Fetal anaemia and its relation with increased concentrations of adenosine

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Abstract

Adenosine concentrations were measured in umbilical venous blood obtained by cordocentesis from 14 fetuses of 19–34 weeks' gestation. The concentration did not change significantly with gestational age, but anaemic fetuses showed significantly increased concentrations of adenosine and there was a positive association with blood oxygen tension. These findings suggest that the fetus responds to tissue hypoxia by increasing blood adenosine concentrations from at least 19 weeks' gestation.

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Postnatal studies of infants who had been subjected to perinatal asphyxia have reported increased concentrations of catecholamines,[1] adenosine, and hypoxanthine in blood samples.[2–5] Cordocentesis, ultrasound guided umbilical venous blood sampling, has now made it possible to investigate the metabolic consequences of intrauterine hypoxia. Previous work, using cordocentesis samples from rhesus isoimmunised fetuses, showed the presence of hypoxaemia and anaemia, and showed that these abnormalities were associated with increased concentrations of catecholamines.[6]

The aim of this study was to measure adenosine concentrations in fetuses and determine their relation with prenatal hypoxia.

Patients and methods

In a cross sectional study of 14 pregnant women, with fetuses between 19 and 36 weeks' gestation, umbilical venous blood was obtained by cordocentesis. The indications for cordocentesis were fetal karyotyping because of advanced maternal age, ultrasonographically detectable fetal defects, investigation of maternal toxoplasmosis infection, or measurement of fetal haemoglobin to assess the severity of rhesus isoimmunisation. Only one fetus was shown to have a chromosomal abnormality. (Turner's syndrome) (table).

Fetal blood (1 ml) was used to determine the haemoglobin concentration (Coulter S Plus counter, Porter Electronics), umbilical venous oxygen tension ($P_{uv}O_2$) (ABL 330, Radiometer) and adenosine concentration. For the latter, blood was collected in precooled syringes containing a stop solution to prevent degradation and formation of adenosine (20 μM dipyridamole, 10 μM erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride, 4 μg indomethacin in 2 ml 0·9% saline). Samples were then further cooled and centrifuged in a microfuge (10 000 g for 1 minute). The supernatant was transferred to a tube with 500 pmol N^2-N^2-dimethylguanosine which was used as an internal standard. The supernatant was titrated with ammonium acetate to a pH of 8·8 and then stored at −20°C until analysis.[7] The coefficient of variation of this assay is less than ±10%, and the lower limit of detection of adenosine is in the range 2·8–13·3 μmol/l.

Clinical details of study group

Gestation (weeks)	*Reason for cordocentesis*
25	Karyotyping (fetal hydronephrosis)
23+4	Rhesus disease
23+3	Rhesus disease
22+2	Karyotyping (advanced maternal age)
34	Karyotyping (polyhydramnios)
36	Rhesus disease
21+3	Rhesus disease
21+5	Maternal toxoplasmosis infection
21+5	Karyotyping (fetal hydrops*)
21+2	Karyotyping (fetal hydronephrosis)
20	Karyotyping (advanced maternal age)
23	Rhesus disease
19	Rhesus disease
23	Karyotyping (fetal growth retardation)

*Turner's syndrome.

STATISTICAL ANALYSIS

In normal pregnancy, blood decreases $P_{uv}O_2$ and the haemoglobin concentration increases with gestational age.[8 9] To allow for this gestational effect, the individual values of $P_{uv}O_2$ and haemoglobin in this study were expressed as SD scores (z scores) using the appropriate normal mean (SD) value for that gestational age.[8 9]

Subsequent regression analysis, fitting an exponential model, and using a computerised statistical package (Statview) has been used to determine the significance of any associations between adenosine concentrations plotted on a logarithmic scale, and the z score for $P_{uv}O_2$ or haemoglobin concentrations. Adenosine concentrations in fetuses with haemoglobin z scores greater and less than −2 were further compared using non-parametric statistics.

Results

Adenosine concentrations ranged from 10·3 to 167·2 nmol/l. There were significant negative correlations between adenosine concentration and haemoglobin z scores ($p=0·0004$, $r^2=0·67$), and between adenosine and $P_{uv}O_2$ z scores (fig 1; $p=0·0061$, $r^2=0·49$), but not between the adenosine concentration and gestational age.

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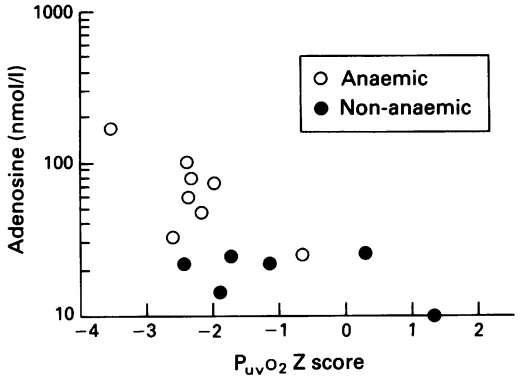

Figure 1 Relation between umbilical vein oxygen tension SD score ($P_{uv}O_2$ zscore) and adenosine concentrations.

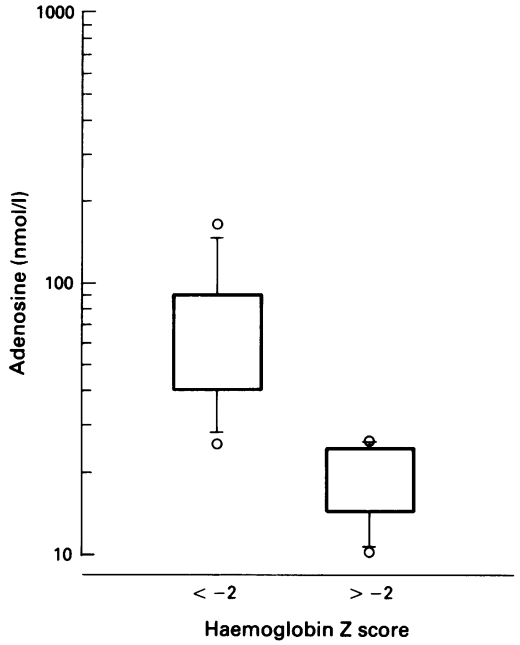

Figure 2 Distribution of adenosine concentrations in patients with haemoglobin z scores greater and less than −2 min. The box and horizontal line mark the median, 25th, and 75th centiles of the data. The error bars mark the 10th and 90th centiles and data outside these limits are plotted individually.

Fetuses with haemoglobin *z* scores below and above −2 had median (range) adenosine concentrations of 67 (25·5–167·2) and 22·05 (10·3–26) nmol/l respectively (fig 2).

Discussion

These data suggest that in the fetus haemoglobin and $P_{uv}O_2$ are related to adenosine concentrations. The relation between $P_{uv}O_2$ and adenosine is expected, as adenosine is a recognised marker of hypoxia. These preliminary results suggest, however, that, in this study group, adenosine concentrations are more closely related to haemoglobin concentration than $P_{uv}O_2$. Although regression analysis of our data implies a direct relation between anaemia and adenosine concentration, the number of patients is small and the possibility remains that the increase in adenosine concentration may only occur in profound anaemia (fig 2).

The findings in this study of an association between fetal blood adenosine concentrations and the degree of fetal anaemia or hypoxaemia, or both, indicate that the fetus is capable of responding to tissue hypoxia from at least 19 weeks' gestation. However, the concentrations of adenosine found in all 14 patients in our study group were markedly lower than in term infants,[3] despite using an identical measurement technique. We found no significant association between adenosine concentration and gestational age, so the most likely explanation for this apparent discrepancy is that the concentration increases during or immediately after delivery.

In fetuses with rhesus isoimmunisation, the reduction in haemoglobin concentration might be proportionately greater than any reduction in blood oxygen tension. Oxygen delivery to the tissues is dependent on these two factors, and so anaemic fetuses may have had reduced tissue oxygenation, despite normal or near normal $P_{uv}O_2$ levels.

These data therefore suggest that tissue hypoxia, as well as blood oxygen concentration, can determine the release of adenosine.

1 Lagercrantz H. The development and functional role of the sympathoadrenal system in the newborn. In: Stern L, Bard H, Friis-Hansen B, eds. *Intensive care of the newborn.* Vol IV. New York: Masson, 1983:67–75.
2 Saugstad O. Hypoxanthine as a measurement of hypoxia. *Pediatr Res* 1975;**9**:158–61.
3 Irestedt L, Dahlin I, Hertzberg T, Sollevi A, Lagercrantz H. Adenosine concentration in umbilical cord blood of newborn infants after vaginal delivery and caesarean section. *Pediatr Res* 1989;**26**:106–8.
4 O'Connor MC, Harkness RA, Simmonds RJ, Hytten FE. Raised hypoxanthine, xanthine and uridine concentrations in meconium stained amniotic fluid and during labour. *Br J Obstet Gynecol* 1981;**88**:375–80.
5 O'Connor MC, Harkness RA, Simmonds RJ, Hytten FE. The measurement of hypoxanthine, xanthine, inosine and uridine concentrations in umbilical cord blood and fetal scalp blood samples as a measure of fetal hypoxia. *Br J Obstet Gynecol* 1981;**88**:381–90.
6 Greenough A, Nicolaides KH, Lagercrantz H. Human fetal sympathoadrenal responsiveness. *Early Hum Dev* 1990;**23**:9–13.
7 Lagercrantz H, Yamamoto BB, Fredholm NR, von Euler C. Adenosine analogues depress ventilation in rabbit neonates. Theophylline stimulation of respiration via adenosine receptors? *Pediatr Res* 1984;**18**:387–90.
8 Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate and small for gestational age fetuses. *Am J Obstet Gynecol* 1989;**161**:996–1001.
9 Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunisation. *Lancet* 1988;**i**:1073–5.