Histological Study on Glycogen Granules and Lipid Droplets in Hepatocytes of Human and Rat Liver Tissues Immediately after Thawing

Akira NARITA*1,4, Masayuki ITO*2, Akiyo SHIGEMATSU*3 and Tetsuo SATOH*4

*1 School of Education, Tokyo University of Social Welfare (Isesaki Campus), 2020-1 San’o-cho, Isesaki-city, Gunma 372-0831, Japan
*2 Advanced Research Institute for Science and Engineering, Waseda University, 3-41-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan
*3 Institute of Whole Body Metabolism, 340-2 Nauchi, Shiroi-city, Chiba 270-1407, Japan
*4 Research Laboratories, Human and Animal Bridging Research Organization, 5-11-13 Sugano, Ichikawa-city, Chiba 272-8513, Japan

(Accepted May 6, 2010)

Abstract: Glycogen granules and lipid droplets in hepatocytes of both human and rat liver tissues immediately after thawing were histologically investigated in a preliminary experiment for medical, pharmacological and biological studies. Human and rat hepatocytes were used immediately before freezing as controls. When human liver tissue sections were incubated using the periodic acid-Schiff method, reddish-purple granules were observed in the hepatocyte cytoplasm. The presence of glycogen granules was confirmed histologically using a salivary test. The glycogen granules were observed in the cytoplasm of most human and rat hepatocytes. Although the amount of glycogen granules was not consistent in each human hepatocyte, many glycogen granules were observed in most rat hepatocytes. Distributions of glycogen granules in the human and rat liver tissues were histologically similar to those of controls. On the other hand, when human liver tissue sections were incubated using the Sudan IV method, reddish-orange lipid droplets were observed in the cytoplasm of hepatocytes located around the central veins in human liver tissues. The lipid droplets in hepatocytes of the human liver tissues were histologically similar to the controls. However, the lipid droplet was not observed in the cytoplasm of both the rat and control hepatocytes immediately after thawing. These results suggest that human and rat liver tissues immediately after thawing are scientifically useful because glycogen granules or lipid droplets as energy sources were preserved in their hepatocytes.

(Reprint request should be sent to Akira Narita)

Key words: Glycogen granule, Lipid droplet, Human, Rat, Hepatocyte, Thawing

Introduction

Mammalian hepatocytes are often used in various medical and pharmacological studies, mainly due to their important metabolic functions. Although there are many cryopreservation methods for mammalian hepatocytes in the literature, there are reports of some post-thaw morphological abnormalities in mammalian hepatocytes (Fuller et al., 1982; Mito and Kusano, 1984a; Mito and Kusano, 1984b; Dou et al., 1992; Suzuki, 1996; Narita et al., 2007; Narita et al., 2008). For examples, the ultrastructure of hepatocyte mitochondria isolated from livers of humans (Dou et al., 1992) and rats (Fuller et al., 1982; Mito and Kusano, 1984b) tended to swell after thawing and develop lower electron density matrices. These mitochondrial changes were also observed in hepatocytes of human (Narita et al., 2007) and rat (Suzuki, 1996; Narita et al., 2008) liver tissues immediately after thawing. Furthermore, we reported that mitochondria of hepatocytes around interlobular connective tissues tended to be less histologically prominent than hepatocytes around central veins in human (Narita et al., 2007) and rat.
(Narita et al., 2008) livers immediately after thawing. These results suggest that mitochondrial functions of the mammalian hepatocytes after thawing are lower than in hepatocytes before freezing. Recently, we reported that mammalian hepatocytes after thawing are lower than in those around the central veins (Narita et al., 2009). Distributions of hepatocytes showing high histochemical activities of those mitochondrial enzymes were the same as in hepatocytes immediately before freezing (Narita et al., 2009). On the other hand, it was reported that the ultrastructure of glycogen granules in isolated rat hepatocytes disappeared after thawing (Mito and Kusano, 1984a). In post-thawed mammalian hepatocytes, however, the relationships between such mitochondrial phenomena and energy sources are essentially unknown.

In this preliminary study, we histologically detected glycogen granules and lipid droplets of hepatocytes in human and rat liver tissues immediately after thawing for further medical, pharmacological or biological research.

Materials and Methods

Human livers

We used livers from 11 brain-dead organ donors of American origin obtained from the Human and Animal Bridging Research Organization in Japan. All patients had provided their consent through organ donor cards. Patient livers were obtained from the National Disease Research Interchange in the United States. Hepatic tissues were preserved at -80 °C for one week, and then thawed at 37 °C in a Krebs-Henseleit buffer solution (pH 7.4). The test tissues were examined immediately after thawing, while the control liver tissues were used immediately before freezing.

Rat livers

Six male Wister strain rats (Clea Japan Co. Ltd., Tokyo) of 9-10 week-old were used. They were kept and fed normally in a room at 24 °C and lit 14 hours a day. They were cannulated with polyethylene tubes (PE-10, Nippon Becton Dickinson Co. Ltd., Tokyo) in the portal vein and the hepatic artery under halothane anesthesia. When the animals had sufficiently recovered from the anesthesia, their livers were perfused through the portal vein and hepatic artery to the hepatic vein with a Krebs-Henseleit buffer solution (pH 7.4) containing 2.5% glucose and 2.0% mannitol. The livers were then perfused with a Krebs-Henseleit buffer solution (pH 7.4) containing 2.5% glucose, 2.0% mannitol and 10% dimethyl sulfoxide. The livers were frozen at a speed of -1 °C/min, preserved for one week at -80 °C, melted with 37 °C in a Krebs-Henseleit buffer solution (pH 7.4), and used immediately after thawing. The control livers were used immediately before freezing.

Confirmation of glycogen granules

The human and rat liver tissues immediately after thawing were fixed in Rossman fluid, embedded in paraffin, sectioned at a thickness of 5 µm, and stained using the periodic acid-Schiff and hematoxylin method (McManus, 1948; Sano, 1985). Glycogen granules in the cytoplasm of hepatocytes in human and rat liver tissues were confirmed with a salivary test. Micrographs for histological observations of glycogen granules were taken using a microscope (8HE, Olympus Co. Ltd., Tokyo).

Confirmation of lipid droplets

We used the Sudan IV and hematoxylin method (Sano, 1985) to demonstrate lipid droplets in hepatocytes of the human and rat liver tissues immediately after thawing. Micrographs for histological observations of lipid droplets were taken using a microscope (8HE, Olympus Co. Ltd.).

Results

Anatomical observation of livers

Eight of the 11 patients had fatty livers. These fatty livers were excluded from the objective observation because they were pathologically unsuitable for this study. On the other hand, all six rat livers were used as they were anatomically normal.

Histological observation of glycogen granules

When sections of human and rat liver tissues were treated with the periodic acid-Schiff and hematoxylin method, reddish-purple granules appeared in the cytoplasm of hepatocytes in both human (Fig. 1) and rat (Fig. 2) liver tissues. These were confirmed as glycogen granules because they disappeared after a salivary test. The amount of glycogen granules was irregular in each human hepatocyte,
Glycogen and Lipid in Human and Rat Hepatocytes

and the characteristic distribution of the hepatocytes containing substantial glycogen granules was not observed in human liver tissues. On the other hand, numerous glycogen granules were observed in most rat hepatocytes. The glycogen granules in the human and rat liver tissues were histologically similar to the controls (Figs. 3 and 4).

Histological observation of lipid droplets

When sections of human liver tissues were incubated using the Sudan IV and hematoxylin method, reddish-orange droplets showing lipid content were observed in the cytoplasm of hepatocytes around the central veins in human liver tissues (Fig. 5). The lipid droplets of hepatocytes in the human liver tissues showed the same as that of the controls (Fig. 6). No lipid droplet was observed in the cytoplasm of hepatocytes in the rat liver tissues immediately after thawing and the controls.

Discussion

In this experiment, the amount of glycogen granules was numerous in most rat hepatocytes, and it was irregular in
Each human hepatocyte. These phenomena may be related to various living environments and eating habits of human.

Some mitochondria of hepatocytes isolated from rat and human liver tissues swelled after thawing (Fuller et al., 1982; Mito and Kusano, 1984b) and developed lower electron density matrices (Fuller et al., 1982; Dou et al., 1992). These phenomena were observed in hepatocytes of rat liver tissues immediately after thawing (Suzuki, 1996; Narita et al., 2008) and in hepatocytes of human liver tissues (Narita et al., 2007). We recognized, however, that the mitochondrial matrices with a low electron density indicate a functional decrease in energy metabolism and are not a fatal phenomenon (Narita et al., 2007). Shiraishi et al. (1982) observed mitochondria with a low electron density in mouse embryos after thawing, indicating that, although the embryos developed into blastocysts on incubation, the speed of development was lower than that of fresh embryos. We previously reported that the electron densities of mitochondrial matrices of stored oocytes in mouse ovaries were lower than grown oocytes (Narita et al., 1990). Furthermore, Mito and Kusano (1984b) succeeded in transplanting liver tissues after thawing into rat spleen. There-
Before, the results of our studies on glycogen granules or lipid droplets in human and rat hepatocytes suggest that energy sources for life activity are maintained in the mammalian liver tissues immediately after thawing.

Recently, we have reported that the mitochondria in hepatocytes around interlobular connective tissues tended to be less prominent than those around central veins in human livers immediately after thawing (Narita et al., 2007). We also reported that cytochrome oxidase and succinate dehydrogenase activities in hepatocytes around interlobular connective tissues in human liver immediately after thawing were higher than those around the central veins (Narita et al., 2009). Therefore, such phenomena in human liver tissues may be related to lipid droplets of hepatocytes around the central veins in this experiment.

It was reported that glycogen granules in isolated rat hepatocytes ultrastructurally disappeared after thawing (Mito and Kusano, 1984a). However, glycogen granules were observed in hepatocytes of human and rat liver tissues immediately after thawing in this experiment. This discrepancy may be due to differences in materials and methods. In their experiment, glycogen granules might flow out

Fig. 5. A human liver tissue immediately after thawing. The central vein is indicated by C, hepatocytes containing lipid droplets by H. Sudan IV and hematoxylin method. The scale indicates 50 \( \mu \) m.

Fig. 6. A human liver tissue immediately before freezing. The central vein is indicated by C, hepatocytes containing lipid droplets by H. Sudan IV and hematoxylin method. The scale indicates 50 \( \mu \) m.
from the cell membranes because the surface of isolated hepatocytes is easily damaged by various outer stimulations. Moreover, when using the method for ultrastructural observations, glycogen granules in the hepatocytes might decrease with alcohol and acetone treatments. On the other hand, in this experiment, we used liver tissues in order to maintain systematic functions and used the Rossman fluid to fix glycogen granules.

Recently, we reported that rats serve as an effective animal model for the study of human liver tissue enzymes such as succinate dehydrogenase (Narita et al., 2009). In our present investigation, glycogen granules were histologically observed in both human and rat hepatocytes. However, lipid droplets were observed in human, but not rat hepatocytes around the central veins. This discrepancy may be due to differences among animal species.

Conclusion

Our histological analysis indicated that glycogen granules are present in hepatocytes of human and rat liver tissues immediately after thawing. Lipid droplets were observed in hepatocytes around the central veins in human liver tissues immediately after thawing. Glycogen granules and lipid droplets were histologically maintained in hepatocytes of liver tissues immediately after thawing because they were found in the controls.

References


解凍直後のヒトおよびラット肝組織における肝実質細胞のグリコーゲン顆粒および脂肪小滴に関する組織学的研究

成田  成”1,4・伊藤政幸”2・重松昭世”3・佐藤哲男”4

”1東京福祉大学 教育学部（伊勢崎キャンパス）
〒372-0831 群馬県伊勢崎市山王2020-1

”2早稲田大学 理工学総合研究センター
〒169-8555 東京都新宿区大久保3-41-1

”3生体科学研究所
〒270-1407 千葉県白井市名内340-2

”4エイチ・エー・ピー研究機構 附属研究所
〒272-8513 千葉県市川市菅野5-11-13

抄録：我々は解凍直後のヒトおよびラット肝組織の肝実質細胞におけるグリコーゲン顆粒および脂肪小滴を組織学的に調査した。グリコーゲン顆粒はほとんどのヒトおよびラットの肝実質細胞の細胞質で認められた。グリコーゲン顆粒の量はそれぞれのヒト肝実質細胞では不均一であったが、ほとんどのラット肝実質細胞では多量であった。ヒトおよびラット肝組織のグリコーゲン顆粒の分布は、組織学的にコントロール（凍結直前のもの）と同様であった。一方、ヒト肝組織の脂肪小滴は、中心静脈付近の肝実質細胞の細胞質で観察され、組織学的にはコントロールと同様であった。しかしながら、脂肪小滴は解凍直後のラット肝実質細胞およびコントロールの両方で認められなかった。これらの結果は、エネルギー源であるグリコーゲン顆粒または脂肪小滴が保たれることを示すことから、解凍直後のヒトおよびラット肝組織における肝実質細胞は科学的に役立つことを示唆している。
（別刷請求先：成田 成）

キーワード：グリコーゲン顆粒、脂肪小滴、ヒト、ラット、肝実質細胞、解凍