Hyaluronidase increases the biodistribution of acid α-1,4 glucosidase in the muscle of Pompe disease mice: An approach to enhance the efficacy of enzyme replacement therapy

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Abstract

Pompe disease (glycogen storage disease type II) is a glycogen storage disease caused by a deficiency of the lysosomal enzyme, acid maltase/acid α-1,4 glucosidase (GAA). Deficiency of the enzyme leads primarily to intra-lysosomal glycogen accumulation, primarily in cardiac and skeletal muscles, due to the inability of converting glycogen into glucose. Enzyme replacement therapy (ERT) has been applied to replace the deficient enzyme and to restore the lost function. However, enhancing the enzyme activity to the muscle following ERT is relatively insufficient. In order to enhance GAA activity into the muscle in Pompe disease, efficacy of hyaluronidase (hyase) was examined in the heart, quadriceps, diaphragm, kidney, and brain of mouse model of Pompe disease. Administration of hyase 3000 U/mouse (intravenous) i.v. or i.p. (intraperitoneal) and 10 min later recombinant human GAA (rhGAA) 20 mg/kg i.v. showed more GAA activity in hyase i.p. injected mice compared to those mice injected with hyase via i.v. Injection of low dose of hyase (3000 U/mouse) or high dose of hyase (10,000 U/mouse) i.p. and 20 min or 60 min later 20 mg/kg rhGAA i.v. increased GAA activity into the heart, diaphragm, kidney, and quadriceps compared to hyase untreated mice. These studies suggest that hyase enhances penetration of enzyme into the tissues including muscle during ERT and therefore hyase pretreatment may be important in treating Pompe disease.

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Pompe disease (glycogen storage disease type II) is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme, acid maltase/acid α-glucosidase (GAA). The enzyme, GAA, hydrolyzes lysosomal glycogen to glucose at the α-1–4 and 1–6 linkages. Deficiency of the enzyme leads to the intra-lysosomal accumulation of glycogen primarily in cardiac and skeletal muscles [1]. Pompe disease is the only glycogen storage disease which is associated with lysosomal storage of glycogen. Deficiency of the enzyme causes cardiomyopathy, respiratory failure, hypotonia, and weakness in infants and is usually associated with death within the first two years of life [2]. Clinical symptoms seen in infantile-onset of the disease include progressive muscle weakness, hypotonia, motor deficits, difficulty in feeding, progressive respiratory failure, growth retardation, progressive cardiomyopathy, and cardiomegaly. In late onset disease, symptoms include proximal muscle weakness, gait...
disturbances, lordosis/scoliosis, hypotonia, low back pain, respiratory difficulties, sleep apnea, and exertional dyspnea [1]. Slowly progressive myopathy and diaphragmatic weakness are observed in the less severe form of the disease [3].

Although enzyme replacement therapy (ERT) has been approved in humans to replace the deficient enzyme activity in Pompe disease, efficacy of the injected enzyme may not be sufficient to clear the skeletal muscle glycogen. Intravenous injection of nonphosphorylated human placenta acid α-glucosidase was less effective for heart and skeletal muscle [4]. This is likely due to the resistant nature of muscle fibers to the therapy [5,6]. Animal [7–10] and human [11–14] studies suggest that recovering the lost function caused by the enzyme defect in Pompe disease is more difficult than anticipated. Hence, it is important to find strategies that enhance the penetration of the enzyme into the muscle during ERT in Pompe disease.

Mammalian hyaluronidases (hyase) (EC 3.2.1.35) are members of a group of glycosidase family 56 [15,16]. Hyaluronidases are present in bacteria [17], fungi [18], and vertebrates [16]. Streptococcus hyaluronidase was termed “spreading factor” because it enhances the spread of bacteria. Hyaluronidase increases tissue permeability and promotes the spread or dispersion of drugs and therefore hyase is used to increase the spread of drug in swellings and ecchymosis. Currently hyase is used in eye surgery. Hyase has also been shown to increase gene transfer efficiency in skeletal muscle [19]. However, the ability of hyase to improve delivery and targeting of recombinant enzyme to various tissues is not known. Therefore, the efficacy of hyase during ERT was examined in the heart, quadriceps, diaphragm, kidney, and brain of a mouse model of Pompe disease.

Materials and methods

**Immunofluorescence study in the mouse tissue.** Fluorescein isothiocyanate-Dextran (FITC-Dextran) (molecular weight 40 kDa) was injected i.v. to wild type mouse 60 min after 10,000 U hyase injection i.p. Twenty-four-hours later, heart, liver, and diaphragm were cryosectioned and fluorescence was determined under fluorescence microscope (Olympus) with digital microscope camera (Olympus), and epifluorescence illuminator with a filter set optimized for FITC. Control mice were treated with saline injection only.

In a second set of wild type mice, 500 µl green fluorescent protein (GFP) was injected i.v. 60 min after 10,000 U of hyase injected i.p. Twenty-four-hours later, heart, liver, and diaphragm were cryosectioned and fluorescence was determined under fluorescence microscope (Olympus) with epifluorescence and the G3 filter set, and photographed using Leica digital microscope camera.

**Pompe disease model mouse.** Pompe mice were obtained from Dr. Nina Raben [20].

**Injection of hyase and GAA.** To examine efficacy of hyase, mice were divided into 9 groups. Human GAA was prepared by Genzyme Corporation [21]. Hyaluronidase (Hyase) was bought from Sigma. Group included: (i) mice were untreated and used as control, (ii) mice receiving only hyase, (iii) mice receiving only GAA 20 mg/kg, (iv) mice that were injected with a single dose of hyase (3000 U/mouse) either i.v. or i.p. and 10 min later they were injected with GAA 20 mg/kg i.v.; (v) mice receiving only GAA (20 mg/kg) i.v.; (vi) mice receiving i.p. injection of 3000 U of hyase/mouse and 20 mL GAA 20 mg/kg i.v.; (vii) mice receiving high dose of hyase (100,000 U/mouse) i.p. and 20 mL later 20 mg/kg GAA i.v.; (viii) mice receiving hyase 3000 U/mouse i.p. and 60 mL later i.v. injection of 20 mg/kg GAA; (ix) mice received hyase 10,000 U/mouse i.p. and 60 mL later mice were given 20 mg/kg GAA i.v. All these mice were sacrificed 24 h after treatment and tissues were collected for the following experiments.

**GAA activity assay.** Tissues were homogenized in a buffer (0.2 M sodium acetate, 0.4 M KCl, pH 4.3, and 0.5% Triton X-100), centrifuged at 14,000 g for 10 min, and supernatant was used to determine GAA activity. Enzyme activity was measured using 0.5 mM 4-methylumbelliferyl-α-glucopyranoside (MUG) substrate as followed earlier [22]. The activity was expressed as nmol/mg/h. GAA activity in the brain fell out of the standard range, so fluorescent absorbance was directly used as arbitrary units as followed earlier [23].

Data were analyzed using ANOVA. A value of p < 0.05 was considered as significant.

**Results and discussion**

Injection of FITC-Dextran 40,000 kDa spread well in heart, diaphragm, and liver even 60 min after hyase injection (Fig. 1A). Injection of GFP i.v. showed penetration and spread into the brain after hyase injection (Fig. 1B).

Recombinant enzyme injection following hyase treatment i.p. significantly enhanced the enzyme activity in heart, quadriceps, and diaphragm compared to their respective untreated control (Fig. 2). The p value was 0.001. Enzyme treatment following i.v. injection of hyase also significantly enhanced the enzyme activity in the heart, quadriceps, triceps, and diaphragm compared to their respective controls (Fig. 2). It is interesting to note that hyase i.p. injection prior to rhGAA injection was more efficient to enhance the enzyme activity during ERT compared to intravenous injection of hyase (Fig. 2). Therefore i.p. administration of hyase was performed in the following experiments in order to investigate time and dose dependent effect of hyase.

In heart, high dose of hyase treatment prior to the recombinant enzyme injection significantly enhanced the enzyme activity. The level of the enzyme activity was more at 20 min hyase treatment interval compared to 60 min interval (Fig. 3). All the mice treated with hyase and rhGAA showed significant enhancement in GAA activity in the heart compared to untreated control (p < 0.001) or hyase only treated mice (p < 0.001).

In quadriceps, hyase injection of both doses significantly enhanced the enzyme activity compared to the untreated control or with the hyase only treated mice. Enzyme treatment showed more activity at 60 min interval of low dose of hyase injection compared to 20 min interval (Fig. 3). Although treatment with a high dose of hyase treatment enhanced the enzyme activity, the level was almost similar at 20 min interval compared to 60 min interval (Fig. 3). Mice injected with hyase and rhGAA showed enhancement in GAA activity in the quadriceps compared to untreated control or hyase only treated mice.

In diaphragm, low dose of hyase injection enhanced the enzyme activity in mice treated with rhGAA 60 min interval following hyase compared to 20 min interval (Fig. 3). Although high dose of hyase treatment enhanced the enzyme activity, the level was higher at 60 min interval.
compared to 20 min interval (Fig. 3). All the mice treated with hyase and rhGAA showed enhancement of GAA activity in the diaphragm compared to the untreated control or hyase only treated mice.

In kidney, injection of rhGAA after treatment with either dose of hyase for 20 or 60 min significantly enhanced the enzyme activity compared to the hyase only treated mice. Significant enhancement of GAA activity was observed in the brain after hyase injection of either doses at both time periods and is shown in Fig. 4.

During postnatal period GAA activity increases dramatically in heart and the lysosomal degradation of glycogen to glucose provides energy to meet the metabolic requirements when there is a demand for large amount of glucose.
Fig. 4. Dose and time dependent effect of hyase in the brain of Pompe disease mouse. Hyase injection increased the enzyme activity compared to hyase uninjected mice. Injection of hyase 10,000 U/mouse by i.p. and after 20 min injection 20 mg/kg rhGAA i.v. increased GAA activity compared to other time and doses applied. The enzyme activity is expressed as absorbance/mg/h.

[6]. When ERT was done to recover symptoms seen in Pompe disease [23–34], the injected enzyme reaching into the muscle is not sufficient. Hyase increases efficacy of gene transfer [19]. Whether hyase enhances enzyme delivery during ERT is not known. Therefore efficacy of hyase during ERT was studied.

Penetration and spread of FITC–Dextran into the heart, liver, and diaphragm and GFP into the brain after hyase treatment observed in the present study suggests that hyase can be used as a potential agent to enhance protein delivery into various tissues including muscle during ERT.

It is interesting to note that injection of hyase i.p. is more efficient in ERT compared to i.v. injection in the mouse with Pompe disease. This is likely due to delivery of hyase by i.p. being absorbed by the intestine spreads well compared to i.v. route.

Injection of low dose of hyase (3000 U/mouse) i.p. and rhGAA increased enzyme activity in the organs including muscle, diaphragm, heart, and brain compared to hyase untreated animals. High dose of hyase (10,000 U/mouse) i.p. injection also showed enhancement of GAA activity compared to hyase untreated mice suggesting that hyase is an important agent which facilitates entry of the enzyme into various tissues including muscle.

In conclusion, pretreatment with hyase during enzyme therapy enhanced delivery of the injected enzyme into the tissues of Pompe mice including muscle. Therefore hyase pretreatment is important in treating Pompe disease.

References


