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Does administration of hydroxychloroquine/amiodarone affect the efficacy of enzyme replacement therapy for Fabry mice?

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ABSTRACT

As a standard therapy for Fabry disease, enzyme replacement therapy (ERT) with recombinant human α-galactosidase A (α-Gal) has been successfully used, and the instructions for this drug state that "it should not be co-administrated with cationic amphiphilic drugs such as hydroxychloroquine (HCQ) and amiodarone (AMI), since these drugs have the potential to inhibit intracellular α-Gal activity". However, there would be cases in which HCQ or AMI is required for patients with Fabry disease, considering their medical efficacy and application.

Thus, we examined the impact of HCQ/AMI on recombinant human α-Gal by *in vitro*, cellular, and animal experiments. The results revealed that HCQ/AMI affected the enzyme activity of α-Gal incorporated into cultured fibroblasts from a Fabry mouse when the cells were cultured in medium containing these drugs and the enzyme, although their direct inhibitory effect on the enzyme is not strong. These lysosomotropic drugs may be trapped and concentrated in lysosomes, followed by inhibition of α-Gal.

On the other hand, no reduction of α-Gal activity incorporated into the organs and tissues, or acceleration of glycoshingolipid accumulation was observed in Fabry mice co-administered with HCQ/AMI and the enzyme, compared with in the case of usual ERT. As HCQ/AMI administered are catabolized in the liver, these drugs possibly do not affect ERT for Fabry mice, different from in the case of cultured cells in an environment isolated from the surroundings.

1. Introduction

Fabry disease (OMIM 301500) is an X-linked genetic disorder caused by variants of the *GLA* gene that result in deficient or decreased enzyme activity of α-galactosidase A (α-Gal, EC 3.2.1.22) [1]. The enzymatic defect leads to accumulation of glycosphingolipids such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (Lyso-Gb3) in body fluids, and various organs and tissues, and it induces systemic manifestations including renal and cardiovascular system ones.

On the other hand, drug-induced phospholipidosis (DIPL) is an acquired lysosomal disorder characterized by excessive accumulation of phospholipids in the kidneys, liver, heart, lungs, brain, cornea, skeletal muscles, and other organs caused by prolonged treatment with cationic amphiphilic drugs (CADs), such as chloroquine, hydroxychloroquine (HCQ), amiodarone (AMI), imipramine, gentamicin, and so on [2–4].

As DIPL exhibits characteristic pathological findings (membranous cytoplasmic bodies, inclusion bodies, Zebra bodies, and myelin figures) and clinical symptoms similar to those of Fabry disease, caution is required for differential diagnosis, especially when sufficient medication history cannot be obtained. Actually, many cases of DIPL with a risk of erroneous diagnosis of Fabry disease have been reported [5–8].

As to a standard therapy for Fabry disease, enzyme replacement therapy (ERT) with recombinant human α-Gals (agalsidase alfa and agalsidase beta) has been introduced and successfully used. It has been

Abbreviations: ERT, enzyme replacement therapy; α-Gal, α-galactosidase A; CAD, cationic amphiphilic drug; HCQ, hydroxychloroquine; AMI, amiodarone; Gb3, globotriaosylceramide; Lyso-Gb3, globotriaosylsphingosine; DIPL, drug-induced phospholipidosis; 4Mu-α-GalPyr, 4-methylumbelliferyl-α-D-galactopyranoside; GalNAc, *N*-acetyl-D-galactosamine; LC, liquid chromatography; MS/MS, tandem mass spectrometry; DMEM, Dulbecco's minimum essential medium; PBS, phosphate-buffered saline; SD, standard deviation; CI-M6PR, cation-independent mannose 6-phosphate receptor.

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reported that ERT is effective for hydrolyzing glycosphingolipids accumulated in organs and tissues, and appears to inhibit the progression of Fabry disease [9–11]. However, product information from the European Medicines Agency warns about the interaction of agalsidases (agalsidase alfa, Replagal®, and agalsidase beta, Fabrazyme®) and other medical products: "Replagal® should not be co-administered with chloroquine, AMI, benoquin or gentamicin since these substances have the potential to inhibit intra-cellular α-Gal activity" [12], and "Tell your doctor if you use any medicines containing chloroquine, AMI, benoquin or gentamicin. There is a theoretical risk of decreased Fabrazyme® activity" [13]. HCQ, which is well known as an antimalarial drug, is also an effective medication widely used for various autoimmune connective diseases [14,15]. On the other hand, AMI is an anti-arrhythmic drug effective for treatment of ventricular and supra-ventricular arrhythmia that is difficult to control [16,17]. As there would be cases in which HCQ or AMI is required for patients with Fabry disease, it is important for clinicians and researchers involved in ERT for Fabry disease to elucidate the interaction between recombinant human α-Gal and HCQ/AMI, and to determine whether HCQ/AMI affect the ERT or not.

In this study, we examined the impact of HCQ/AMI on agalsidase beta by in vitro, cellular, and animal experiments.

2. Materials and methods

2.1. Reagents, cultured cells, and animals

Recombinant human α-Gal produced in Chinese hamster ovary cells (agalsidase beta, Fabrazyme®) and AMI hydrochloride (Ancaron®) were purchased from Sanofi Co. (Tokyo, Japan). HCQ sulfate was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). 4-Methylumberiferyl-α-D-galactopyranoside (4Mu-α-GalPyr) and *N*-acetyl-D-galactosamine (GalNAc) were purchased from Calbiochem (La Jolla, CA) and Sigma-Aldrich (St. Louis, MO), respectively. Stable isotope-labelled Lyso-Gb3 with one ^{13}C and three deuterium atoms [18] was synthesized by Nard Institute Ltd. (Kobe, Japan), and Gb3 (C17:0) was purchased from Matreya L.L.C. (Pleasant Gap, PA). All other reagents used in this study were of analytical grade.

HEK293 cells derived from kidney tissue of a human embryo and fibroblasts from skin tissue of a Fabry mouse (F666) [19] were used for cell experiments in this study.

Fabry model mice (*Gla* knock-out mice, C57BL/6 × 129SvJ hybrid background) denoted by A.B. Kulkarni and T. Oshima (National Institute of Dental Research, National Institute of Health) [20]. This study was approved by the animal ethics committee of our university.

2.2. Measurement of α-Gal activity

Fluorometric measurement of α-Gal activity in samples was performed using 4Mu-α-GalPyr as a substrate, and GalNAc as an inhibitor of α-*N*-acetylgalactosaminidase, as described previously [21]. Briefly, 10 μL aliquots of samples were mixed with 40 μL of the substrate solution comprising 5 mmol/L 4Mu-α-GalPyr and 117 mmol/L GalNAc in 0.1 mol/L citrate-phosphate buffer, pH 4.6, and each mixture was incubated at 37 °C for 30 min. Then, the reaction was stopped by adding 950 μL of 0.2 mol/L glycine buffer, pH 10.7. The fluorescence of 4-methylumbelliferone released on the enzyme reaction was measured with a Varioskan LUX (Thermo Scientific, Rockford, IL) at excitation and emission wavelengths of 365 nm and 450 nm, respectively.

2.3. Protein determination

Determination of protein in samples was performed with a Micro BCA Protein Assay kit (Thermo Scientific), using bovine serum albumin as the standard.

2.4. Measurement of Gb3 and Lyso-Gb3

The contents of Gb3 and Lyso-Gb3 in samples were determined by means of liquid chromatography (LC)-tandem mass spectrometry (MS/MS), according to the method described previously [22]. Briefly, 10 μL of a sample was mixed with a 70 μL aliquot of chloroform:methanol (1:2), and then a 10 μL aliquot of 5 μg/mL Gb3 (C17:0) and a 10 μL aliquot of 500 nmol/L stable isotope-labelled Lyso-Gb3 were added as the internal standards. The mixture was centrifuged, and then LC was performed using the supernatant as a sample. For LC, a Unison UK-C8 column (20 × 3 mm I.D., 3 μm; Imtakt Co., Kyoto, Japan) was used, and the column oven was kept at 30 °C. Chromatographic separation was performed with a binary gradient. Then, Gb3 isoforms and Lyso-Gb3 in the samples were detected by MS/MS with a LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization interface in the positive-ion mode. The total Gb3 content in a sample was calculated from the sum of the Gb3 isoforms.

2.5. In vitro experiment

The direct impact of HCQ/AMI on the enzyme activity of recombinant human α-Gal was examined. Five μL of a HCQ or AMI solution (0.25, 0.5, 1.0, 5.0, 10, and 100 mmol/L in water) was first mixed with 40 μL of the substrate solution for α-Gal assaying, and then 5 μL of 1 μg/mL agalsidase beta in 20 mmol/L MES buffer, pH 6.0, was added to the mixture, followed by α-Gal assaying.

The α-Gal enzyme activities in the samples containing HCQ/AMI at the indicated concentrations (final concentrations: 0.025, 0.05, 0.1, 0.5, 1.0, and 10 mmol/L) were calculated as the percentages compared to that when water was added instead of the HCQ/ AMI solution.

2.6. Cell experiments

2.6.1. Examination of the impact of HCQ/AMI on α-Gal in cultured HEK293 cells

HEK293 cells were distributed into the wells of a commercially available 12-well culture plate at the concentration of 1.0×10^5 cells/well, and then cultured in Dulbecco's minimum essential medium (DMEM) containing 10% fetal bovine serum and antibiotics for 24 h in a humidified incubator flushed continuously with a 5% CO_2-95% air mixture. Then, the culture medium was changed to DMEM containing HCQ or AMI to give concentrations of 6.25, 12.5, and 25 μmol/L, respectively, and the cells were further cultured for 24 h. After the culture, the cells were harvested by trypsinization, washed three times with phosphate-buffered saline (PBS), pH 7.4, and then collected as a pellet by centrifugation. An appropriate amount of 20 mmol/L MES buffer, pH 6.0, including proteinase inhibitors was then added to the pellet, and the cells were sonicated and centrifuged, the resulting supernatant being used for the α-Gal assay. The enzyme activity in samples was calculated as the percentage compared to that when the cells were cultured in the medium without HCQ or AMI.

2.6.2. Examination of the impact of HCQ/AMI on recombinant human α-Gal incorporated into cultured F666 cells

To examine the impact of HCQ/AMI on recombinant human α-Gal incorporated into F666 cells, the cells were cultured under the same culture conditions using Ham's F-10 medium instead of DMEM. After culture in the medium including 1.0 μg/mL agalsidase beta and HCQ or AMI, at final concentrations of 6.25, 12.5, and 25 μmol/L for 24 h, respectively, the cells were harvested, washed, sonicated and centrifuged. Then, α-Gal assaying was performed using the supernatants as samples. The α-Gal enzyme activity in samples was calculated as the percentage compared to that when the cells were cultured in the medium containing agalsidase beta but not HCQ/AMI.

2.7. Animal experiments

2.7.1. Examination of the impact of HCQ/AMI on recombinant human α-Gal incorporated into organs and tissues of Fabry model mice

Fabry model mice (31– 32-week-old males) were used in this experiment. One mg/kg body weight of agalsidase beta and 50 mg/kg body weight of HCQ or AMI were simultaneously injected into the Fabry model mice via a tail vein and intraperitoneally, respectively. The mice were sacrificed 24 h after administration of the drugs, and their livers, kidneys and hearts were removed after perfusion with PBS. Then, tissue samples were homogenized in 20 mmol/L MES buffer, pH 6.0, including proteinase inhibitors. Then, the α-Gal activity in the organs and tissues was measured using the supernatants of the homogenates as samples.

As controls, two Fabry model mouse groups, one treated with 1 mg/kg body weight agalsidase beta only and the other without treatment, were used.

2.7.2. Examination of the impact of HCQ/AMI on cleavage of Gb3 and Lyso-Gb3 accumulated in organs and tissues of Fabry model mice that had received ERT with recombinant human α-Gal

Fifty mg/kg body weight HCQ or AMI was administered intraperitoneally to Fabry model mice (11-week-old males) every day for 14 days, 1.0 mg/kg body weight of agalsidase beta being injected via a tail vein on the 8th day. These mice were sacrificed 24 h after the last administration of HCQ/AMI, and their organs and tissues were removed after perfusion with PBS, followed by homogenization. Then, the contents of Gb3 and Lyso-Gb3 in the liver, kidneys, and heart were measured by LC-MS/MS using the homogenates as samples.

As controls, Fabry model mice without any treatment and ones treated with agalsidase beta only were used.

2.8. Statistical analysis

Data are expressed as means ± standard deviation (SD). Statistical analyses were performed using Student's *t*-test. Values were considered statistically significant at $P < 0.05$. JMP pro 13 was used as the statistical software.

3. Results

3.1. In vitro experiment

To examine the direct impact of HCQ/AMI on cell-free recombinant human α-Gal, HCQ and AMI were independently added to the α-Gal assay system including agalsidase beta (specific enzyme activity: 2.8 ± 0.1 mmol/h/mg protein [$n = 4$]) and the enzyme substrate, followed by α-Gal assaying. The results are shown in Fig. 1. When the concentration of HCQ/AMI in the mixture was low (0.025–0.1 mmol/L), only a moderate or no decrease in the α-Gal enzyme activity was observed. However, apparent inhibition of the enzyme activity by HCQ/AMI was observed when the concentration of these drugs was >0.5 mmol/L in the mixture, and the enzyme activity decreased to about 50% of that in the case without addition of these drugs to the mixture, when the concentration of these drugs was 10 mmol/L.

3.2. Cell experiments

3.2.1. Impact of HCQ/AMI on intracellular α-Gal activity in cultured HEK293 cells

To examine the impact of HCQ/AMI on native α-Gal in cultured HEK293 cells (specific α-Gal activity: 284 ± 16 nmol/h/mg protein), HCQ and AMI were independently added to the culture medium, and the cells were cultured for 24 h, followed by quantification of the intracellular α-Gal activity. The results are shown in Fig. 2. A dose-dependent decrease in the intracellular α-Gal activity was observed when the concentration of the drugs in the culture medium was 6.25–25 μmol/L (treated with HCQ/AMI vs. not treated with HCQ/AMI, $P < 0.05$). The enzyme activity decreased to 60–80% of that in the case without addition of HCQ/AMI to the culture medium, when the concentration of these drugs was 25 μmol/L.

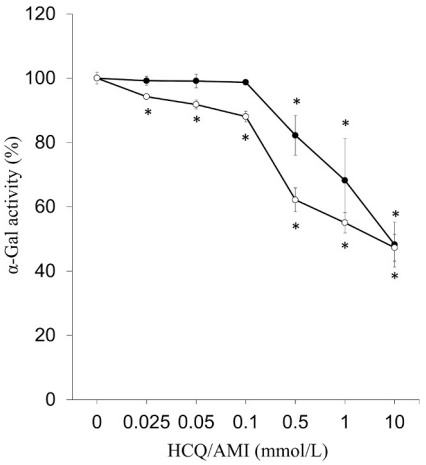

Fig. 1. Direct impact of HCQ/AMI on agalsidase beta.
The vertical axis represents the α-Gal activity, and the horizontal one the concentration of HCQ/AMI in the assay mixture. The α-Gal activities in the samples containing HCQ/AMI at the indicated concentrations were calculated as the percentages compared with that when water was added instead of HCQ/AMI. The black (HCQ) and white (AMI) circles exhibit the means and the bars standard deviations [$n = 4$]. *$P < 0.05$ (treated with HCQ/AMI vs. not treated with HCQ/AMI). α-Gal: α-galactosidase A, HCQ: hydroxychloroquine, AMI: amiodarone.

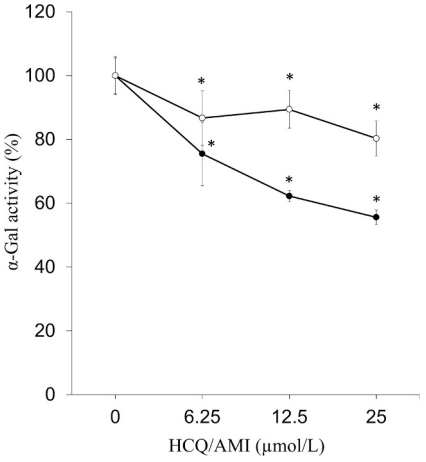

Fig. 2. Impact of HCQ/AMI on intracellular α-Gal activity in cultured HEK cells.
The vertical axis shows the α-Gal activity in the cultured HEK293 cells, and the horizontal one the concentration of HCQ/AMI in the cultured medium. The α-Gal activities in the samples with the indicated concentrations of HCQ/AMI were calculated as the percentages compared to that when the cells were cultured in the medium without HCQ/AMI. The black (HCQ) and white (AMI) circles are the means and the bars standard deviations [n = 4]. *P < 0.05 (treated with HCQ/AMI vs. not treated with HCQ/AMI). α-Gal: α-galactosidase A, HCQ: hydroxychloroquine, AMI: amiodarone.

3.2.2. Impact of HCQ/AMI on recombinant human α-Gal incorporated into F666 cells when the cells were cultured in the medium containing agalsidase beta and HCQ/AMI

To examine the impact of HCQ/AMI on recombinant human α-Gal incorporated into the cultured F666 cells (native α-Gal activity: < 1 nmol/h/mg protein), agalsidase beta and HCQ/AMI were added to the

culture medium, and the cells were cultured for 24 h, followed by enzyme assaying using the supernatants of cell homogenates as samples. The results are shown in Fig. 3. The α-Gal enzyme activity in the cells after the culture in the medium containing agalsidase beta but not HCQ/AMI was 2467 ± 124 nmol/h/mg protein [$n = 4$]. The enzyme activity decreased according to the concentration of HCQ/AMI in the culture medium, and it reached the level of 3–20% of that in the case without addition of HCQ/AMI to the culture medium, when the concentration of these drugs was 25 μmol/L.

3.3. Animal experiments

3.3.1. Impact of HCQ/AMI on α-Gal enzyme activity in organs and tissues of Fabry model mice that had received ERT with agalsidase beta

The impact of HCQ/AMI on α-Gal enzyme activity in organs and tissues of Fabry model mice (native enzyme activity in the liver, kidneys, and heart: < 1 nmol/h/mg protein) that had received ERT with agalsidase beta was examined. The results are shown in Fig. 4. The enzyme activity of agalsidase beta incorporated into the livers, kidneys, and hearts of Fabry model mice was not affected by the co-administration of HCQ/AMI (treated with HCQ/AMI vs. not treated with HCQ/AMI, $P \geq 0.05$).

3.3.2. Impact of HCQ/AMI on cleavage of Gb3 and Lyso-Gb3 accumulated in organs and tissues of Fabry model mice that had received ERT with agalsidase beta

The impact of HCQ/AMI on cleavage of Gb3 and Lyso-Gb3 accumulated in organs and tissues of Fabry model mice that had received ERT with agalsidase beta was examined. The results are shown in Fig. 5a and b. Apparent cleavage of Gb3 and Lyso-Gb3 accumulated in the organs and tissues was observed in the Fabry model mice that had received ERT with agalsidase beta (treated with agalsidase beta vs. not treated, $P < 0.05$), and there were no differences in the contents of Gb3 and Lyso-Gb3 in the liver, kidneys, and heart between the Fabry model mouse group co-injected with agalsidase beta and HCQ/AMI and that injected with agalsidase beta only ($P \geq 0.05$).

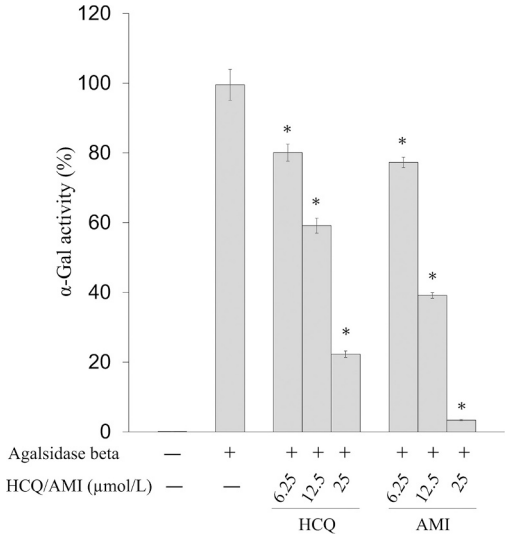

Fig. 3. Impact of HCQ/AMI on α-Gal activity of agalsidase beta incorporated into cultured F666 cells.
The vertical axis shows the α-Gal activity in the cultured F666 cells, and the horizontal one substrates added to the culture medium. The α-Gal activities in the samples under the culture conditions indicated were calculated as the percentages compared to that when the cells were cultured in the medium containing agalsidase beta only. The columns and bars show the means and standard deviations [n = 4], respectively. *P < 0.05 (treated with HCQ/AMI vs. not treated with HCQ/AMI). α-Gal: α-galactosidase A, HCQ: hydroxychloroquine, AMI: amiodarone.

4. Discussion

CADs are lysosomotropic agents, which have a hydrophilic amine head group and a hydrophobic tail consisting of an aromatic or aliphatic ring structure. It has been reported that they are trapped in lysosomes, where they interfere with negatively charged intralysosomal vesicles, the major platforms of cellular sphingolipid degradation [4]. They inhibit the activities of phospholipases, and bind to phospholipids, followed by the formation of a complex resistant to degradation, leading to phospholipidosis [2,23]. Furthermore, CADs incorporated into cells from the culture medium have various impacts on cell function, i.e., they inhibit the function of saposin B [24], disturb autophagy [25], increase the intralysosomal *pH* [26,27], impair mannose 6-phosphate receptor recycling, and inhibit lysosomal enzyme transport [28].

As to the impact of CADs on α-Gal, de Groot et al. reported that a reduction in intracellular α-Gal activity was observed when human skin fibroblasts were cultured in the presence of chloroquine [29], and Inagaki et al. also demonstrated specific reduction of α-Gal activity in transformed endothelial cells on chloroquine treatment [30].

On the basis of these reports, we previously administered 50 mg/kg of HCQ/AMI to wild-type and Fabry mice every day for 14 days, and then examined the effects of these drugs on the α-Gal activity and glycolipids accumulated in the organs and tissues [22]. The results revealed no reduction in α-Gal activity in the wild-type mice or acceleration of storage of Gb3/Lyso-Gb3 in the Fabry mice on treatment of HCQ/AMI, although accumulation of a large amount of phosphatidylcholine was observed in both the mouse groups. This suggests that HCQ/AMI do not have any significant impact on the catabolism of Gb3/Lyso-Gb3 in organs and tissues of both wild-type and Fabry mice.

In this study, we examined the impact of HCQ/AMI on agalsidase beta incorporated into Fabry mouse fibroblasts from the culture medium. The examination revealed that HCQ/AMI affected the enzyme activity of agalsidase beta incorporated into the cultured fibroblasts derived from a Fabry mouse, and the enzyme inhibition was observed under the condition of a much lower drug concentration compared with that in the *in vitro* experiment. This suggests that these lysosomotropic drugs, incorporated into the cultured cells, are possibly concentrated in the lysosomes and increase the *pH* value, leading to suppression of the enzyme activity, although their direct inhibitory effect on the enzyme is not strong. Furthermore, the results of cell experiments in this study revealed that the inhibitive effect is greater when the α-Gal-deficient cells were cultured in the medium containing agalsidase beta and HCQ/AMI than that in the case of HEK293 cells grown in the medium containing HCQ/AMI. As the main pathway for delivery of recombinant human α-Gal in cultured fibroblasts is endocytosis via the cation-independent mannose 6-phosphate receptor (CI-M6PR) and clathrin-associated pathway, and it is known that chloroquine acts as a clathrin inhibitor [31], HCQ/AMI may not only affect agalsidase beta incorporated into the lysosomes but also have an impact on its transportation from the medium to the lysosomes.

However, no inhibitory effect of HCQ/AMI on the catalytic activity of agalsidase beta, incorporated into the organs and tissues, or acceleration of Gb3/Lyso-Gb3 deposition was observed in the animal experiments involving Fabry model mice that had received ERT with 1 mg/kg agalsidase beta. It has been reported that administered HCQ/AMI are catabolized in the liver via various metabolic pathways and their catabolized metabolites are excreted in the urine and feces (Japan Pharmaceutical Interview Forms on HCQ sulfate [32] /AMI hydrochloride [33]). Thus, HCQ/AMI may not affect ERT for Fabry model mice, different from in the case of cultured cells in an environment isolated from the surroundings. Furthermore, it is known that various types of membrane receptors involved in endocytosis of α-Gal, such as mannose receptors, megalin, and sortilin [34–36] other than CI-M6PR, are expressed in cells comprising organs and tissues, although CI-M6PR is

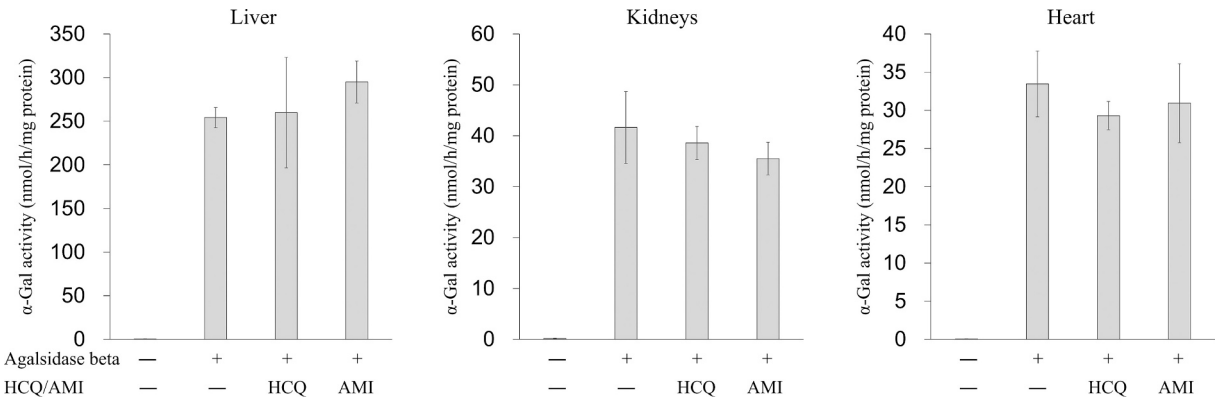

Fig. 4. Impact of HCQ/AMI on α-Gal activity in organs and tissues of Fabry mice that had received ERT with agalsidase beta.
The vertical axis shows α-Gal activity in the livers, kidneys, and hearts of Fabry mice that had received ERT with agalsidase beta, and the horizontal one the substrates injected into the mice. The α-Gal activities in the samples are presented as nmol of the artificial enzyme substrate cleaved/h/mg protein. The columns and bars show the means and standard deviations [n = 4], respectively. cf.) The α-Gal activities in the livers, kidneys, and hearts of wild-type mice were 26 ± 4, 17 ± 1, and 4 ± 0 nmol/h/mg protein [n = 4], respectively [22]. α-Gal: α-galactosidase A, HCQ: hydroxychloroquine, AMI: amiodarone, ERT: enzyme replacement therapy.

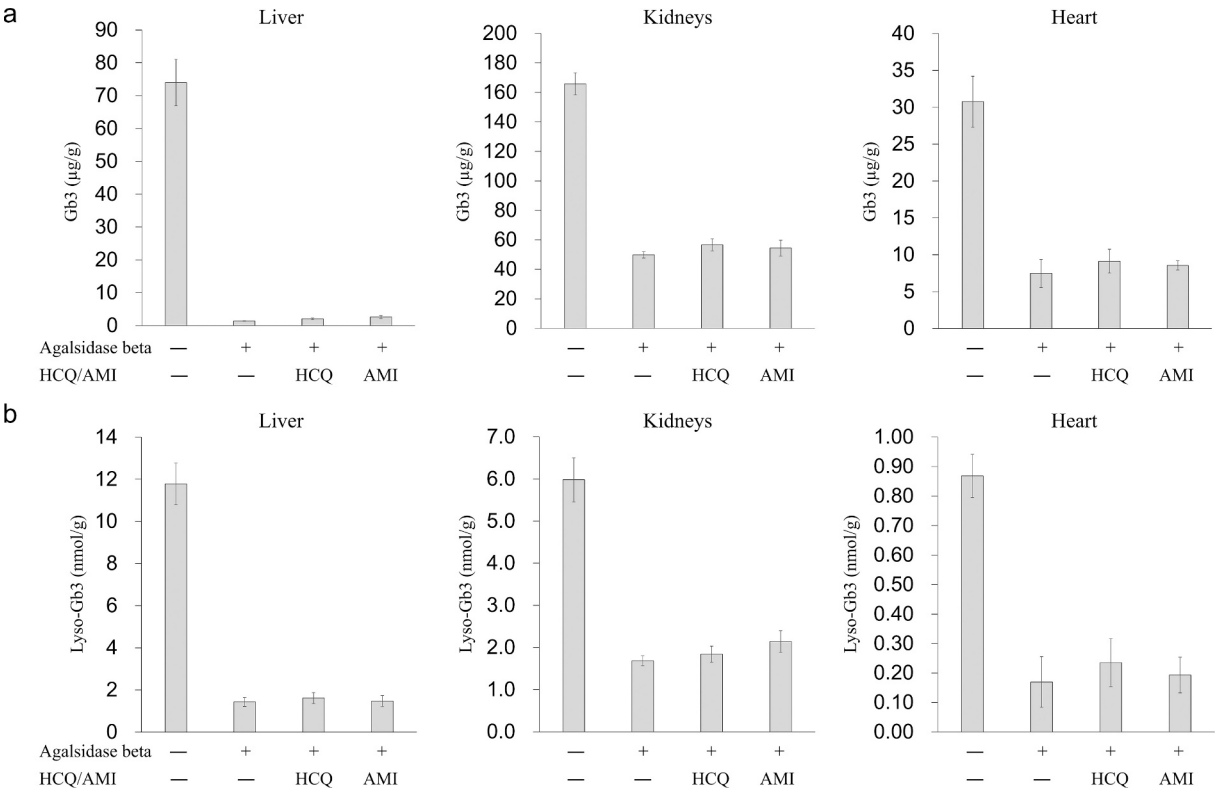

Fig. 5. Impact of HCQ/AMI on cleavage of Gb3 (a) and Lyso-Gb3 (b) in organs and tissues of Fabry mice that had received ERT with agalsidase beta.
The vertical axis shows the contents of Gb3 (a) and Lyso-Gb3 (b) in the livers, kidneys, and hearts of Fabry mice that had received ERT with agalsidase beta, and the horizontal one the substrates injected into the mice. The Gb3 and Lyso-Gb3 contents in the organs and tissues are presented as μg/g wet weight and nmol/g wet weight, respectively. The columns and bars show the means and standard deviations [n = 4], respectively. cf.) The Gb3 contents in the livers, kidneys, and hearts of wild-type mice were 1.0 ± 0.0, 15 ± 3, and 0.5 ± 0.0 μg/g [n = 4], respectively [22]. The Lyso-Gb3 contents in the livers, kidneys, and hearts of wild-type mice were 0.08 ± 0.01, 0.08 ± 0.01, and 0.09 ± 0.02 nmol/g [n = 4], respectively [22]. HCQ: hydroxychloroquine, AMI: amiodarone, Gb3: globotriaosylceramide, Lyso-Gb3: globotriaosylsphingosine, ERT: enzyme replacement therapy.

the predominant one in cultured fibroblasts [19]. Differences in the distribution of these receptors between cells comprising organs and tissues and cultured fibroblasts may also influence the sensitivity to HCQ/AMI.

This study has several limitations. The experimental target in this study was mice, not humans. For confirmation of the results obtained, it will be necessary to perform further experiments using clinical samples such as blood and urine from patients with Fabry disease treated with ERT and HCQ/AMI. The experiments were performed under fixed conditions, although the doses and method of administration of the drugs used were according to the papers previously reported [22,37,38].

5. Conclusion

We examined whether administration of HCQ/AMI affects the efficacy of ERT by cell and animal experiments. The results revealed that

administration of HCQ/AMI did not affect the catalytic activity of agalsidase beta toward Gb3/Lyso-Gb3 in the livers, kidneys, and hearts of Fabry model mice, although these drugs inhibited the enzyme activity of agalsidase beta incorporated into cultured cells.

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CRediT authorship contribution statement

Takahiro Tsukimura: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Koki Saito:** Investigation. **Tomoko Shiga:** Investigation. **Yasuhiro Ogawa:** Investigation. **Hitoshi Sakuraba:** Conceptualization, Writing – original draft, Writing – review & editing. **Tadayasu Togawa:** Conceptualization, Writing – review & editing.

Declaration of competing interest

We declare that none of the authors have any competing interests.

Data availability

The authors do not have permission to share data.

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