Tricaprin Rescues Myocardial Abnormality in a Mouse Model of Triglyceride Deposit Cardiomyovasculopathy

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Abstract: Triglyceride deposit cardiomyovasculopathy (TGCV) is an intractable cardiovascular disease for which a specific treatment is urgently required. In TGCV, adipose triglyceride lipase (ATGL) deficiency results in the abnormal intracellular metabolism of long-chain fatty acid (LCFA) which leads to TG deposition. Medium-chain triglycerides have been used as an important functional food for various human diseases. To address the potential activities of tricaprin, a medium-chain triglyceride, on cardiac dysfunctions of TGCV, we examined the effects of tricaprin diet on \(\text{Atgl}^\) knockout (KO) mice, an animal model for TGCV. Cardiac imaging tests showed that the tricaprin diet reduced TG accumulation, resulting from improvement of LCFA metabolism, and improved left ventricular function in \(\text{Atgl}^\) KO mice compared to that in mice fed the control diet. In conclusion, tricaprin improved myocardial abnormality in the TGCV model, thus, it may be useful for the treatment of patients with TGCV.

Key words: adipose triglyceride lipase, capric acid, iodine-123-\(\beta\)-methyl iodophenyl-pentadecanoic acid, medium-chain triglyceride, tricaprin, triglyceride deposit cardiomyovasculopathy

1 Introduction

Triglyceride deposit cardiomyovasculopathy (TGCV) is a rare and intractable cardiovascular disease that was first reported among patients requiring cardiac transplantation in Japan\(^1\). TGCV is characterized by a massive accumulation of triglyceride (TG) in the myocardium\(^2\) and coronary arteries\(^3\) which leads to severe congestive heart failure and angina pectoris. TGCV is classified into primary and idiopathic types. Primary TGCV is caused by genetic mutations

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Abbreviations: ATGL, adipose triglyceride lipase; BMIPP, iodine-123-\(\beta\)-methyl iodophenyl-pentadecanoic acid; CT, computed tomography; FA, fatty acid; HE, hematoxylin and eosin; KO, knockout; LCFA, long-chain fatty acid; LVEF, left ventricular ejection fraction; MCFA, medium chain fatty acid; ROI, region of interest; SPECT, single-photon emission computed tomography; TG, triglyceride; TGCV, triglyceride deposit cardiomyovasculopathy; WOR, washout rate; WT, wild type
in PNPLA2 which encodes adipose triglyceride lipase (ATGL), a rate-limiting enzyme in the hydrolysis of intracellular TG and the release of free long-chain fatty acids (LCFAs)\textsuperscript{4,5}. Idiopathic TGV is not due to PNPLA2 mutations\textsuperscript{6}, but is associated with a marked reduction in ATGL activity in peripheral leukocytes\textsuperscript{7}. 

Clinically, TGV with ATGL deficiency causes severe heart failure with a poor prognosis\textsuperscript{1,8,9}. Hence, the development of specific treatment for TGV is an urgent requirement. Our previous in vitro experiments on fibroblasts derived from patients with TGV showed that LCFAs, the major energy source for the normal heart, accumulate as TG in cytoplasmic lipid droplets owing to ATGL deficiency\textsuperscript{10,11}. To resolve the above-mentioned toxicity of LCFAs, we explored the development of nutritional therapeutic agents from medium-chain fatty acids (MCFAs) for the following reasons: 1) MCFAs have a long and safe history as they have been used clinically for 50 years in patients with metabolic and neurological disorders\textsuperscript{12-14}; 2) MCFAs are an alternative energy source to LCFA and glucose\textsuperscript{15,16}; and 3) the intracellular metabolism of MCFAs was shown to be different from that of LCFA\textsuperscript{17}. It was shown that MCFAs activated FA oxidation enzymes in the rat liver when oils containing medium-chain triglycerides (MCTs) were administered orally to rats\textsuperscript{18}. Moreover, the effects of MCTs on body fat have been evaluated and it was suggested that an MCT diet reduced body weight in humans\textsuperscript{19,20}.

Previous studies have shown that TG deposition in cardiomyocytes in Atgl KO mice resulted in cardiac phenotypes that were similar to those observed in patients with TGV\textsuperscript{5,10}. In the present study, the potential beneficial effects of tricaprin, the TG form of caprylic acid (a type of MCFA) on the cardiac TG deposition and dysfunctions were examined in Atgl KO mice.

2 Experimental procedure

2.1 Tricaprin

Purified tricaprin (99%) was obtained from Yashiro Co. Ltd (Osaka, Japan). Our recent study confirmed the safety of tricaprin in canine toxicity tests\textsuperscript{21}.

2.2 Mouse model

Atgl KO mice were kindly provided by Professor Rudolf Zechner (University of Graz, Austria)\textsuperscript{9}. Four-week-old Atgl KO mice were divided into tricaprin (+) and tricaprin (−) diet groups. In the tricaprin (+) diet, 8% of the natural fat was replaced with tricaprin (Yashiro, Osaka, Japan) whereas the tricaprin (−) diet contained 10% natural fat. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka and Hamamatsu University School of Medicine. All experiments were performed in accordance with the guidelines for animal use approved by the Institutional Animal Care and Use Committee of Osaka University Graduate School of Medicine (approval number: 27-070-001) and Hamamatsu University School of Medicine (approval number: 2012010). Wild-type (WT) C57Bl/6 mice fed the tricaprin (−) diet were used as the control.

2.3 Animal experiments

All analyses described in this section were performed on 7-week-old Atgl KO mice, because the KO mice over 8 weeks of age could not tolerate anesthesia owing to heart failure.

2.3.1 Myocardial iodine-123-β methyl iodophenyl-pentadecanoic acid (BMIPP) scintigraphy by single-photon emission computed tomography (BMIPP-SPECT)

LCFA clearance at the cellular level can be detected by in vivo myocardial scintigraphy using BMIPP, a radiolabeled analog of LCFA\textsuperscript{22-24}. We recently reported that patients with TGV showed a defective washout of BMIPP in a scintigraphic analysis\textsuperscript{2}. To evaluate LCFA metabolism in the heart of Atgl KO mice in this study, BMIPP-SPECT was used. The Atgl KO mice fed the tricaprin (+) or tricaprin (−) diet (n = 4 in each group) were evaluated by using myocardial BMIPP-SPECT. BMIPP was obtained from Nihon Medi-Physics Co. Ltd., Tokyo, Japan, and injected into the jugular vein of mice under isoflurane anesthesia. BMIPP was then traced by SPECT/CT (Flex X-SPECT/CT, Gamma Medica-Ideas Inc., CA, USA). The SPECT images were obtained at 1 h and 4 h after the injection of BMIPP. The regions of interest (ROIs) were placed on the myocardium-based on CT images, and the decay-corrected radioactivity uptake in each ROI was obtained. Washout rates (WORs), used to evaluate LCFA metabolism, were calculated from the formula \([\text{BMIPP uptake (1 h)} - \text{remaining BMIPP (4 h) }] \)/BMIPP uptake (1 h). 

2.3.2 Measurements of myocardial lipid deposition and left ventricular ejection fraction (LVEF) by CT

Micro-CT has been used to measure cardiac function in rodents\textsuperscript{25,26}. In the present experiment, micro-CT (Latheta LCT-200, Hitachi-Aloka Medical, Tokyo, Japan) was used to measure cardiac lipid deposition and function in the mouse heart. The myocardial lipid deposition was examined in KO mice fed the tricaprin (+) or tricaprin (−) diet (n = 5 in each group). The CT values (in Hounsfield units, HU) were measured in four regions (the septum and the anterior, posterior, and lateral walls of the heart) and the mean CT value was calculated from the CT values of four points.

The KO mice that were fed the tricaprin (+) or tricaprin (−) diet (n = 7 in each group) were examined by using a previously reported method for measurement of LVEF\textsuperscript{27}. After the mice were anesthetized, 30 μL of contrast agent (ExiTron nano 12000, Miltenyi Biotec, Germany) was injected into the subclavian vein of the mice. The systolic...
and diastolic heart volumes were examined by using micro-CT. Images were obtained after every 96 µm, which separated the LV into segments; the volume of each segment was calculated by multiplication of the area by 96. The total LV volume was calculated from addition of all segmental volumes. LVEF was determined after the systolic and diastolic volumes of the whole heart were calculated. EF was calculated from the following formula:

$$EF = \frac{LVEDV - LVESV}{LVEDV} \times 100\%,$$

where LVEDV is the LV end-diastolic volume, and LVESV is the LV end-systolic volume.

2.3.3 Histological analysis

The mice hearts were fixed in formalin. The paraffin block sections were stained with hematoxylin and eosin (HE) and Masson’s trichrome, and examined by using a microscope (Olympus, Tokyo, Japan).

2.4 Statistical analysis

The data are presented as the mean ± standard error. A p value of < 0.05 was considered to indicate statistical significance.

3 Results

3.1 Tricaprin diet improved LCFA WOR in the heart of Atgl KO mice

Similar to that observed in patients with TGCV\(^{21}\), the WORs of BMIPP were considerably reduced in the KO mice fed the tricaprin (−) diet (Fig. 1A and 1B). In contrast, the WORs of BMIPP in KO mice fed the tricaprin (+) diet were markedly higher than those in mice fed the tricaprin (−) diet (Fig. 1A and 1B). As BMIPP is known to be incorporated into the cytoplasmic TG pool and oxidized by the cardiac mitochondria of rodents, similar to native LCFA\(^{27}\), the increased WOR indicated that the tricaprin (+) diet might facilitate LCFA metabolism in the heart of the KO mice.

3.2 Tricaprin diet ameliorated lipid accumulation in the heart of Atgl KO mice

The myocardial CT values shown in Fig. 2A and 2B reflect the tissue TG content in patients with TGCV\(^{9,29}\). The CT value in the WT group was 125 ± 9.6 HU. In contrast, the CT values of the KO mice fed the tricaprin (−) diet were low. The tricaprin (+) diet-fed group showed significantly higher CT values than the tricaprin (−) diet-fed group (−27.5 ± 5.7 HU vs. 8.1 ± 5.5 HU, \(^*p < 0.01\)) (Fig. 2B). This suggested that the tricaprin (+) diet reduced fat accumulation in the heart of Atgl KO mice.

Histological analysis was performed to confirm the observations obtained by using micro-CT. In 7-week-old KO mice, massive vacuoles were present in cardiomyocyte and fibrotic changes were observed after Masson’s trichrome staining (Fig. 2C), as shown in previous results\(^3\). These features were attenuated in KO mice fed the tricaprin (+) diet (c and f in Fig. 2C).

3.3 Tricaprin diet improved LV function in the heart of Atgl KO mice

We assessed the effect of the tricaprin (+) diet on cardiac function (Fig. 3). The LVEF in the KO mice fed the tricaprin (+) diet was significantly higher than that in the KO mice fed the tricaprin (−) diet (30 ± 12% vs. 15 ± 9%, \(p < 0.01\)). LVEF in the KO mice fed the tricaprin (+) diet was comparable with that of WT mice fed tricaprin (−) diet, which indicated that LV function was restored in the KO mice fed the tricaprin (+) diet.

4 Discussion

There are several hypotheses that may explain the beneficial effects of the tricaprin (+) diet in ATGL-deficient conditions. First, capric acid can be used and oxidized as an alternative energy source, as reported in patients with other rare genetic disorders, such as very long-chain acyl-CoA dehydrogenase and malonyl-CoA decarboxylase deficiencies; in contrast, very long chain fatty acids and LCFA, respectively, cannot be metabolized owing to defects in the corresponding enzymes\(^{28,29}\). Second, capric acid might facilitate TG hydrolysis via certain MCFA-specific pathways at the cellular level, as previously suggested by a detailed biochemical analysis\(^7\). Third, the tricaprin (+) diet contains relatively low (2%) natural fat, and it is potentially, not tricaprin, but the low natural fat content, that contributes to the present observations; however, this is unlikely, because a low-fat diet paradoxically induced fat accumulation\(^8\) and shortened the life span of Atgl KO mice (data not shown).

Capric acid has been reported to increase the capacity for fatty acid oxidation in mouse skeletal muscle\(^{31}\), and reduce the body weight of high-fat diet-fed mice through an increase in ATGL level\(^{32}\).

This study has certain limitations. First, MCFAs include caprylic acid, capric acid and lauric acid. We examined the beneficial effects of capric acid on mouse cardiac functions; however, other MCFAs such as caprylic acid and lauric acid may have similar effects that are not examined in the study. Second, the detailed molecular mechanisms of the capric acid-mediated improvement in LCFA metabolism were not clarified in this study.

5 Conclusion

In summary, the tricaprin (+) diet improved the myocar-
dial metabolism of LCFAs, reduced myocardial lipid deposition (Fig. 4), and improved left ventricular function in a mouse model of TGCV. We believe that the present data are sufficient to translate the use of tricaprin into clinical trials as nutritional therapeutic agents for patients with severe and critical symptoms of TGCV.
Fig. 3  Tricaprin diet improved cardiac dysfunction in Atgl KO mice
(A, B, C) Representative bar graphs indicate the volumes in each LV section from the base to the apex of the heart. Red and blue bars indicate segmental LV volumes at end-systole and end-diastole, respectively, in A (WT mice that were fed tricaprin (-) diet), B (KO mice that were fed tricaprin (-) diet), and C (KO mice that were fed tricaprin (-) diet). (D) The mean value of LVEF in KO mice that were fed tricaprin (+) diet was significantly higher than that in mice fed tricaprin (-) diet (p < 0.01) (n = 7 for each group).

Fig. 4  Scheme of tricaprin regulated LCFA metabolism.
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Conflict of interests

Ken-ichi Hirano received grants from Nihon Medi-Physics Co., Ltd., during the conduct of the study. Other authors declare that they have no conflicts of interest.

References


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