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Effects of glutamate dehydrogenase, choline oxidase, and glucose-6-phosphatase on ^{67}Ga accumulation in lysosome

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AIM: To clarify the effects of the activities of hepatic enzymes in liver, hepatoma, and malignant tumor on ^{67}Ga accumulation in lysosome. **METHODS:** ^{67}Ga-citrate solution was prepared from carrier-free ^{67}Ga-citrate solution 0.08 mol·L^{-1} and sodium citrate solution 0.08 mol·L^{-1}, and was injected iv 0.4 ml to the rats. Subcellular fractions of the liver were measured for radioactivity of ^{67}Ga by a well-type scintillation counter (Aloka JDC-701). Glutamate dehydrogenase, choline oxidase, and G-6-P activities were calculated as described by Shimizu H, Ikuta S, and Baginski E, respectivily. **RESULTS:** ^{67}Ga radioactivity in normal liver lysosome (55 %) was significantly higher than those in hepatoma AH 109A (32 %) and Yoshida sarcoma (18 %). Glutamate dehydrogenase activities were 1830±*s* 320 U·L^{-1} in normal liver while 23±*s* 6 U·L^{-1} in hepatoma AH 109A, and 7 ±*s* 2 U·L^{-1} in Yoshida sarcoma. Choline oxidase activities were 46±*s* 10 U·L^{-1} for normal, 25.0±*s* 0.4 U·L^{-1} for hepatoma AH 109A, and 2.0±*s* 0.4 U·L^{-1} for Yoshida sarcoma. G-6-P activities were 2550±*s* 180 U·L^{-1} in normal, 84±*s* 14 U·L^{-1} in hepatoma AH 109A, and 78±*s* 13 U·L^{-1} in Yoshida sarcoma. **CONCLUSION:** Lysosome of normal rat liver in which hepatic enzymes work actively played a major role in the tissue concentration of ^{67}Ga, but the role diminishes with the neoplastic transformation into hepatoma. The lysosome of Yoshida sarcoma does not play any role in ^{67}Ga accumulation because it does not possess any features of liver.

KEY WORDS gallium radioisotopes; liver; glutamate dehydrogenase; choline oxidase; glucose-6-phosphatase; lysosomes

Gallium-67 has widely been used for malignant tumor imaging. Some investigators[1-3] reported that intracellular ^{67}Ga in normal and neoplastic tissues were localized in lysosome-like bodies. Others[4-6] asserted that the lysosome did not play a major role in the accumulation of ^{67}Ga in neoplastic tissues. The activities of glucose-6-phosphatase (G-6-P), glutamate dehydrogenase, and choline oxidase were related to the degree of differentiation of the experimental hepatoma[7]. ^{67}Ga accumulated into the lysosome in the mitochondrial fraction of liver and hepatoma[2]. To clarify the effects of the activities of hepatic enzymes in liver, hepatoma, and malignant tumor on ^{67}Ga accumulation in lysosome, this study was carried out.

MATERIALS AND METHODS

Rats Normal Donryu rats (♂ weighing 172±*s* 19 g). Donryu rats (♂ weighing 180±*s* 17 g) underwent subcutaneous implantation of hepatoma AH 109A (4×10^{11} cells·L^{-1}) or Yoshida sarcoma (4×10^{11} cells·L^{-1}) in the right thighs. After 5-7 d the livers of normal ♂ Donryu rats and the tumors were quickly removed and were homogenized in 1:6 Tris-HCl buffer 0.01 mol·L^{-1} (pH 7.6). The homogenate was ultrasonicated at 20 kHz for 2 min in ice bath, repeated 5 times to disrupt completely all the micro-organelles.

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Then a part of the homogenate was centrifuged (5 000 × g) for 15 min and the supernatant was used for the assay of G-6-P. An other part of the homogenate was centrifuged at 105 000 × g for 60 min and the supernatant was used for the assay of glutamate dehydrogenase and choline oxidase.

Radioactive compound ^{67}Ga-citrate solution (about 40 MBq·L^{-1}) was prepared from carrier-free ^{67}Ga-citrate solution (Daiichi Radioisotope Laboratories Ltd) 0.08 mol·L^{-1} and sodium citrate solution 0.08 mol·L^{-1}.

Subcellular fractionation ^{67}Ga-citrate solution (0.4 ml) was injected iv into the rats. After 24 h the rats were killed under sodium pentobarbital anesthesea (40 mg·kg^{-1}, ip). The livers of normal rats and the tumors were excised. Subcellular fractionation was carried out at 4 C. Fractions were measured for radioactivity of ^{67}Ga by a well-type scintillation counter (Aloka JDC-701). Let the radioactivities (dpm) of ^{67}Ga in nuclear (A), mitochondrial (B), microsomal (C) and supernatant fractions (D) be expressed. Calculate the % of ^{67}Ga in each fraction out of the total (A+B+C+D).

Enzyme assay Glutamate dehydrogenase[8], Choline oxidase[9] and G-6-P[10] activities (U·ml^{-1}) were calculated.

RESULTS AND DISCUSSION

Subcellular distribution of ^{67}Ga In normal liver, about 55 % of ^{67}Ga accumulated in the mitochondrial fraction (the lysosome is contained in this fraction), small amounts were localized in other 3 fractions. In hepatoma AH109A, about 32 % of total radioactivity was found in the mitochondrial fraction and the accumulation in each of other 3 fractions was less than that in the mitochondrial fraction. In Yoshida sarcoma, most of ^{67}Ga was localized in the supernatant fraction, and 18 % in the mitochondrial fraction (Fig 1).

^{67}Ga radioactivity in the lysosome of the normal liver was significantly higher ($P < 0.01$) than those of the 2 tumors.

Glutamate dehydrogenase The enzyme activities were 1830±s 320 U·L^{-1} of normal liver, (23 ± s 6 U·L^{-1}) of hepatoma AH109A, and (7±s 2 U·L^{-1}) of Yoshida sarcoma (Fig 2).

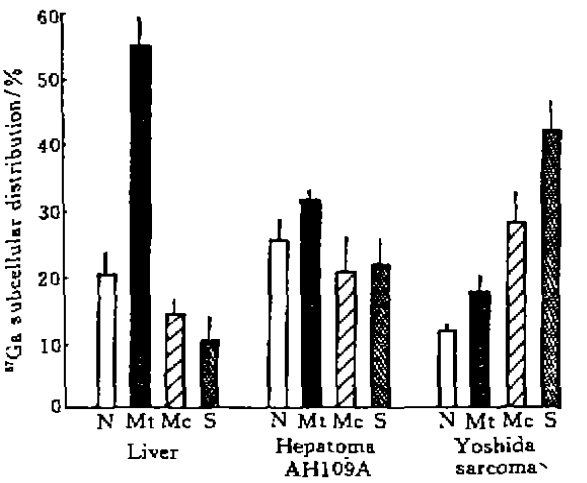

Fig 1. Subcellular distribution (%) of ^{67}Ga 24 h after iv ^{67}Ga-citrate. N: nuclear fraction. Mt: mitochondrial fraction. MC: microsomal fraction. S: supernatant fraction. n=5 rats, $\bar{x}\pm s$.

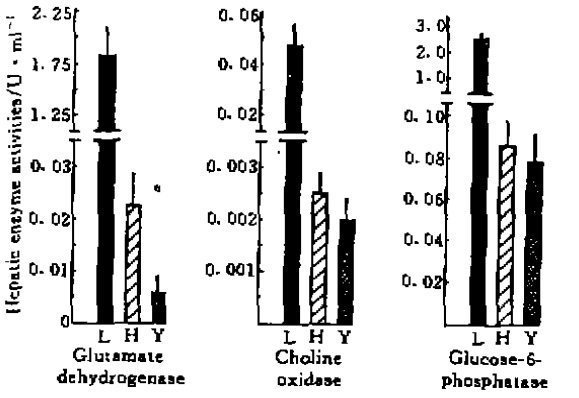

Fig 2. Hepatic enzyme activities (U·ml^{-1}) of liver, hepatoma AH109A and yoshida sarcoma. L: liver. H: hepatoma AH109A. Y: Yoshida sarcoma. n=5 rats, $\bar{x}\pm s$.

Choline oxidase The activities were 46 ±s 10 U·L^{-1} for normal liver, 2.5±s 0.4 U·L^{-1} for hepatoma AH 109A, and 2.0±s 0.4 U·L^{-1} for Yoshida sarcoma (Fig 2).

G-6-P The activities were 2550±s 180

U·L^{-1} for normal liver, 84±s 14 U·L^{-1} for hepatoma AH 109 A, and 78±s 13 U·L^{-1} for Yoshida sarcoma.

The normal liver exhibited a high level of all hepatic enzymes studied. The activities of these enzymes became extremely weak after the neoplastic transformation into hepatoma. Yoshida sarcoma which did not originate from the liver cell showed the lowest value. All 3 enzymes in normal liver had the activities higher ($P<0.01$) than those in the 2 tumors.

In normal liver in which hepatic enzymes work actively, the lysosome played a major role in the tissue concentration of ^{67}Ga. The lysosome did not play an important role in the concentration of ^{67}Ga in the tumor in which hepatic enzymes hardly work.

In conclusion, (1) Lysosome plays a major role in ^{67}Ga concentration in normal liver, (2) The role of lysosome in ^{67}Ga concentration diminishes with the neoplastic transformation into hepatoma. (3) The lysosome of Yoshida sarcoma does not play any important role in ^{67}Ga accumulation because it does not possess any features of liver tissue.

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谷氨酸脱氢酶，胆碱氧化酶和葡萄糖-6-磷酸酶对溶酶体中^{67}Ga 积累的影响

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目的：比较正常肝组织与肝癌 AH 109A，吉田肉瘤中谷氨酸脱氢酶，胆碱氧化酶和葡萄糖-6-磷酸酶的活力对^{67}Ga 摄取与积累的影响；**方法**：制备^{67}Ga 枸橼酸溶液给大鼠静注后处死大鼠，制备亚细胞悬液，液闪计数器测定放射活度。**结果**：^{67}Ga 的放射活性在正常肝组织溶酶体中（55 %积聚）显著高于肝癌 AH 109A（32 %积聚）和吉田肉瘤（18 %）积聚。谷氨酸脱氢酶的活力在正常肝组织，肝癌和吉田肉瘤分别是1830±s 320 U·L^{-1}，23±s 6 U·L^{-1}和7±s 2 U·L^{-1}；胆碱氧化酶的活力分别是46±s 10 U·L^{-1}，25.0±s 0.4 U·L^{-1}，2.0±s 0.4 U·L^{-1}；葡萄糖-6-磷酸酶活力分别是2550±s 180 U·L^{-1}，84±s 14 U·L^{-1}，78±s 13 U·L^{-1}。**结论**：正常肝组织中溶酶体酶活力很强，对^{67}Ga 的积累起较大作用。癌变组织酶活力降低而作用减弱。吉田肉瘤细胞无肝细胞特点，其溶酶体对^{67}Ga 积累作用不大。

关键词 镓放射性同位素；肝；谷氨酸脱氢酶；胆碱氧化酶；葡萄糖-6-磷酸酶；溶酶体

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Effects of pineal body and melatonin on lymphocyte proliferation and dinoprostone production in rat spleen

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AIM: To determine the effects of pineal body and melatonin (Mel) on lymphocyte proliferation and dinoprostone production in rat spleen. **METHODS:** Pinealectomy (PE), lymphocyte proliferation assay and dinoprostone radioimmunoassay were used. **RESULTS:** A circadian rhythm of splenic lymphocyte proliferation which peaked at 22:00 was obliterated by PE in rats. PE led to an impairment of lymphocyte proliferation and an increase of dinoprostone production, which were restored by ip Mel 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$ at 16:00 for 7 d. Mel promoted lymphocyte proliferation and inhibited dinoprostone production in intact rats. A negative correlation between the change in lymphocyte proliferation and dinoprostone production was seen. **CONCLUSION:** The pineal body and its main hormone Mel play a regulatory role in circadian lymphocyte proliferation which is related to dinoprostone production in rat spleen.

KEY WORDS pineal body; melatonin; spleen; lymphocyte; dinoprostone

The pineal body has been recognized as an important component of the neuroendocrine system regulating circadian rhythmicity. The release of norepinephrine from postganglionic sympathetic fibers originating in the superior cervical ganglion stimulates the production of the pineal hormone melatonin (Mel) via its binding to adrenoceptors located on pinealocytes. Mel conveys the influence of the light-dark cycle on body physiology, thus affecting the regulation of the neuroendocrine and immune systems[1,2]. As shown by several studies, abrogation of the cyclicity of the pineal secretion by pinealectomy (PE) or by permanent lighting leads to impairment of cellular and humoral immune responses, whereas exogenous Mel enhances antibody production, helper inducer T lymphocyte activity and IL-2 production[3-5]. We have demonstrated that the pineal body and Mel play an important role in inflammatory and immune respones[6,7]. But their effects on circadian rhythm of lymphocyte proliferation have not been found. In this article, we studied the effects of the pineal body and Mel on lymphocyte proliferation and dinoprostone production in the spleen of adult male rats to determine whether there is a circadian rhythm of lymphocyte proliferation controlled by pineal body and this immunoregulatory effect is mediated by dinoprostone changes in the spleen.

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MATERIALS AND METHODS

Animals Sprague-Dawley rats (♂, $n = 110$, 3-4 month old, 251 ± s 50 g) were provided by the Animal Center of Anhui Medical University. Rats were maintained under laboratory conditions with 12-h light (6:00-18:00) and 12-h darkness at 22 ± 1 C with free access to food and tap water.

Reagents and chemicals Mel, purchased from Sigma Chemical Co (St Louis MO), was dissolved in ethanol and then diluted with normal saline to the final concentration of 2 % ethanol. Concanavalin A (Con A) and lipopolysaccharides (LPS), from Sigma, were prepared at a concentration of 1 $g \cdot L^{-1}$ and stored at -20 C. Medium RPMI-1640 (Gibco Laboratories) was supplemented with HEPES buffer 10 $mmol \cdot L^{-1}$,