A modified perfusion system for dual in vitro perfusion of the human placenta

Mehmet Ali ONUR, Aşkın TÜMER

Hacettepe University, Faculty of Science, Department of Biology, Beytepe, ANKARA, TURKEY

A modified system for dual in vitro perfusion of the human placenta is developed and tested by measuring antipyrine clearance. For this a perfusion chamber consisting of 4 parts is manufactured and placed in the system. Placentas obtained following vaginal deliveries are used. Isolated cotyledones instead of whole placentas are perfused. Flow rates and perfusion pressures are recorded and antipyrine clearances are calculated. Obtained values are compared with those in the literature. [Turk J Med Res 1992, 10(3):121-125]

Keywords: Human placenta, In vitro perfusion

A safe way of studying placental transfer characteristics of various substances in humans is perfusing the placenta in vitro. Such studies have begun with perfusing the whole placenta (1,2). Subsequently single or a group of cotyledones have been perfused (1). In one of their studies Schneider et al. (3) perfused the fetal side through the fetal artery and vein supplying the cotyledon and they perfused the maternal side by means of 2 glass cannulas inserted in to the intervillous space. Brandes et al. (4) were able to extent the duration of the perfusion up to 2 hrs by recirculating the perfusate. Miller et al (5) have repoted a prolonged perfusion time of 12 hrs. Kaufmann (6) has pointed out the structural changes occured in the placenta during perfusions. In the present study we developed and tested a slightly modified perfusion system for the human placenta.

MATERIALS AND METHODS

In this study a slightly modified perfusion system of Schneider et al (3) is used (Fig 1).

Geliş Tarihi: 7.4.1992 **Kabul Tarihi:** 21.4.1992

Yazışma Adresi: Mehmet Ali ONUR
Hacettepe University, Faculty of Science, Department of Biology, Beytepe, ANKARA, TURKEY

Term placentas obtained immediately following vaginal deliveries after 38-42 week normal pregnancies are used. Chorionic artery and vein of a non-peripheral and undamaged cotyledon of approximately 4-6 cm. in diameter are cannulated. Fetal perfusion is initiated with a flow rate of 6-12 ml/min and with a pressure of 50-90 mmHg. Perfused cotyledon is then trimmed away from the rest of the placenta and placed in the perfusion chamber with its chorionic side down. Five steel cannulas of 15 mm long and 200 urn internal diameter are inserted into the decidual layer to a depth of approximately 8 mm in order to perform the perfusion of the maternal side. Maternal perfusion is then initiated with a flow rate of 10-15 ml/min and with a pressure of 80-140 mm Hg.

Bicarbonate-buffered Earle's solution containing 4% of dextrane (MW 40.000) and heparine (2500 u/L) is used as the perfusate. The perfusate is continuously aerated with a mixture of 5% CO_2 and 95% O_2 starting 30 min prior to the perfusion. The perfusate is circulated with two separate peristaltic pumps. The pressures are monitored with mercury manometers connected to the system and the possible air bubles are trapped (Fig 1). The system was kept at 37 ± 1°C throughout the perfusions.

Antipyrine is used as the material and this is added to the initial maternal pool at a concentration of 100 ng/ml. Antipyrine content of the samples taken from the fetal pool at time intervals is analysed spectrophotometrically according to the method of

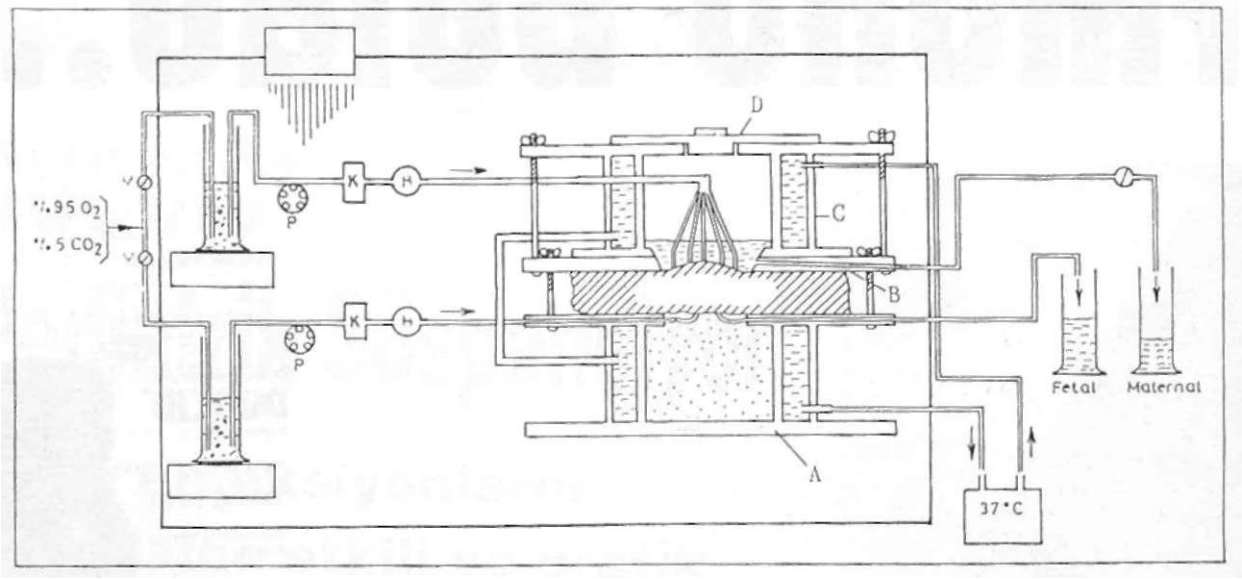

Figure 1. Scheme of the placenta perfusion system.
(P=pump; K=bubble trap; H=mercury manometer; WB=water bath; V=clips; A=lower part; B=middle part; C=upper part; D=cover).

Brodie et al (7). Antipyrine clearance of the maternal pool is calculated taking into account the initial concentration, transferred amount and flow rates. For this the following equation developed by Schneider et al (8) is used:

$$CI = (CFV-CFA) \times QF / (CMA-CFA)$$

in which:

CI = antipyrine cleared from the maternal perfusate in each minute (ml/min)

CFV - antipyrine concentration in the fetal return reservoir

CFA = antipyrine concentration in the fetal initial reservior (this is equal to zero when the perfusate is not recirculated)

QF = fetal flow rate

CMA = antipyrine concentration in the maternal initial reservoir

RESULTS

Variables and the clearance index obtained in 7 seperate perfusions are presented in Table 1. According to this the mean wet weight of the perfused cotyledons is 16.3 ± 1.2 g. The mean fetal and maternal perfusion pressures are 53.9 ± 5.2 mmHg and 127 ± 8.9 mmHg respectively. The mean fetal and maternal flow rates 5.7 ± 0.32 ml/min. and 13.6 ± 1.3 ml/min. respectively. The mean QF is 0.41 ± 0.03 ml/min. The mean clearance index of antipyrine from the maternal side in 7 perfusions is found as 1.58 ± 0.9.

DISCUSSION

The most problematic aspect of the in vitro perfusions of the human placenta is the cessation of the blood circulation following delivery and consequently necrosis caused by the ischemia. The only way to avoid this is to shorten the time between the delivery and the initiation of the perfusion. Kaufmann (6) have reported that the optimum time is between 15 and 30 minutes. In another study, Kaufmann (9) also points out that mitochondrial changes and changes in the endoplasmic reticulum within the first minutes of the ischemia exceeding 20 minutes, are irreversible and anoxia is a pathological factor in this ischemia. He also suggests that ischemic period should not exceed 30 minutes. In our system the independence of the two cannulas which are connected to the fetal arterial and venous cannulas has enabled us to shorten this period to as low as 10 minutes. Another modification brought about by our system is the usage of cannulas with an internal diameter of 1.19 mm. This in turn has enabled us to decrease the volume of the fetal and maternal perfusates to as low as 50 ml. This volume has been claimed by Brandes et al (4) as 70-100 ml for the fetal and 170-200 ml for the maternal circulations. This slight modification of ours offers an economical advantage considering the prices of the material used in the perfusate. The antipyrine clearance results obtained in our system are in good accordance with those obtained by the others (3,10). In conclusion, with this study we are presenting a modified perfusion system for perfusing the

Table 1. Results of antipyrine perfusions
(SD=standard deviation, F=Fetal, M=maternal, FFR=fetal flow rate, MFR=maternal flow rate, Cl=clearance)

Perfusions	Fetal pressure (mmHg)	Felal flow rate (ml/min.)	Maternal pressure (mmHg)	Maternal flow rate (ml min.)	FFR.MFR	Clearance (Cl)	Weight (g.)
1	49.6	5.45	123	11.8	0.47	1.35	17.30
2	51.3	6	137.6	15.2	0.39	1.29	14.37
3	56.5	5.2	1 10	12.5	0.41	1.56	16.30
4	52.1	6	130	13.0	0.46	1.71	17.50
i-lâfa-'	54.4	5.8	132	14.2	0.40	1.75	16.72
6	52.0	6	129	14.0	0.42	1.81	17.00
-	53.0	5 5	132	14.9	0.36	1.63	15.01
MHAX±SD =	53.98+5 2	5.7=0.3	127.6=8.9	13.6+1.3	0.41 ±0 03	1.58+0 19	16.31+1.18

human placenta in vitro which is suitable for studying placental transfer kinetics of various substances (including particularly drugs).

İnsan plasentasının çift taraflı in vitro perfüzyonu için modifiye bir sistemin kurulması ve denenmesi

Bu çalışmada insan plasentasının in vitro perfüzyonunu sağlayacak modifiye bir deney sistemi kurulmuş ve bunun işlerliği denenmiştir. Bu amaçla, 4 kısımdan oluşan birperfüzyon cihazı yapılmış ve bu cihazın yer aldığı bir perfüzyon sistemi düzenlenmiştir. Deneylerde, vajinal doğumlardan elde edilen plasentalar kullanılmıştır. Perfüzyonlar tüm plasentayla değil, izole kotiledonlarla yapılmıştır. Çalışmalar esnasında akım hızı, basınç ve antipirin temizlenmesi kaydedilerek daha önceki çalışmalar ile karşılaştırılmıştır.

[Türk Tıp Araştıma 1992, 10(3): 121-125]

Anahtar Kelimeler: İnsan plasentası, in vitro perfüzyon

REFERENCES

Panigel M. Placental perfusion experiments. Am J Obstet Gynec 1962; 84(11):1164-72.

Krantz, KE, Panos TC, Evans J. Physiology of maternal-fetal relationships through the extracorporeal circulation of the human placenta. Am J Obstet Gynec 1962; 83:1214-28.

3. Schneider H, Panigel M, Dancis J. Transfer across the perfused human placenta of antipyrine, sodium, and leucine. Am J Obstet Gynec 1972; 114(6):822-8.
4. Brandes JM, Tavoloni N, Potter BJ, Sarkozi L, Shepard MD, Berk PD. A new recycling technique for human placental cotyledon perfusion, Application to studies of the fetomaternal transfer of glucose, inulin and antipyrine. Am J Obstet Gynecol 1983; 146:800-6.
5. Miller RK. Wier PJ, Maulik D, Santagnese PA. Human placenta in vitro: characterization during 12h of dual perfusion. Contr Gynec Obstet, H.Schneider and J.Dancis (Eds), Kargel-Basel 1985; 13:77-84.
6. Kaufmann P. Influence of ischemia and artificial perfusion on placental ultrastructure and morphometry. Contr Gynec Obstet, H.Schneider and J. Dancis (Eds.), Kargel-Bsel 1985; 13:18-26.
7. Brodie B, Axelrod J, Soberman R, Levy BB. The estimation of antipyrine in biological materials. J Bio Chem 1949;179:25-9.
8. Schneider H, Challier JC, Dancis J. Transfer and metabolism of glucose and lactate in the human placenta studied by a perfusion system in vitro. Placenta 1981 (2):129-38.
9. Kaufmann P. Basic morphology of the fetal and maternal circuits in the human placenta. Contr Gynec Obstet, H.Schneider and J. Dancis (Eds.), Kargel-Basel 1985; 13:5-17.
10. Schneider H, Huch A. Dual in vitro perfusion of an isolated lobe of human placenta. Contr Gynec Obstet, H. Schneider and J. Dancis (Eds.), Kargel-Basel 1985; 13:40-7.