FOSFATASA ALCALINA PLACENTARIA DE ALTO PESO MOLECULAR EN PLASMA DE MUJERES EMBARAZADAS DURANTE EL ÚLTIMO TRIMESTRE DE GESTACIÓN.

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Resumen

En el plasma humano pueden encontrarse las isoenzimas ósea, hepática e intestinal de fosfatasa alcalina (EC 3.1.3.1). En el plasma de mujeres embarazadas, durante el último trimestre de gestación puede encontrarse otra isoenzima, la fosfatasa alcalina placentaria. Además, en extractos butanólicos de tejido placentario se ha encontrado una isoenzima unida a membrana, la fosfatasa alcalina placentaria de alto peso molecular. En suero de mujeres embarazadas se ha determinado la actividad de fosfatasa alcalina placentaria soluble pero, hasta el momento, no se ha detectado la presencia de la isoenzima de alto peso molecular. En nuestro laboratorio hemos desarrollado un método que permite la detección de fosfatasa alcalina de alto peso molecular en el pellet de plasma centrifugado a 100.000xg.

Utilizando el mencionado método hemos determinado la actividad de fosfatasa alcalina placentaria de alto peso molecular en plasma de mujeres embarazadas durante el último trimestre de gestación.

Palabras claves: Fosfatasa alcalina placentaria de alto peso molecular. Gestación. Ultimo trimestre

Abstract

Human blood plasma contains alkaline phosphatase (EC 3.1.3.1) isoenzymes from bone, liver and intestine. Blood of pregnant women in the third trimester of gestation also contains an isoenzyme of alkaline phosphatase from placenta, placental-AP (PLAP). Serum from pregnant women in the last trimester of gestation shows activity of soluble placental alkaline phosphatase (sol-PLAP). It is also known that a membrane bound high molecular weight placental-AP (high Mr-PLAP) is present in butanol extracts from placental tissue. Nevertheless, up to now, high Mr-PLAP has not been founded in human plasma. A method developed in this laboratory allows detection of membrane-bound alkaline phosphatase in pellet of plasma centrifuged at 100,000xg. By applying this method we have detected high molecular weight placental alkaline phosphatase in plasma of healthy pregnant women in the third trimester of pregnancy.

Key words: Plasmatic high molecular weight placental alkaline phosphatase. Pregnancy. Third trimester.

Introduction

Human blood plasma contains alkaline phosphatase (EC 3.1.3.1) isoenzymes from bone, liver and intestine. Blood of pregnant women also contains an isoenzyme from placenta, placental-AP (PLAP) (1). PLAP activity is expressed in human placenta beginning in the first trimester and increase throughout pregnancy. This fact suggests that placental-AP would play a functional role in fetus-maternal relationship and placental differentiation (2). Serum from pregnant women shows activity of soluble placental alkaline phosphatase (sol-PLAP). Also a membrane bound high molecular weight placental-AP (high Mr-PLAP) is present in butanol extracts from placental tissue. Nevertheless, up to now, high Mr-PLAP has not been founded in human plasma. (3).

Previous works developed in our laboratory demonstrated the presence of high Mr-AP in pellets of plasma centrifuged at 100,000xg (4). This method allowed us to determine fetal intestinal-AP, another membrane

bound isoenzyme, in amniotic fluid (5). In the present investigation we will apply the above mentioned method to detect activity of high Mr-PLAP in plasma of healthy pregnant women during the third trimester of gestation.

Material and methods

Human term placenta was frozen at -23°C immediately after delivery. Human liver and intestine tissues were obtained within 24h post-mortem at autopsy of adult individuals free of liver or intestine pathology, and were stored at -23°C. Placenta, liver and intestine mucosae were homogenized and extracted by butanolic treatment. Two purifications of placental-AP were performed: a) with Tris-HCl pH 9 (alkaline medium) and b) with 0.1M Na acetate buffer pH 5 (acid medium) (6). After extraction at pH 5, most of the activity corresponds to sol-PLAP, whereas at pH 9 most of the activity corresponds to high Mr-PLAP (2). Plasma samples were obtained from 21 healthy individuals (Group 1), 12 healthy pregnant women in the last trimester of pregnancy (Group 2) and 7 patients with extensive burns (Group 3). The last group was included in order to compare membrane-bound AP activities, because it is known that plasma of patients with extensive burns shows high activity of membrane bound liver-AP (4). A 10mL citrated blood sample was centrifuged immediately after extraction at 4,000xg for 10 min. The plasma was centrifuged at 13,000xg for 15 min to separate the remaining cells and debris, and a 4mL aliquot was then ultracentrifuged at 100,000xg for 2 hours. The supernatant was decanted and pellets were resuspended in 200µL of 40mM Tris-HCl buffer pH 7.8. There was measured AP activity in supernatant and pellet suspension at 37°C by the method of Bessey modified to have a final assay volume of 2mL (7). Inhibition tests were performed to determine organ of origin of AP: a) liver-AP with 10mM L-homoarginine (8) and 0.1mM levamisole (9); b) PLAP with 5mM L-phenylalanine-glycylglicine (10) and c) PLAP and intestine-AP, with 5mM L-phenylalanine (8). Aliquots of pellet suspensions and tissue extracts were heat-inactivated by placing a 1mL aliquot into a water bath at 65°C during 5 min and then into water at 4°C (2). To determine the presence of membranes there was determined the gamma-glutamyltranspeptidase (γ-GT) activity in plasma and pellet suspensions (11). Tissue extracts and pellet suspensions were electrophoresed in 6.5% polyacrylamide gel after the addition of 1% Triton X-100 to all preparations (12). ANOVA was used to test for significant differences among means

Results

Table 1 shows the activity of AP and γ-GT in supernatant and 100,000xg pellet obtained from plasma of the studied groups. The mean activity of AP differs significantly among the three groups ($p<0.05$). The highest values correspond to patients with extensive burns (Group 3), and the lowest to control (Group 1). Group 2 (healthy pregnant women) PLAP activity is significantly different from that corresponding to control. Supernatant and pellet from Group 3 show the highest values of γ-GT, while there are not significantly differences between control and pregnant women (Groups 1 and 2).

Table 2 shows percentages of inhibition of AP isoenzymes. The greatest percentage of liver-AP inhibition (L-homoarginine and levamisole) is observed in pellets from plasma of patients with extensive burns (Group 3). The highest percentage of inhibition of PLAP (L-phenylalanine-glycylglicine) is found in pellet from plasma of pregnant women (Group 2), while the highest percentage of inhibition of placenta and intestine AP (L-phenylalanine) corresponds to placenta and intestine extracts and to pellet of Group 1. On the other hand, heating at 65°C during 5min produce total inactivation of AP activity in liver and intestine extracts and in pellets of plasma from patients with extensive burns (Group 3), while percentage of inhibition is significantly lower in pellets from pregnant women (Group 2), because PLAP is a thermoresistant isoenzyme.

Figure 1 shows that PLAP purified at pH 5 (lane A) and PLAP from the heat-inactivated supernatant of pregnant women plasma (lane C) show the same electrophoretic mobility. The placenta AP purified at pH 9 (lane B) and the suspension of 100,000xg pellet from Group 2 (lane D), also have the same

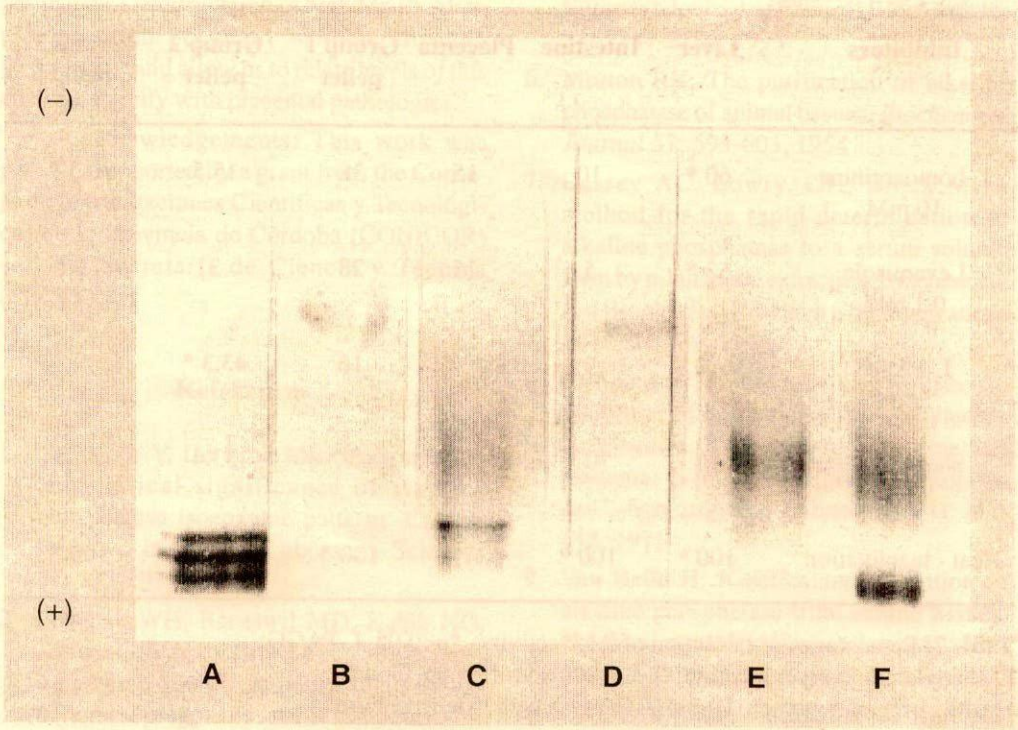

Figure 1: Alkaline phosphatase (AP) electrophoretic patterns in polyacrylamide gel. All the samples were treated with Triton X-100. **A**: placenta-AP purified at pH 5. **B**: placenta AP purified at pH 9. **C**: supernatant from pregnant women plasma centrifuged at 100,000xg and heat-inactivated. **D**: suspension of 100,000xg pellet from pregnant women plasma. **E**: AP purified from intestine. **F**: AP purified from liver.

GROUPS	Alkaline phosphatase		γ-glutamyltranspeptidase	
	Supernatant mU/mL	Pellet mU/mL	Supernatant mU/mL	Pellet mU/mL
Group 1 (21)	31.5±3.8	0.5±0.1	17.8±3.7	0.9±0.13
Group 2 (12)	107.5±15.8*	2.8±1.0*	17.9±3.1	0.8±0.1
Group 3 (7)	188.6±35.7*	12.9±1.7*	95.4±13.6*	145±4.7*

Table 1: Activity of alkaline phosphatase and gamma-glutamyltranspeptidase in supernatant and pellet from plasma centrifuged at 100,000xg.
Number of cases between parentheses. *$p<0.05$. Values are mean±SE.
Group 1: Control. Group 2: pregnant women. Group 3: patients with extensive burns

Inhibitors	Liver	Intestine	Placenta	Group 1 pellet	Group 2 pellet	Group 3 pellet
L-homoarginine 10 mM	60 *	10	15	38	15.5	70.5 *
Levamisole 0.1 mM	53 *	5	15	28	3	71 *
L-PAGG 5mM	10	9	60 *	16	43.3 *	9
L-phenylalanine 5 mM	10	70 *	60 *	65 *	33.4	21.2
Heat - inactivation	100 *	100 *	17,5	100 *	57.3	100 *

Table 2: Percentages of inhibition of ALP activity. *p<0.05. L-PAGG: L-phenylalanine-glycylglycine. Group 1: Control group. Group 2: Healthy pregnant women. Group 3: Patients with extensive burns.

electrophoretic mobility. The mobility of liver and intestine extracts (lanes E and F) differs from that of placenta extracts (lanes A and B).

Discussion

Results presented show that there is high activity of AP in the supernatant from plasma of pregnant women (Group 2). Supernatant and pellet from plasma of patients with extensive burns (Group 3) show the highest AP activity. As shown by Moreno et al (1992) the activity of AP found in the 100,000xg pellet, corresponds to high Mr-AP that is a membrane bound enzyme (4). The highest values of γ-GT (a membrane marker enzyme) and AP activities showed by supernatant and pellet in Group 3 indicate that AP activity corresponds to high Mr-AP isoenzyme.

According to the inhibition tests, AP activity found in pellet of Group 2 corresponds to placental alkaline phosphatase, since it is mostly inhibited by L-phenylalanine-glycylglicine, which is a specific inhibitor of PLAP. Heat-inactivation confirms these results, because AP was scarcely inhibited in pellets from pregnant women, whereas pellets from controls, patients with extensive burns, liver and intestine extracts were entirely inhibited. The aforementioned data indicate that AP present in pellet of plasma from pregnant women centrifuged at 100,000xg, is the thermoresistant, membrane bound high Mr-weight-PLAP. On the other hand, the pellet of patients with extensive burns, shows the greatest percentages of inhibition with levamisole and L-homoarginine, which indicates that the membrane bound high Mr-AP is the liver isoenzyme. Electrophoretic zymogram shows that, enzyme from heat inactivated 100,000xg plasma supernatant, behaved just as that from placenta purified at pH 5, which corresponds to soluble-AP. The enzyme from 100,000xg pellet shows the same mobility than that from placenta purified at pH 9, which is high Mr-PLAP. We emphasize that up to now high Mr-PLAP has not been founded in human plasma (3).

According to obtained results we conclude that, the method developed in our laboratory allow us to determine the membrane bound high Mr-PLAP, in plasma of healthy pregnant women, in the third trimester of

pregnancy. Determination of high Mr-PLAP in plasma from pregnant women, through gestation, would allow us to relate levels of this enzyme activity with placental pathologies.

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