min)	Tmax (sec)	Abs. Rate (ug/min)	Elim. Rate (ug/min)	Cot. Wt.(g)
Vanadium (V)	1600.98 ± 659.2*	0.0053 ± 0.0*	152.5±13.54	0.00013±0.00003 *	0.003±0.001 *	31.84±1.92
Antipyrine (A)	4677.1± 771.35	0.006± 0.0009	150.0±10.95	0.084± 0.014	1.124±0.179	31.84±1.92

Mean± SEM of 12 perfusions ; PK= Pharmacokinetic Index; AUC=Area Under the Curve; Cl=Clearance; Abs= Absorption; Elim= Elimination (Statistical Analysis was done by ANOVA Test ; * $p<0.05$)

Table 4: Transport Fractions of Vanadium and Antipyrine

Perfusion	Transport Fraction (V)	Transport Fraction Antipyrine (B)	Transfer ratio V/B
1	0.453	0.355	1.276
2	0.071	0.811	0.088
3	0.224	0.236	0.949
4	0.101	0.309	0.327
5	0.237	0.309	0.767
6	0.213	1.154	0.185
7	2.253	1.556	1.448
8	2.27	4.193	0.541
9	0.31	0.879	0.353
10	0.165	0.289	0.571
11	0.192	0.321	0.598
12	0.099	0.321	0.308
Mean±SEM	0.549 ± 0.23*	0.89 ± 0.0.32	0.618 ± 0.12

Mean± SEM of 12 perfusions; TF=Transport Fraction; V=Vanadium; A= Antipyrine; (Statistical Analysis was done by Student's t- test ; * $p<0.05$)

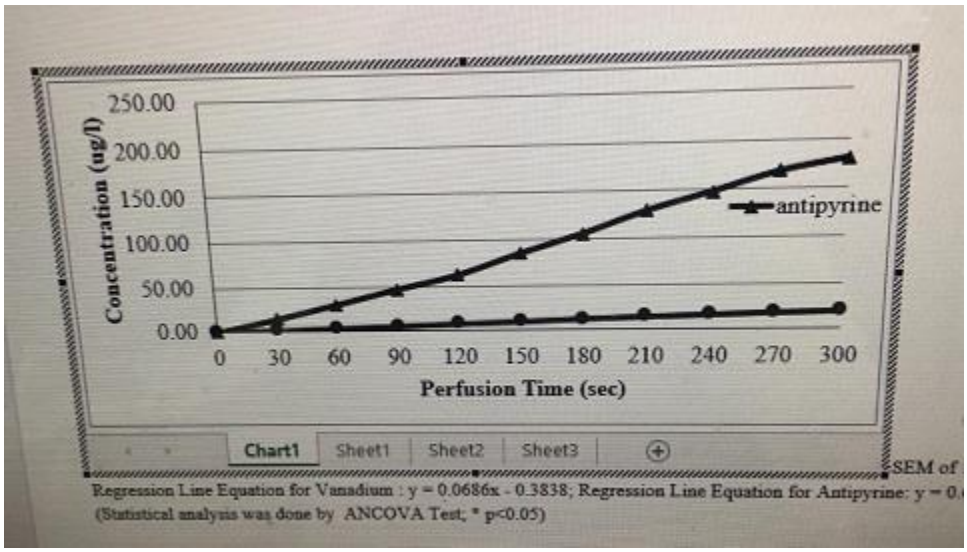

Figure 3: Cumulative concentration of Vanadium and Antipyrine into the Fetal Vein Mean±SEM of 12 perfusions; Regression Line Equation for Vanadium: y = 0.0686x - 0.3838; Regression Line Equation for Antipyrine: y = 0.6293x - 6.3765 (Statistical analysis was done by ANCOVA Test; * p<0.05)

DISCUSSION & CONCLUSION

Transport data on antipyrine the reference marker are consistent with our earlier findings in human placentae and confirm free and uninhibited transport like titrated water across the human placentae[22,23,24]We report for the first time original new data on maternal-fetal transport of an essential trace element, Vanadium in human placentae in vitro and detail for the first time in literature, maternal-fetal transport kinetics of this trace element in human placenta in vitro.

Our data show that Vanadium is transferred to the extent of only about 61.80% of reference marker transport. Considering such a restricted transport despite being a small atomic weight trace element, it is likely that the element is transported actively across the human placental membrane Our in vitro study supports the notion that Vanadium is actively transported across the human placental membrane. There have been no reported studies to date on relation of vanadium levels with antioxidant enzyme, Studies done in our laboratory on experimentally induced diabetic rats showed that vanadium supplementation raises activities of anti-oxidant enzymes, SOD and GPX in vanadium treated animals. However, there are reports of vanadium toxicity in experimental on reproductive and fetal development (25) The above report and side-effects need to be considered and analyzed more carefully when supplementation of manganese in diet is recommended in clinical situations.

Previous studies done in our laboratory have shown that vanadium transport is impaired from maternal to fetal circulation in hyperglycemic diabetic

model human placental lobules though limitation of in vitro study does not permit us to conclude whether the same ffect could be replicated in vivo situations,

We hypothesize that in diabetic patients vanadium transport may be compromised and that better clinical management of pregnant diabetic women and their new born may be possible with adequate dietary supplementation in of vanadium in diet and or as supplements in compromised patients, taking extra care to minimize the undesirable toxicity of over-loading of this essential trace element in needy patient population. Further studies are under way to assess the effect of hyperglycemia on maternal-fetal disposition of this trace element in insulin dependent as well as Type 2 diabetic pregnancies in humans.

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