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Effect of *L*–4–oxalysine on ultrastructures of liver cells in mice[1]

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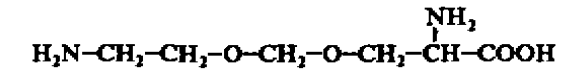
ABSTRACT Mice were given ig *L*–4–oxalysine (I–677) 10, 50, and 100 mg · kg^{-1} · d^{-1} for 7 d. On d 8 the hepatocytes showed accumulation of lipid droplets followed by loss of matrices in cytoplasm. The total area of lipid droplets was far less than 25% of mean section of hepatocytes. The injury of mitochondria and RER was only found in the groups of medium and high dose. The lipidoses and regional topolysis of cytoplasm graduated away at same pace. After 4 wk the hepatocytes were restored to normal. Such finding suggests that the site of action of I–677 be at the cytoplasmic ground substance. The inhibition of protein synthesis causes a decrease in albumin carrier, that may be the main mechanism of steatosis of liver cells induced by I–677.

KEY WORDS lysine; liver; lipids; steatosis; electron microscopy

L–4–oxalysine (I–677) was found to be a new antibiotic isolated from the culture media of *Streptomyces roseovirdofuscus* n sp obtained from the soil in Dalian region of Liaonin Province in China by the Department of Antibiotics of our Institute[1]. Prior works revealed that I–677 lowered the elevated SGPT and SGOT levels in acute and chronic hepatic injuries[2]. I–677 inhibited many solid tumors in rats and mice[3]. Pathological examination, however, indicated that 160 mg · kg^{-1} · d^{-1} of I–677 administered for 8 wk caused swelling of hepatocytes and steatosis[2]. Steatosis showed multifarious evidences with different mechanisms of morphosis[4,5]. Accumulation of lipid droplets induced by some drugs tended to abatement upon discontinuing the drug[6], and the steatosis might be reversible[7]. So from the ultrastructural level, we observed the selective action of I–677 on the hepatocytes in mice and the possible changes of hepatocytes after stopping treatment, in order to approach the reversibility of lipidoses induced by I–677.



L–4–oxalysine

MATERIALS AND METHODS

Drug I–677 was provided by the Department of Antibiotics of our Institute. It is white needle–like crystal, soluble in water and insoluble in organic solvents. I–677 is dissolved in saline for use.

Mice Kun Ming strain ♀ mice weighting 19 ± SD 2 g, and its forages were obtained from the Experimental Animal Center of Chinese Academy of Sciences Shanghai Branch. Prior to the experimental treatment, the mice did not fast overnight.

Methods Sixty mice were randomly and equally divided into 4 groups. One of the groups was given ig saline and the other were given ig 10, 50, 100 mg · kg^{-1} · d^{-1} for 7 d. On d 1, 7, 14, and 28 respectively after terminating the treatment, 3 mice of each group were killed by cervical dislocating. The right lobe of liver was minced into small 1 mm^3 fragments, then forefixed in 2.5% glutaraldehyde in 0.1 mol · L^{-1} cacodylate buffer at 4℃ for 3 h, postfixed in 1% osmium tetraoxide in 0.1 mol · L^{-1} cacodylate buffer at 4℃ for 1 h, dehydrated in a graded series of acetone and embedded in epoxy resin 618.

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Thin sections were cut on a LKB ultratome and stained with uranyl acetate and lead citrate. Finally the samples were examined under transmission electron microscope. In order to count the number and its maximum diameter of lipid droplets in hepatocytes. We observed more than 100 hepatocytes. Aforementioned experiment repeated twice.

RESULTS

Ultrastructural features of hepatocytes in control mice Hepatocytes, which usually appeared polygonal and larger in volume, had three different types of surfaces. The intercellular surface adjoining on the adjacent hepatocytes was closely approximated to a distence of about 20 nm. The desmosomes might be seen near the surface of bile capillary. The sinusoidal surface was studded with numerous irregular microvilli. Most of the hepatocytes had single nucleus, spherical or egglike with a mean diameter about 10 μm. Nucleoli might be seen 1–3, the greater part showed netlike or ring and frequently nestled up against nuclear membrane. The intranuclear chromatin was mainly euchromatin. The nuclear vacuoles with diameter less than 1 μm were found occasionally. Other intranuclear inclusion body was seldom seen. In the cytoplasm, the smooth endoplasmic reticulum was less in number. The rough endoplasmic reticulum was well-developed and often located in proximity of the mitochondria. There was abundant of mitochondria in cytoplasm. Most of them had distinct inner cristae but a few had so denser electronic density that looked cloudy. The cytoplasm contained less free ribosomes and glycogens mainly to be β-mode particles. The scondary lysosomes usually showed in myelin figure. Sometimes several lipid droplets might be examined in cytoplasm. Their outline with a homogenous darkish or transparent content was similar to circular or ellipse, which is not more than 1 μm in diameter (Fig 1a, Plate 1). Above mentioned ultrastructural features of hepatocytes of Kun Ming strain mice were generally in accord with the reports of literature about other strain mice, except for sparse glycogen. Moreover, less free lipid droplets were found in cytoplasm *vs* fasting mice.

Effect of I-677 on hepatocytes in mice One day after ig I-677 10 mg · kg^{-1} · d^{-1} for 7 d, most of the hepatocytes remained in normal morphologic character. But some ultrastructural features of part of hepatocytes showed noticeable changes. In the cytoplasm, the lipid droplets were increased in number. They might be irregular in outline measured 0.8–2.0 μm in diameter with a darkish tone and usually presented in clustered fashion. The regional topolysis of local cytoplasm was seen markedly around the lipid droplets. The cytoplasmic matrices were lost and the areas with sparse cytoplasmic ground substance were formed. Some degradative products of organelles, which were like fragmentary catkin, presented with the areas (Fig 1b, Plate 1). However, rough endoplasmic reticulum (RER) did not show distinguishable changes. Most of mitochondria were normal. Individual mitochondrion with obscurity or disappearance of cristae or ruptured outer membrane was seen in one or two examined fields. Different kinds of lysosomes were frequently found in the cytoplasm. The nuclei did not show obvious alteration. One week after stopping treatment, the lipid droplets which presented in cytoplasm similar to circular or ellipse were decreased in number and distributed in dispersed tendency. The phenomenon of regional topolysis of local cytoplasm was seen occasionally. Other cellular organelles such as RER and mitochondria remained normal in all respects (Fig 1c, Plate 1). Two weeks after stopping

the regional topolysis of local cytoplasm was unable to be seen. The free lipid droplets were hardly detected. All the hepatocellular ultrastructures were back to normal.

One day after ig I–677 50 mg · kg^{-1} · d^{-1} for 7 d. Part of hepatocytes presented accumulation of lipid droplets, of which the shape and distribution were similar to those of low dosage group. The areas with sparse cytoplasmic matrices were usually nearby the lipid droplets (Fig 1d, Plate 1). Parts of mitochondria showed dilation. Some of them with obscurity or disappearance of cristae, or even only a swelling remains of cristae, were seen. The cisternae of RER differed in dilation and part of them were serious and accompanied degranulation. Various secondary lysosomes (myelin figure, multivesicular body, lipofuscin) were seen frequently. The nuclei showed tendency of irregular shape and enlarged perinulear space (Fig 1e, Plate 1). One week after stopping treatment, the lipidoses lightened and the areas with sparse cytoplasmic matrices decreased obviously. In addition, the damage of mitochondria was reduced, of which most cristae became normal. But the swelling cisternae of RER and degranulation still presented. A part of perihuclear space was still seen enlargement (Fig 1f, Plate 1). Two weeks after stopping treatment, the areas with sparse matrices disappeared. The lipid droplets were hardly found in the cytoplasm. The mitochondria almost became normal, but a few of them showed slight dilation. The RER and perinuclear space still differed in dilation (Fig 2a, Plate 2). Four weeks after stopping treatment, the ultrastructural features of hepatocytes showed no noticeable difference *vs* control group.

One day after ig I–677 100 mg · kg^{-1} · d^{-1} for 7 d, the hepatocytes which presented accumulation of lipid droplets were seen frequently, but the shape and distribution of lipid droplets in cytoplasm were similar to those of median and low dose groups. The maximum number of lipid droplets detected in individual hepatocyte was a little bit over forty with diameter less than 2.5 μm. The regional topolysis of cytoplasm might be seen in cytoplasm. Sometimes a large patch with sparse matrices adjoined in the nucleus. Most of mitochondria showed injury of cristae, it was often found that the inner cristae was agglutinated and looked obscurity or was broken even disappeared. The cisternae of RER dilated obviously, of which some showed vacuolization and the ribosomes detached from RER sacs. In addition, various secondary lysosomes were seen frequently. The nuclei showed irregular shape and enlarged perinuclear space (Fig 2b, c, Plate 2). One week after stopping treatment, the lipid droplets decreased and the regional topolysis of cytoplasm was still seen. The injury of mitochondria was less. Some of mitochondria even became normal. But the cisternae of RER still dilated severely and the degranulation of RER was obvious (Fig 2d, Plate 2). Two weeks after stopping treatment, the lipid droplets were hardly examined in cytoplasm. The areas with sparse matrices disappeared. The damage of inner cristae and outer membrane of mitochondria was scarce, but the enlarged part of perinuclear space and dilational cisternae accompaning degranulation were still found (Fig 2e, Plate 2). Four weeks after stopping treatment, the ultrastructural features of hepatocytes were restored to normal (Fig 2f, Plate 2).

DISCUSSION

The ultrastructural effects of different doses of I–677 on hepatocytes in mice were most serious at d 1 after stopping treatment. These observations were in accordance with the literature and previous experimental results

of our Department[2,8]. The lipidoses induced by I–677 might be extended with the increasing dose, but even in the group of high dosage (about 8 times of clinical available dose) the number of lipid droplets within a hepatocyte was less than fifty with the largest diameter less than 2.5 μm, and the total area of lipid droplets was far less than 25% of mean section of hepatocytes. In accordance with the diagnostic standard of hepatitis and relative data in literature[9], it was classified as slight steatosis. The results of our observations indicated that the slight steatosis induced by I–677 did not cause hepatocellular necrosis.

The steatosis induced by I–677 is a block in lipids export owing to the lack of protein carrier[2]. Someone also suggested that the steatosis induced by I–677 is in connection with its interfering on oxidation of fatty acid in mitochondria, and decrease of protein synthesis as the result of the shortage of energy[10]. Our experiments here revealed that the hepatocytes already presented accumulation of lipid droplets in low dose group. Meantimes, the regional topolysis of cytoplasm might be seen, but the ultrastructural morphology of mitochondria and other organelles did not show obvious changes. In the convalescence after terminating the treatment of each group, the lipidoses and regional topolysis of cytoplasm almost graduated away at same pace, but the RER and mitochonchdria were restored to normal, always one to two weeks earlier or later than remission of lipidoses. So a block in later phase of protein synthesis induced by dilational cisternae and degranulation of RER is not the main reason of steatosis. Mitochondria damage and decreased fatty acid oxidation causing accumulation of lipid droplets within hepatocytes may only collaborate with other causes[11]. The site of original action of I–677 should be hepatocellular cytoplasm which is also the main position of subcellular distribution of I–677[12], the principal reason of I–677 causing steatosis. The regional topolysis of cytoplasm and loss of matrices indicate that various free synthesis enezymes in the cytoplasm and both number and function of soluble tRNA were affected. As a result, amino acid was activated with some mistakes, and the protein synthesis was inhibited at primary phase. Thereby the albumin transporting the lipid molecules became lack and the steatosis appeared.

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氧代赖氨酸对小鼠肝细胞的超微结构影响

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提要 小鼠 po I-677 10，50，100 mg·kg^{-1}·d^{-1} 连续 7 d 能引起肝细胞轻度脂变．中、高剂量组停药后 2 wk，脂肪变性逐渐消退，4 wk 后，肝细胞超微结构恢复正常．电镜观察提示，I-677 的作用起始部位是肝细胞的胞浆部分．胞浆局部区域性溶解，基质丢失，使蛋白质合成的初始阶段受阻，脂质载体蛋白缺乏，可能是 I-677 所致肝脂变的主要原因．

关键词 赖氨酸；肝；脂类；脂肪变性；电子显微镜检查

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大鼠脾淋巴细胞 β 肾上腺素受体的放射配位体测定

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Radioligand assay of beta adrenoceptors in lymphocytes isolated from rat spleen

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ABSTRACT [^{3}H]Dihydroalprenolol ([^{3}H]DHA) was used to label β-adrenoceptors in intact lymphocytes isolated from rat spleen. The binding conditions were investigated by the methods of L_9 (3^4) orthogonal analysis, the results showed the optimum binding conditions were pH 7.7, 1×10^6 lymphocytes·ml^{-1} and 10 min at 25℃ for incubation. On these conditions the [^{3}H]DHA binding to β-adrenoceptors was rapid ($T_{\frac{1}{2}}$ 2 min) and readily reversed by propranolol 1 mmol·L^{-1} ($T_{\frac{1}{2}}$ 4 min). [^{3}H]DHA saturation experiments indicated a single class of site with a K_D of 7.2±2.2 nmol·L^{-1} and B_{max} of 81±28 fmol/10^7 lymphocytes (n=3). Computer analysis of competition experiments with isoproterenol and epinephrine revealed two classes of sites. One site had high affinity for the [^{3}H]DHA and comprised 60±5% of the total number of sites, whereas the other site had a lower affinity. The affinity of the high affinity site was about 3 orders of magnitude higher than that of the lower affinity site.

KEY WORDS spleen; lymphocytes; beta adrenergic receptors; radioligand assay; dihydroalprenolol; propranolol; research design; competitive binding; binding site

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提要 用 L_9 (3^4)型正交设计确立受体结合实验的最佳条件为：pH 7.7，细胞浓度 1×10^6 个·ml^{-1}，25℃ 水浴 10 min．[^{3}H]二氢烯丙洛尔与大鼠脾淋巴细胞 β 受体结合是快速($T_{\frac{1}{2}}$ 2 min)、可逆($T_{\frac{1}{2}}$ 4 min)的，饱和实验表明 β 受体只存在一种类型，有高度亲和力(K_D：7.2±SD 2.2 nmol·L^{-1})，可饱和性(B_{max}：81±SD 28 fmol/10^7 淋巴细胞) (n=3)．在异丙肾上腺素和肾上腺素竞争实验中，β 受体存在两种状态，高亲和力状态占总受体数的 60±5%，低亲和力状态占总受体数的 40±5%，两种状态 β 受体亲和力相差 3 个数量级．

关键词 脾；淋巴细胞；β 肾上腺素能受体；放射配位体测定；二氢烯丙洛尔；普萘洛尔；研究设计；竞争性结合；结合位点

脾脏淋巴细胞 β 受体是神经免疫调节的重要环节，用放射配位体测定其特征，虽有零