J. Clin. Chem. Clin. Biochem.
Vol. 18, 1980, pp. 611–614

β -Glucuronidase Activity in Amniotic Fluid

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(Received November 15, 1979/April 17, 1980)

Summary: A bimodal distribution of the β -glucuronidase activities was observed within a group of amniotic fluid samples obtained from women by transabdominal amniocentesis in the third trimester of complicated gestations: lower enzyme activities were found in the larger subgroup and extremely high enzyme activities in the smaller subgroup. There was no relation between the enzyme activity and pregnancy complications. However, a sex-relationship was observed, in that all the children from all the pregnancies showing very high enzyme activities were boys. This may be due to the known stimulating effect of androgens on the specific activity of β -glucuronidase.

Die Aktivität der β -Glucuronidase im Fruchtwasser

Zusammenfassung: Eine bimodale Verteilung der β -Glucuronidase-Aktivitäten wurde in Fruchtwasser-Proben, welche von Frauen im Laufe des dritten Trimesters einer komplizierten Schwangerschaft mittels transabdominaler Amniocentese entnommen wurden, ermittelt: niedrigere Werte der Enzym-Aktivitäten in der ersten größeren Untergruppe und erheblich erhöhte Enzym-Aktivitäten in der zweiten kleineren Untergruppe. Es wurde kein Verhältnis zwischen der Enzym-Aktivität und den Schwangerschaftskomplikationen gefunden, doch scheint ein suspektes Verhältnis zwischen den erheblich hohen β -Glucuronidase-Aktivitäten und dem Geschlecht der aus diesen verfolgten Schwangerschaften geborenen Kinder zu bestehen. Der bekannte Stimulationseffekt der Androgene auf die spezifische Aktivität der β -Glucuronidase könnte die erheblich erhöhte Enzym-Aktivität im Fruchtwasser der Schwangerschaften, aus welchen die Kinder männlichen Geschlechtes stammen, erklären.

Introduction

The activities of amniotic fluid enzymes in the course of pregnancy were recently studied with a view to using the data either to determine fetal maturity, or for prognosis in complicated pregnancies. Ogita et al. (1) reported that the ratio of the pancreatic type isoamylase to the salivary type major isoamylase was highly correlated with fetal age, regardless of levels of total amylase activity. Kowalewski (2) found lowered levels of lactate dehydrogenase isoenzymes composed of B polypeptide subunits in amniotic fluids from pregnancies complicated by erythroblastosis. Beckman et al. (3) found a decreased activity of heat stable alkaline phosphatase in isoimmunisation between the 36th and 40th week, but no differences in the levels of heat labile and heat stabile alkaline phosphatase in

pregnancies complicated with toxemia and placental insufficiency.

In our study we followed the total activities of β -glucuronidase (β -D-glucuronide glucuronosohydrolase EC 3.2.1.31) from women with complicated pregnancies of the second and third trimester, in order to determine the average levels and the trend of enzyme activity during gestation. β -Glucuronidase catalyses the hydrolysis of β -glucuronides, and the transfer of glucuronyl radicals to acceptor alcohols. β -Glucuronidase belongs to the group of non-specific enzymes that show pregnancy-dependent changes. Bardin et al. (4) stated that progestins as well as androgens can stimulate β -glucuronidase in mouse kidney, and the enzyme protein serves as useful marker for study of androgen action.

Materials and Methods

Subjects

Ninety-nine samples of amniotic fluid were obtained by transabdominal amniocentesis in second and third trimester from women with complicated pregnancies due to isoimmunisation, diabetes mellitus or toxemia. We also examined the activities of β -glucuronidase in a group of 37 amniotic fluid samples obtained at normal term as a quasi comparative group, although it is clear that the two-groups are not strictly comparable.

Procedure

The amniotic fluid was filtered through a nylon sieve and then centrifuged at 3000 g and 5 °C for 30 minutes. The enzyme activity estimations were performed immediately, or the material was stored at 4–5 °C and analysed within the next three days. Within this time no changes in activities were observed. The activities were measured according to the method of Fishman (5) with phenolphthalein glucuronide as substrate (Calbiochem, Los Angeles, California, U.S.A.). The released phenolphthalein was measured spectrophotometrically at 540 nm in a PYE-UNICAM SP 1750 Ultraviolet Spectrophotometer. The coefficient of variation with 10 parallel determinations on amniotic fluids with the normal lower activity was 6.8%; with extremely high activity 1.9%.

Results

The β -glucuronidase activity levels in amniotic fluid samples from second and third trimester of gestation are shown in figure 1. Ten specimens of amniotic fluid obtained before 26 weeks of gestation showed a mean activity of 129.5 ± 49 mU/l. Eighty-nine specimens of amniotic fluid obtained in the third trimester of gestation revealed two distinctly different groups of β -glucuronidase activities. Seventy-seven specimens exhibited lower activities with a mean value of 131.3 ± 71.1 mU/l, and 12 specimens showed extremely high β -glucuronidase activities with wide variations, ranging from 429 to 1224 mU/l. There was no relation of amniotic fluid β -glucuronidase activity to the particular complications of the pregnancy. However, a sex

Fig. 1. Levels of β -glucuronidase activity in amniotic fluid with fetal age (n = 99).

relationship was observed. In 63 of the 89 pregnancies followed we were able to learn the sex of born children. The distribution of β -glucuronidase activity levels in these amniotic fluid samples is evident from figure 2. The normal lower β -glucuronidase activities in amniotic fluid with mean values of 131.3 ± 71.1 mU/l were determined in pregnancies resulting in 32 girls and 19 boys. In 12 remaining pregnancies with extremely high activities all the children were boys. The distribution of amniotic fluid β -glucuronidase activities at term (n = 37) also reveals two groups with lower and extremely high activities (fig. 3). One group (n = 24) showed a mean value of 224.4 ± 80.7 mU/l; the other group (n = 13) showed wide variations from 428 to 5633 mU/l. The children born within the group with lower activities of β -glucuronidase were 14 girls and 10 boys, in the group with high activities 4 girls and 9 boys. The repeatedly examined amniotic fluid β -glucuronidase activities in various periods of gestation were performed in 10 pregnancies from the group with the lower enzyme levels (tab. 1). Similarly repeated activity

Fig. 2. Distribution of β -glucuronidase activity in 63 amniotic fluids obtained by transabdominal amniocentesis from women with complicated pregnancies in the 27th–43rd week. Sex of born children from these pregnancies is indicated. □ = females ▨ = males.

Fig. 3. Distribution of β -glucuronidase activity levels in 37 amniotic fluids obtained at term. Sex of born children is indicated. □ = females ▨ = males.

Tab. 1. Trends of β -glucuronidase activities in amniotic fluid samples repeatedly examined in various gestation ages.

Child Sex	Weeks of gestation												
	28	29	30	31	32	33	34	35	36	37	38	39	40
β -glucuronidase (mU/l)													
JA. ♀						204			108	97			
KO. ♀						123							
PR. ♀												200	
LU. ♀												86	69
BO. ♀							114	82	49				
RE. ♀				131			93					309	
MI. ♂									82		93		
LA. ♀				169		101				144			
SK. ♀		236		160			129						
HA. ♀	219			183	144		108

Fig. 4. Trends of β -glucuronidase activities with extremely high final values in amniotic fluid samples obtained from women repeatedly examined during gestation. All the children born were male.

determinations were performed in five amniotic fluid samples of pregnancies with final extremely high β -glucuronidase levels. The trends of these gradually increasing activities during pregnancy examined in 5 cases are shown in figure 4.

Discussion

Bardin et al. (4) reported from experiments on animals that the regulatory gene for β -glucuronidase influences the rate of glucuronidase synthesis in response to androgens and progestins. The effect of androgens on β -glucuronidase activity in mouse kidney is expressed in two ways. One group of mice (with allele GUR^a) had a short lag period with a rapid rise of β -glucuronidase activity, the other group (with allele GUR^b) showed a longer lag period and slower rise to a lower maximum enzyme activity. This hypothesis may explain the extremely high activities of β -glucuronidase in amniotic fluid samples at the third trimester of gestation from which all the twelve (0.39) borne children were the boys. The other 19 (0.61) boys were from pregnancies in which amniotic fluid samples usually showed lower

activities of the enzyme. In total 31 (1.0) boys were born from supervised pregnancies. This group originated from 63 pregnancies, the other 32 children being girls (sex ratio 1:1). The whole group ($n = 63$) was obtained randomly from the total number of 99 originally followed pregnancies.

The source of β -glucuronidase in amniotic fluid is not yet clear. The kidney of the fetus is a possible source, the enzyme entering the amniotic fluid from the fetal urine. From experiments in animals Bardin et al. (4) reported that β -glucuronidase comprises only 0.003 of total kidney protein when fully induced, and its usefulness for the study of hormone action is derived from the fact that androgens produce a 20-50-fold increase in enzyme specific activity. The amniotic fluid content of androgens probably reflects fetal sex steroid production. Giles et al. (6) measuring unconjugated testosterone in amniotic fluid in early and late pregnancy found that the concentration was significantly higher in pregnancies with male fetuses in comparison with those with female fetuses.

The amniotic fluid β -glucuronidase activity increase in prolonged gestation or at the term could be caused by the enzyme originating from the placenta. This source is probably involved in amniotic fluid obtained at term. Leung et al. (7) stated very high activities in first trimester and term placenta. Progressive degeneration and insufficiency of placental cells at the term result in the release of β -glucuronidase into the amniotic fluid. This could explain the higher mean values of activities of β -glucuronidase in a quasi control-group ($n = 37$) of amniotic fluid obtained at term from pregnancies giving birth to 9 male and 4 female newborns. Similarly meconium admixtures significantly elevate the β -glucuronidase activities in amniotic fluid obtained at term. The amniotic fluid cells also contain a very low β -glucuronidase activity. The disintegrated cells from the volume of 20 ml amniotic fluid (taken in 33rd week of gestation) increased the originally determined enzyme activity (209 mU/l) by about 1-2 mU/l (own observation).

The detailed study of β -glucuronidase isoenzymes in amniotic fluid could help in the further elucidation of the enzyme source.

Acknowledgement

We are greatly indebted to Mrs. *Ludmila Vostrejšová*, *Helga Mašaráková* and *Anna Vaverková* for skilful technical assistance.

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