Activity of Alkaline Phosphatase and Its Histo-cellular Location in Liver, Kidney and Small Intestine of Mouse during Growth

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Abstract: A multidisciplinary approach, colorimetry, non-denatured electrophoresis, histochemistry and EM-cytochemistry, was used to investigate the activity changes of alkaline phosphatase (AKP) and its histo-cellular location in mouse's liver, kidney and small intestine during growth after birth. The results showed that the activities of AKP in the three organs were: small intestine \( > \) kidney \( > \) liver. The AKP activity ascended at first and then declined in small intestine and liver, while ascended constantly in kidney during mouse growth. In small intestine, AKP activity was mainly distributed over four areas of epithelial cells: cytomembrane, cytoplasm, microvillus and the carbohydrate-riched cell coat. In liver, it was mainly located in canaliculi. In kidney, it mainly existed in such zones as brush border and membrane of epithelial cells of proximal tubule, and cracks between kinds of tubules. 3 AKP isoenzymes in liver, 2 in small intestine and 3 in kidney were determined during the mouse growth and each AKP isoenzyme activity changed with the mouse growth.

Key words: mouse; liver; small intestine; kidney; alkaline phosphatase

AKP is a kind of widely distributed enzyme mainly in intestinal mucosa, as well as in skeletal muscle, heart and brain. It is an important enzyme in the catalysis of the absorption of phosphate in the intestinal tract and the mineralization of bone. In the present study, a multidisciplinary approach was used to investigate the activity changes of alkaline phosphatase (AKP) and its histo-cellular location in mouse's liver, kidney and small intestine during growth after birth. The results showed that the activities of AKP in the three organs were: small intestine \( > \) kidney \( > \) liver. The AKP activity ascended at first and then declined in small intestine and liver, while ascended constantly in kidney during mouse growth. In small intestine, AKP activity was mainly distributed over four areas of epithelial cells: cytomembrane, cytoplasm, microvillus and the carbohydrate-riched cell coat. In liver, it was mainly located in canaliculi. In kidney, it mainly existed in such zones as brush border and membrane of epithelial cells of proximal tubule, and cracks between kinds of tubules. 3 AKP isoenzymes in liver, 2 in small intestine and 3 in kidney were determined during the mouse growth and each AKP isoenzyme activity changed with the mouse growth.

1 Materials and methods

1.1 Experimental animals

The experimental animals were adult male mice (ICR strain, purchased from the Experimental Animal Center of Henan Normal University). All experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals. The animals were fasted for 12 hours before being sacrificed by cervical dislocation. The liver, kidney, and small intestine were removed and immediately frozen in liquid nitrogen. The tissues were then stored at \(-80^\circ\text{C}\) until use.

1.2 Methods

1.2.1 Activity determination

The activity of AKP was determined using the method of Gomori. The reaction mixture contained 0.5 ml of the homogenate, 0.5 ml of 0.2 M sodium acetate buffer (pH 5.0), and 0.5 ml of 1 M 4-nitrophenyl phoshate (PNP). The reaction was initiated by the addition of PNP and the absorbance at 405 nm was measured spectrophotometrically every 5 minutes for 30 minutes. The enzyme activity was expressed as \(\text{U}\) (unit) per gram of tissue. A unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 nmol of 4-nitrophenol per minute at 37\(^\circ\text{C}\) and pH 5.0.

1.2.2 Location determination

The location of AKP was determined using histochemical techniques. The sections were incubated with 3% hydrogen peroxide for 30 minutes at room temperature to block endogenous peroxidase activity. Then, the sections were incubated with a 1:100 dilution of anti-AKP antibody for 1 hour at room temperature. After washing, the sections were incubated with a 1:100 dilution of biotinylated secondary antibody for 30 minutes. Finally, the sections were incubated with a 1:100 dilution of streptavidin-peroxidase complex for 30 minutes. The peroxidase activity was visualized by incubating the sections with 3,3-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide. The sections were counterstained with hematoxylin.

1.3 Statistical analysis

The data obtained were expressed as the mean \(\pm\) standard deviation (SD). Differences among the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test. The significance level was set at \(p<0.05\) for all comparisons.

1.4 Results

1.4.1 Activity determination

The activity of AKP in different organs is shown in Table 1. The activity of AKP in the liver was significantly higher than that in the kidney and small intestine, while the activity in the kidney was significantly higher than that in the small intestine. The activity of AKP decreased gradually with age in the liver, while it increased gradually in the kidney and small intestine.

1.4.2 Location determination

The location of AKP in different organs is shown in Figure 1. AKP was mainly located in the cytoplasm of hepatocytes in the liver, in the brush border of enterocytes in the small intestine, and in the proximal tubules of the kidney. The staining intensity of AKP in the liver was significantly higher than that in the small intestine and kidney, while the staining intensity in the kidney was significantly higher than that in the small intestine.

1.5 Discussion

The results of the present study indicate that the activity and location of AKP in different organs changes with age. The activity of AKP in the liver is significantly higher than that in the kidney and small intestine, while the activity in the kidney is significantly higher than that in the small intestine. The activity of AKP decreases gradually with age in the liver, while it increases gradually in the kidney and small intestine. The location of AKP in the liver is mainly in the cytoplasm of hepatocytes, in the brush border of enterocytes in the small intestine, and in the proximal tubules of the kidney. The staining intensity of AKP in the liver is significantly higher than that in the small intestine and kidney, while the staining intensity in the kidney is significantly higher than that in the small intestine.

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1994)，肝、肾的上样量为每版 80 μg 总蛋白，小肠的上样量为 50 μg 总蛋白。电泳结束后，经洗涤固定，用 Biep II/NBT 显色剂显色。

1.5 AKP 的组织化学测定

用 Gomori 钙-铬法显色（杜卓民，1998）。

1.6 AKP 的超微化学鉴定

用羧铵 β-甘油磷酸钠的保温液作阴性对照，保温液组成（张振杰等人，1988）：0.1 M β-甘油磷酸钠 5 ml；0.1 M pH 9.4 巴比妥缓冲液 20 ml；0.5 M 氯化镁 5 ml；0.2 M 氯化钙 20 ml。保温液的最终 pH 为 9.4。

2 结果

2.1 AKP 活性的比色测定结果（金氏单位 / 100 ml 组织匀浆液）（图 1）

3 种器官内的 AKP 活性依次为：小肠 > 肾 > 肝。小肠 AKP 活性显著高于肝和肾。在大鼠成熟过程中，小肠和肝 AKP 活性先上升后下降，小肠中以 D1 活性最强，D2 依次之，成年最弱；肝中 AKP 活性以 D1 最强，D2 依次之，D3 和 Da 活性最低。肾内 AKP 活性总体呈上升趋势，D3 活性达到最强，成年后略有下降。

2.2 AKP 的原位复性电泳结果（图 2）

2.3 AKP 活性的组织化学测定结果（图 3）

AKP 活性在组织化学测定结果与比色测定结果基本一致。小肠内 AKP 活性显著高于肝和肾。小肠和肝内 AKP 活性先上升后下降，小肠内 AKP 活性总呈上升趋势，肝内 AKP 活性在肝区较弱，中间有分支，因此在肝小叶内连接成网络状（图 3 ①）。小肠内 AKP 活性主要分布在黏膜层，大肠 AKP 活性主要分布在肠腔外，小肠部见酶活性（图 3 ②③）。肾内 AKP 活性主要分布在皮质部，髓质部未见酶活性（图 3 ④⑤⑥）。

2.4 AKP 的超微化学测定结果（图 4）

肝 AKP 活性主要分布于肝小管内膜上，尤于胆小管处最多（图 4 ①）。小肠内 AKP 活性主要分布在上皮细胞的 4 个部位：微绒毛表面的糖衣上，微绒毛，细胞膜和胞浆内（有的位于肠腔侧的胞浆内，有的位于基底侧的胞浆内）（图 4 ④⑤⑥）。肾内 AKP 活性主要分布在以下几个部位：近曲小管的刷状缘，近曲小管上皮细胞的细胞膜（尤其在基底膜上有着较强的酶活性），肾小管的基膜和各种管状结构之间的腔隙内。

3 讨论

根据组织切片可以看出，AKP 活性沿肝板呈放射状走向肝小叶周边，在小叶内连接成网络状（图 3 ①），这种分布与胆小管在肝小叶内的分布一致。因此推测，肝内 AKP 活性可能主要分布在胆小管。通过透射电镜观察到，肝内 AKP 活性确实主要分布在相邻两肝细胞之间的细胞膜，特别是在胆小管处酶活性显著（图 4 ①）。因此，肝内 AKP 可能与胆
图4 AKP活性的细胞化学定位

1. 为肝实质细胞；N为细胞核，M为细胞膜，→所指的深黑色颗粒为AKP活性染色，位于两个相邻细胞间的胆小管；2. 为肾小管上皮细胞；M为细胞膜，SB为上皮细胞纹状缘，BL为基底膜，→所指的深黑色颗粒为AKP活性染色，位于上皮细胞的纹状缘，细胞膜及基底膜；3. 为肾小管上皮细胞，SB为上皮细胞纹状缘，BL为基底膜，→所指的深黑色颗粒为AKP活性染色，位于上皮细胞的纹状缘，细胞膜及基底膜；4. 为小肠上皮细胞，M为细胞核，→所指的深黑色颗粒为AKP活性染色

汁的分泌有密切关系。在小鼠生长过程中肝内共出现3种AKP同工酶。gAKP1只在D1时出现，以后便消失，可能这是一胚脂质同工酶，出生时尚未完全消失；gAKP2从D0开始出现，一直保持到成年，可能是一种成年型同工酶，参与成年鼠的胆汁分泌；gAKP3从D1持续到D2，一直保持较高活性，但在成年时消失，说明是一种参与小鼠生长发育的AKP同工酶。小肠AKP活性主要分布在小肠绒毛上皮细胞。根据组织切片可以看出，D1由于小肠尚未发育成熟，绒毛很细，绒毛腔很小，只有在粘膜处可看到明显的活性分布，以后随着小肠的发育，绒毛逐渐增大，可以看到D1和D3的酶活性主要分布在小肠绒毛上皮细胞（图3②），到了D3和D4，小肠发育成熟，绒毛达到最大，绒毛腔也明显增大，可以看出酶活性集中分布在小肠绒毛上皮细胞（图3③）。出生后小肠AKP可能参与细胞衣糖蛋白的生物合成及分泌（高金等，1995），并可能与某些物质的吸收和分泌有关（赵保荣等，1982）。图4④⑤⑥均存在，可以显示复活性主要分布在小肠绒毛上皮细胞（图4④），经过同工酶的消化，绒毛上皮细胞的刷状缘及绒毛表面的糖衣上，小肠绒毛上皮细胞内，有时分布在肠腔侧的胞浆内，有有时分布在结肠侧的胞浆内，因小肠绒毛上皮细胞的消化吸收功能，使绒毛上皮细胞的酶活性，有力的证明了AKP可能参与小鼠的生长发育，也可参与消化吸收功能

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