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Novel Isozyme-Specific Quantitation Method for Alpha-Amylases in Human Plasma Revealed Possible AMY2B Induction by Alpha-Amylase Inhibitor, CS-1036

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Abstract

In a clinical trial of an α-amylase inhibitor (CS-1036), which was developed for the treatment of patients with type 2 diabetes, the half-life of plasma CS-1036 concentration was prolonged with the increase of the dose level. The prolonged half-life was assumed to be associated with alternation in the plasma level of any one of the α-amylase isozymes from the treatment. Human α-amylase is classified into 3 isozymes encoded by AMY1A, AMY2A, and AMY2B. Due to high sequence homology between α-amylase isozymes and the low plasma level, it is extremely challenging to quantify individual isozymes. A mass spectrometry-based approach, Multiple Reaction Monitoring (MRM) using the Absolute Quantification (AQUA) strategy, has been applied to quantification of target proteins, and this approach provides high selectivity and specificity. Here we report a novel quantitation method of α-amylase isozymes, which is a combination of purification of α-amylase from plasma by starch affinity adsorption and LC-MRM-MS. This method was applied to the plasma samples from the clinical trial of CS-1036. As a result, only AMY2B showed a statistically significant increase in a time-dependent manner. This result suggested that AMY2B might be related to the prolonged half-life of CS-1036.

Keywords: Amylase; Amylase inhibitor; Diabetes; CS-1036; Mass spectrometry; MRM; Targeted proteomics; Plasma; Clinical sample; Quantitation

Introduction

Alpha-amylase hydrolyzes internal α-1, 4 glycoside bonds in oligosaccharides and polysaccharides, such as starch and glycogen. Human α-amylases are mainly classified into 2 types, salivary and pancreatic amylases. Salivary amylase is encoded by AMY1A and pancreatic amylases are encoded by AMY2A and AMY2B. These amylases are very similar with about a 97% homology overall [1]. Alpha-amylase is responsible for the digestion of carbohydrates and glucose absorption in the intestine and plays a key role in the management of postprandial hyperglycemia that is closely associated with type 2 diabetes. Hence, α-amylase is considered to be a therapeutic target to decrease postprandial hyperglycemia in type 2 diabetes patients [2-4].

(2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidin-3-yl-4-O-(6-deoxy-β-d-glucopyranosyl)-α-d-glucopyranoside (CS-1036), an α-amylase inhibitor, was developed for the treatment of patients with type 2 diabetes [5]. In single and multiple ascending dose (SAD and MAD) studies of CS-1036 in healthy male subjects, CS-1036 half-lives in MAD were more prolonged than those in SAD. Moreover, the half-lives on Day 8 at higher doses were more than 2-times longer than those at 20 mg per dose. Experiments using rats indicated that CS-1036 dose not bind albumin but binds predominantly to α-amylase in plasma [6]. Based on these results, we hypothesized that following the MAD of CS-1036, especially at higher doses, plasma α-amylase is induced and increased and the plasma protein binding ratio for CS-1036 increases in accordance with the plasma α-amylase level. Consequently, the change of CS-1036 binding to α-amylase was assumed to prolong the half-life of CS-1036. To confirm the hypothesis that one or more α-amylase isozymes were induced by CS-1036 administration, isozyme specific quantitation of plasma amylases was necessary.

Alpha-amylase activity in serum, plasma, and urine is applied to the diagnosis of pancreatic disorders, diabetes, parotitis, and so forth. In general, in clinical practice total amylase activity is measured for diagnosis of the diseases. To identify a failed organ, it is necessary to measure each individual amylase. Several methods are described for the separate detection of salivary and pancreatic amylases based on electrophoresis and assays using selective inhibitors or substrates and anti-salivary amylase antibodies [7-13]. However, these methods are unsupported as being able to determine AMY2A and AMY2B separately.

Omichi and Hase reported the detection of AMY2A and AMY2B separately in urine [14,15]. They introduced two methods. In the first, AMY1A was removed from urine by using an anti-salivary amylase antibody. Then AMY2A and AMY2B were separately detected using a fluorogenic substrate [14]. In the second, a mixture of AMY1A, AMY2A, and AMY2B was purified from urine by affinity adsorption to starch, and then α-amylases were separated by ion-exchange chromatography [15]. However, because these methods use antibodies or ion-exchange chromatography to separate AMY2A and AMY2B, it would be highly challenging to have good separation for highly homologous proteins

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such as α-amylases (more than 95% identity between AMY2A and AMY2B).

Recently, a mass spectrometry-based approach using Multiple Reaction Monitoring (MRM) with a strategy of Absolute Quantification (AQUA), has been applied to quantitation of a variety of target proteins [16]. This approach provides high selectivity and specificity; therefore it could be applicable to the quantification of highly homologous isozymes [17]. However, due to the complexity of the plasma proteome and the limited dynamic range of mass spectrometry, it is generally necessary to enrich targeted proteins from plasma for detection of those proteins below the microgram per milliliter concentration in plasma [18]. The depletion of highly abundant proteins in plasma and the immunoaffinity purification of targeted proteins are effective and commonly used as pre-treatment methods [19,20]. Since α-amylase concentration in human plasma has been estimated as being below one microgram per milliliter (data not shown), α-amylase enrichment was required by some means.

Here we developed an efficient and high-throughput method for isozyme specific quantitation of α-amylase in plasma. This method consists of 2 steps; first, purification of α-amylase from plasma by starch affinity adsorption; second, quantification by LC-MRM-MS using an AQUA strategy. We then applied the method to quantify the individual α-amylase isozymes of plasma samples from the MAD clinical study of CS-1036 and verified our hypothesis that one or more α-amylase isozymes was induced by CS-1036 administration.

Experimental Procedures

Plasma samples

Plasma samples were obtained from a clinical trial titled, "A randomized, double-blind, placebo-controlled multiple ascending dose study of CS-1036 with open-labeled acarbose to assess the safety and tolerability in healthy Chinese male subjects" (MAD study of CS-1036). The study was approved by the ethics committee/institutional review board, and was conducted in compliance with ethical principles that originated in the Declaration of Helsinki and are in accordance with the International Conference on Harmonization Guidelines for good clinical practice and other applicable local regulations. Briefly, blood samples for analysis were collected into 4-mL heparin sodium blood collection tubes. Plasma was separated via centrifugation (1,800g for 10 min, 4°C) within 30 min of blood collection. The plasma samples at pre-dose, 4 h after administration on Day 1 and 8 h after administration on Day 8 (Day 1-pre, Day 1-4 h, and Day 8-8 h) at doses of 20 mg and 80 mg in this study (n=6 in each group) were analyzed. Plasma concentrations of CS-1036, several pharmacokinetic parameters of CS-1036, and total α-amylase activity were previously evaluated. Pooled plasma from healthy donors (human heparin sodium plasma pool of donors, batch number: PLA008082-100ML, BIOPREDIC, Rennes, France) was used for assay development and external quality control reference.

Purification of α-amylase by starch affinity adsorption method

Plasma was subjected to α-amylase purification in a 96-well format. Each well of the inner 60 wells of the 96-well filter plate (Multiscreen Filter Plate, catalog number: MSHVN4510, Billerica, MA, USA) contained 50 mg potato starch (catalog number: 37326-01, Kanto Chemical, Tokyo, Japan). Purification and wash steps were accomplished either by low-speed centrifugation or a vacuum system. Initially the wells were washed three times with 20 mM Tris-HCl, pH 7.0 containing 150 mM NaCl (TBS). Then, 100 µL of plasma was diluted 2-fold with TBS and applied to the wells. After incubation of the plasma samples for 10 min at room temperature, the wells were washed three times with TBS.

A new collection plate was placed under the filter plate and α-amylase bound to the potato starch was eluted with 100 µL of elution buffer (8 M urea, 50 mM Tris pH 8.0, 10 mM EDTA, 0.005% dodecyl maltoside (DM; Nacalai Tesque, Kyoto, Japan)).

Sample preparation for LC-MRM-MS analysis

In solution protein digestion for mass spectrometry was conducted as described in our previous studies [21-23]. Briefly, proteins in the eluate were reduced by 100 mM dithiothreitol (DTT; Wako, Osaka, Tokyo) in 100 mM ammonium bicarbonate (AB; SIGMA, St. Louis, MO, USA) for 20 min at 37°C, and then alkylated by 200 mM iodoacetoamide (IAA; Wako, Osaka, Japan) in 100 mM AB for 20 min at room temperature in the dark. After the addition of 680 µL of 0.005% DM in 50 mM Tris pH 8.0 to dilute urea, 1 µg of sequence grade modified trypsin (Promega, Madison, WI, USA) was added to the reaction mixtures. Proteins were digested overnight at 37°C.

Peptide standards, the [$^{13}C_6$/^{15}N] leucine-labeled forms of proteotypic peptides corresponding to the AMY1A, AMY2A and AMY2B (Supplementary Table 1), were synthesized by Scrum (Tokyo, Japan) and stored at -20°C until use. Peptide standards (50 fmol of AMY1A and 2A, and 10 fmol of AMY2B) were spiked to the digested peptides, followed by adding 80 µL of 50% formic acid (FA; Wako, Osaka, Japan) in water to stop digestion. The digested peptides were desalted by a modified C18 StageTips protocol as described previously [24]. Desalted peptides were dried with a centrifuge evaporator and dissolved with 16 µL of 5% FA.

LC-MRM-MS analysis

All MRM experiments were performed using a triple quadruple mass spectrometer (TSQ Vantage, Thermo Fisher Scientific) coupled with a nano-HPLC system (UltiMate 3000 RSLCnano system, Dionex) via a nano-ESI source interface. A nanospray capillary needle packed with BEH C18 beads (1.7 µm particle size, Waters) in-house was used as an analytical column. The nano-HPLC system had a dual-gradient pump (NC pump and Loading pump). Solvent A (0.1% FA, 5% CH_3CN) and solvent B (0.1% FA, 95% CH_3CN) were used in the NC pump, and solvent C (0.1% trifluoroacetic acid (TFA) in water) and solvent D (0.1% TFA in CH_3CN) were used in the Loading pump. Four microliters of the samples were loaded into the column with 20% Solvent D at 1 µL/min for 21 min using the Loading pump, and were then separated with the gradient of 20% to 45% Solvent B for 19 min at 300 nL/min. MRM operating conditions were as follows: an ion source temperature was set to 200°C, and the source voltage was set to 2000 V. A scan width of 1.000 m/z and a scan time of 50 ms were used. Both Q1 and Q3 were set to 0.7 FWHM. Two MRM transitions per peptide were monitored. The collision energy and S-Lens value for each transition were optimized using the isotope-labeled peptide standards (Supplemental Table 1). The MS RAW data have been submitted to a mass spectrometry data repository, jPOSTrepo (https://repository.jpostdb.org) with the data set identifier JPST000241 [25]. Data analysis was performed using an Xcalibur Quan Browser (Thermo Fisher Scientific). Peak integration and detection were performed using the Genesis algorithm with automated determinations using the following parameters: a signal-to-noise threshold of 0.5 and minimum peak height of 3.0. The peak area

ratio of the endogenous α-amylase peptide to spiked AQUA peptide was used to calculate the measured concentration.

Samples for the standard curve were prepared by serial dilution of the isotope-labeled peptide standards in 50 μg/mL Bovine Serum Albumin (BSA; SIGMA, St Louis, MO, USA) solution. Between 100 amol and 1 pmol of each peptide was loaded onto the column for LC-MRM-MS analysis (n=5).

Statistical analysis

Statistical analysis was performed using SAS System software (Release 8.2, SAS Institute Inc.). The change of each α-amylase isozyme concentration in plasma from Day 1-pre (%) to Day 1-4 h or Day 8-8 h was calculated. The ratios of change at each dose were compared between the time points using a Tukey's test for equal variances.

Results and Discussion

Development LC-MRM-MS analysis for α-amylase isozymes

Commercially available α-amylases purified from human saliva and pancreas was analyzed to find each proteotypic peptide of α-amylase isozymes for LC-MRM-MS analysis. Tryptic digests of the α-amylases were analyzed using LC-MS/MS (LTQ Orbitrap) and proteotypic peptides were identified. Since the sequence homology between α-amylase isozymes was extremely high (>95%) (Table 1), we observed the only one set of representative proteotypic peptides corresponding to residues 177 to 187 fulfilling the following criteria: 1) High signal intensities; 2) No post-translational modifications listed in the Swiss-Prot database; 3) No cysteine and methionine residues, though it is highly recommended to utilize at least 3 proteotypic peptides.

Therefore, the residues 177-187 of the α-amylase isozymes were chosen as AQUA peptides of the isotopically labeled synthetic peptides by incorporating 13C/15N in one leucine residue (Figure 1). Then an LC-MRM-MS assay was developed using the synthetic peptide standards. Two transitions per peptides were selected based on intensity and stability of MS data of the synthetic peptides (Supplemental Table 1). The LC-MRM-MS assay of each AQUA peptide showed good linearity in the range of 100 amol to 1 pmol (Figure 2).

Purification of α-amylases from plasma by starch affinity adsorption

We attempted to detect α-amylase isozymes directly in human plasma using the MRM approach, but it did not work out as hoped. Therefore, we developed a method to enrich α-amylase from human plasma and established a protocol using potato starch affinity based on Deutsch et al's study [26]. Several conditions of the sample preparation for LC-MRM-MS analysis were optimized. The amount of trypsin for the digestion of proteins in solution was confirmed to be sufficient. AQUA peptides were spiked prior to the desalting step to correct recovery rates during and after desalting step. To assess inter-day and inter-plate reproducibility of purification, the same human normal plasma was purified by starch affinity adsorption on each day, separately, and two plates (random 6 wells per plate) were prepared at one time (Figure 3). Subsequently, recovery of each isozyme was estimated. Plasma of healthy donors depleted of endogenous α-amylases by the starch adsorption method was prepared. Purified commercial available α-amylases (the concentrations were unknown) in a 5-point dilution series were spiked into the α-amylase depleted plasma, and subjected to purification, digestion by trypsin, and LC-MRM/MS. Average spike recoveries (%) and Pearson's correlation coefficient R^2 of AMY1A, AMY2A and AMY2B were 88.6% and 0.9967, 101.3% and 0.9996, and 90.4% and 0.9983, respectively (Figure 4). Thus, quantification of α-amylase isozymes in human plasma by this method showed good linearity as spiked-in α-amylase increased across a wide concentration range. These results indicated that this novel method enabling the distinctive quantification of α-amylase isozymes with high specificity was successfully developed for the first time.

Quantitation of the α-amylase isozymes in CS-1036 clinical samples

Our method was next applied to clinical plasma samples derived from the MAD study of CS-1036. Alpha-amylase isozymes were measured at pre-dose (Day 1-pre), 4 h after administration on Day 1 (Day 1-4 h) and 8 h after administration on Day 8 (Day 8-8 h) at doses of 20 mg and 80 mg (n=6 in each group). Each sample was prepared in triplicates. The samples that didn't meet the following criteria were re-measured: 1) Peak intensities of both standard and endogenous peptides were more than 10^5; 2) A coefficient of variation (CV (%)) of triplicate technical replicates was less than 30. Figures 5A, 6A and 7 shows the measured concentrations of AMY1A, AMY2A, and AMY2B, respectively. It was revealed that AMY1A was the most abundant isozyme in human plasma. We performed a correlation analysis between measured concentrations of each α-amylase isozyme and total α-amylase biological activity in plasma using data of Day 1-pre. Total α-amylase biological activity was highly correlated with the concentration of AMY1A (R^2=0.7069) On the other hand, the total α-amylase activity was not correlated with the concentrations of AMY2A (R^2=0.0102) and AMY2B (R^2=0.0006) (Figure 8A-8C). This was consistent with the finding that AMY1A was the most abundant α-amylase. Changes from the concentration on Day 1-pre to Day 1-4 h or Day 8-8 h were calculated (Figures 5B, 6B and 7B, respectively). As compared with Day 1-pre, AMY1A and AMY2A did not show statistically significant increases, and only AMY2B showed a statistically significant increase in a time-dependent manner.

	AMY1A NP_004029.2	AMY1B NP_001008219.1	AMY1C NP_001008220.1	AMY2A NP_000690.1	AMY2B NP_066188.1
AMY1A* NP_004029.2**	-	100	100	96.9	97.5
AMY1B NP_001008219.1	100	-	100	96.9	97.5
AMY1C NP_001008220.1	100	100	-	96.9	97.5
AMY2A NP_000690.1	96.9	96.9	96.9	-	98.8
AMY2B NP_066188.1	97.5	97.5	97.5	98.8	-

Table 1: Comparison of amino acid sequences of human a-amylase from salivary glands and pancreas by BLASTP 2.5.1+. *: Gene symbol, **: RefSeq protein ID.

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CLUSTAL O(1.2.3) multiple sequence alignment

AMY1A       MKLFWLLFTIGFCWAQYSSNTQQGRTSIVHLFEWRWVDIALECERYLAPKGFGGVQVSPP
AMY2A       MKFFLLLFTIGFCWAQYSPNTQQGRTSIVHLFEWRWVDIALECERYLAPKGFGGVQVSPP
AMY2B       MKFFLLLFTIGFCWAQYSPNTQQGRTSIVHLFEWRWVDIALECERYLAPKGFGGVQVSPP
            **:* ************ ************************************************

AMY1A       NENVAIHNPFRPWWERYQPVSYKLCTRSGNEDEFRNMVTRCNNVGVRIYVDAVINHMCGN
AMY2A       NENVAIYNPFRPWWERYQPVSYKLCTRSGNEDEFRNMVTRCNNVGVRIYVDAVINHMCGN
AMY2B       NENVAIHNPFRPWWERYQPVSYKLCTRSGNEDEFRNMVTRCNNVGVRIYVDAVINHMSGN
            ******:*************************************************.**

AMY1A       AVSAGTSSTCGSYFNPGSRDFPAVPYSGWDFNDGKCKTGSGDIENYNDATQVRDCRLSGL
AMY2A       AVSAGTSSTCGSYFNPGSRDFPAVPYSGWDFNDGKCKTGSGDIENYNDATQVRDCRLTGL
AMY2B       AVSAGTSSTCGSYFNPGSRDFPAVPYSGWDFNDGKCKTGSGDIENYNDATQVRDCRLVGL
            ************************************************************ **

AMY1A       LDLALGKDYVRSKIAEYMNHLIDIGVAGFRIDASKHMWPGDIKAILDKLHNLNSNWFPEG
AMY2A       LDLALEKDYVRSKIAEYMNHLIDIGVAGFRLDASKHMWPGDIKAILDKLHNLNSNWFPAG
AMY2B       LDLALEKDYVRSKIAEYMNHLIDIGVAGFRLDASKHMWPGDIKAILDKLHNLNSNWFPAG
            ***** ***********************:************************** *

AMY1A       SKPFIYQEVIDLGGEPIKSSDYFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWG
AMY2A       SKPFIYQEVIDLGGEPIKSSDYFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWG
AMY2B       SKPFIYQEVIDLGGEPIKSSDYFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWG
            ************************************************************

AMY1A       FMPSDRALVFVDNHDNQRGHGAGGASILTFWDARLYKMAVGFMLAHPYGFTRVMSSYRWP
AMY2A       FVPSDRALVFVDNHDNQRGHGAGGASILTFWDARLYKMAVGFMLAHPYGFTRVMSSYRWP
AMY2B       FMPSDRALVFVDNHDNQRGHGAGGASILTFWDARLYKMAVGFMLAHPYGFTRVMSSYRWP
            *:************************************************************

AMY1A       RYFENGKDVNDWVGPPNDNGVTKEVTINPDTTCGNDWVCEHRWRQIRNMVNFRNVVDGQP
AMY2A       RQFQNGNDVNDWVGPPNNNGVIKEVTINPDTTCGNDWVCEHRWRQIRNMVIFRNVVDGQP
AMY2B       RQFQNGNDVNDWVGPPNNNGVIKEVTINPDTTCGNDWVCEHRWRQIRNMVNFRNVVDGQP
            * *:**:**********:*** *************************** *********

AMY1A       FTNWYDNGSNQVAFGRGNRGFIVFNNDDWTFSLTLQTGLPAGTYCDVISGDKINGNCTGI
AMY2A       FTNWYDNGSNQVAFGRGNRGFIVFNNDDWSFSLTLQTGLPAGTYCDVISGDKINGNCTGI
AMY2B       FTNWYDNGSNQVAFGRGNRGFIVFNNDDWTFSLTLQTGLPAGTYCDVISGDKINGNCTGI
            ****************************:*******************************

AMY1A       KIYVSDDGKAHFSISNSAEDPFIAIHAESKL
AMY2A       KIYVSDDGKAHFSISNSAEDPFIAIHAESKL
AMY2B       KIYVSDDGKAHFSISNSAEDPFIAIHAESKL
            ********************************
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Figure 1: Comparison of amino acid sequences of human AMY1A (NP_004029.2), AMY2A (NP_000690.1) and AMY2B (NP_066188.1). Selected proteotypic peptide sequences are underlined.

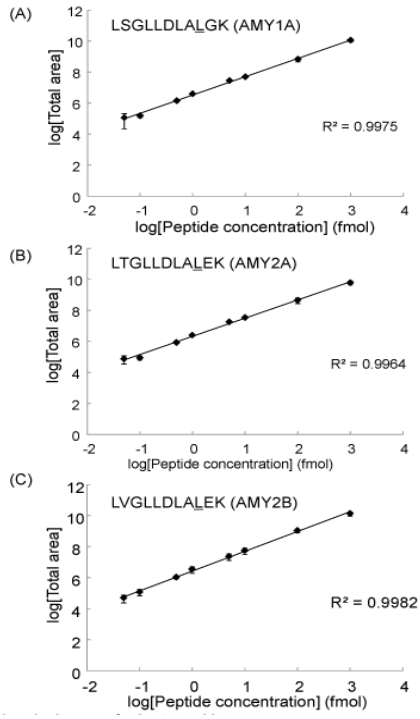

Figure 2: Standard curve of AQUA peptides. AQUA peptides of AMY1A (A), AMY2A (B), and AMY2B (C) spiked into 50 μg/mL BSA solution were analyzed in 5 replicates by LC-MRM. Error bars indicate standard deviation. Letters under bar refer to stable isotope-labeled amino acids.

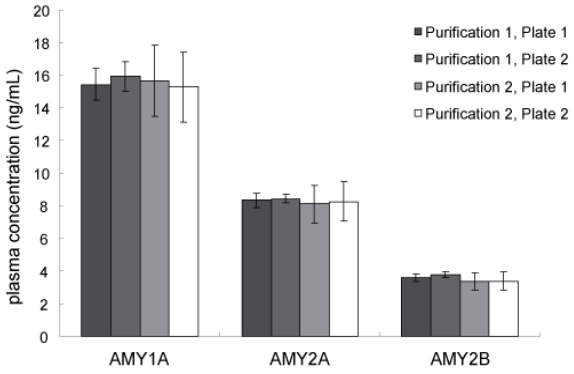

Figure 3: Inter-day and inter-plate reproducibility. Purification of the same plasma by starch affinity adsorption on two different plates (Plate 1 and 2, n=10), was repeated on different days (Purification 1 and 2). Error bars indicate standard deviation.

Conclusion

In conclusion, we developed a novel quantitation method that can distinguish α-amylase isozymes by using affinity purification of α-amylase from plasma coupled with MRM-MS, and applied the method to the plasma samples from the clinical trial of an α-amylase inhibitor, CS-1036. The plasma AMY2B level was statistically increased by the treatment of CS-1036, and affected the plasma protein binding of CS-1036. This change was assumed to be related to the prolonged half-life of CS-1036 in human plasma at higher doses.

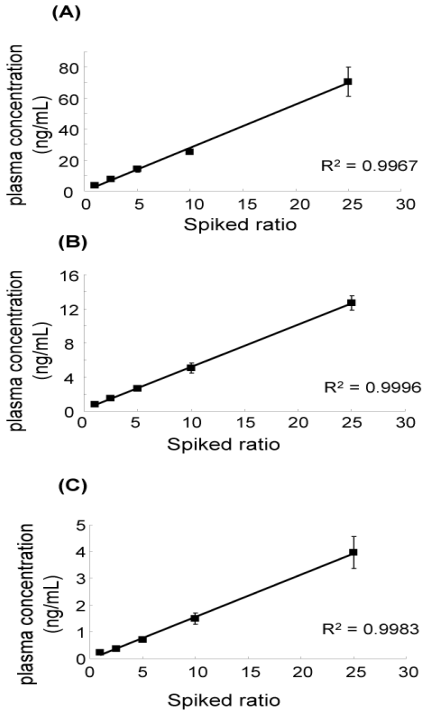

Figure 4: Dilution curve of α-amylase isozymes spiked into plasma. AMY1A (A), AMY2A (B), and AMY2B (C) at five dilution points spiked into the α-amylase-depleted human plasma were subjected to potato starch treatment, and subsequently analyzed in triplicate by LC-MRM. The mean of measurements on triplicate experiments is represented. Error bars indicate standard deviation.

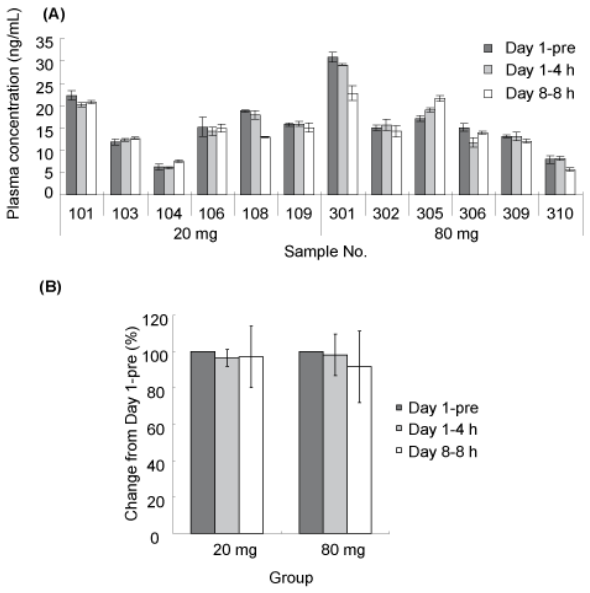

Figure 5: Results of the quantification of AMY1A in plasma of CS-1036 MAD study. (A) Plasma concentration. The mean of triplicate technical replicates is represented. Error bars indicate standard deviation. The horizontal axis represents donor numbers. (B) Change ratio of the concentration to Day 1-pre (%). The mean of the change ratio (n=6) is represented. Error bars indicate standard deviation

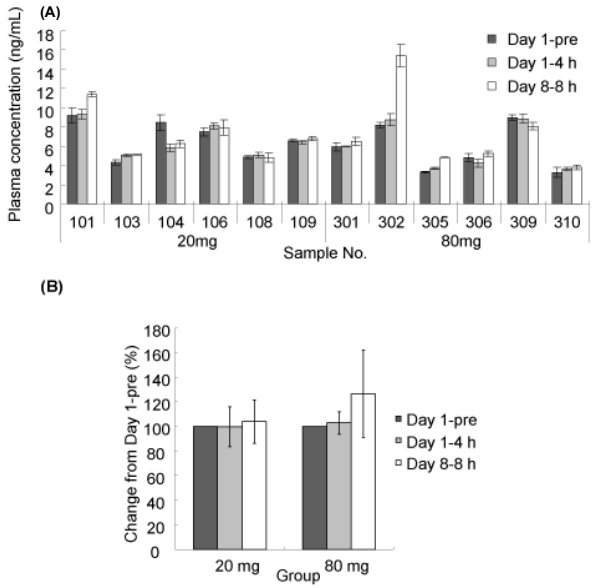

Figure 6: Results of the quantification of AMY2A in plasma of CS-1036 MAD study. (A) Plasma concentration. The mean of triplicate technical replicates is represented. Error bars indicate standard deviation. The horizontal axis represents donor numbers. (B) Change ratio of the concentration to Day 1-pre (%). The mean of the change ratio (n=6) is represented. Error bars indicate standard deviation.

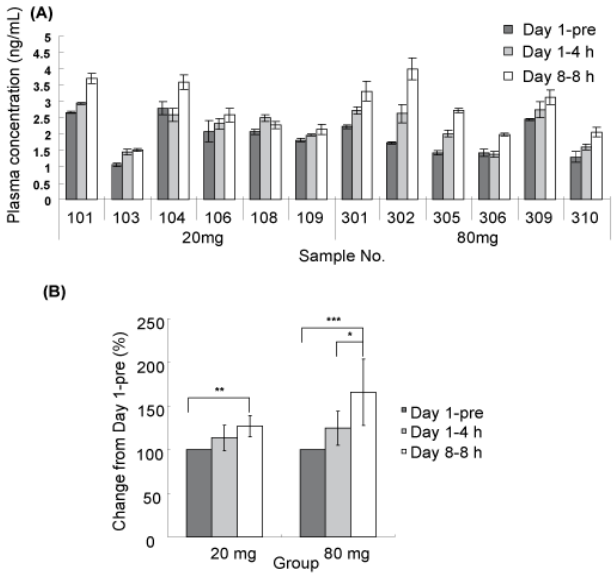

Figure 7: Results of the quantification of AMY2B in plasma of CS-1036 MAD study. (A) Plasma concentration. The mean of triplicate technical replicates is represented. Error bars indicate standard deviation. The horizontal axis represents donor numbers. (B) Change ratio of the concentration to Day 1-pre (%). The mean of the change ratio (n=6) is represented. Error bars indicate standard deviation. Asterisks indicate statistically significant differences (*P<0.05, **P<0.01, ***P<0.001, Tukey's test).

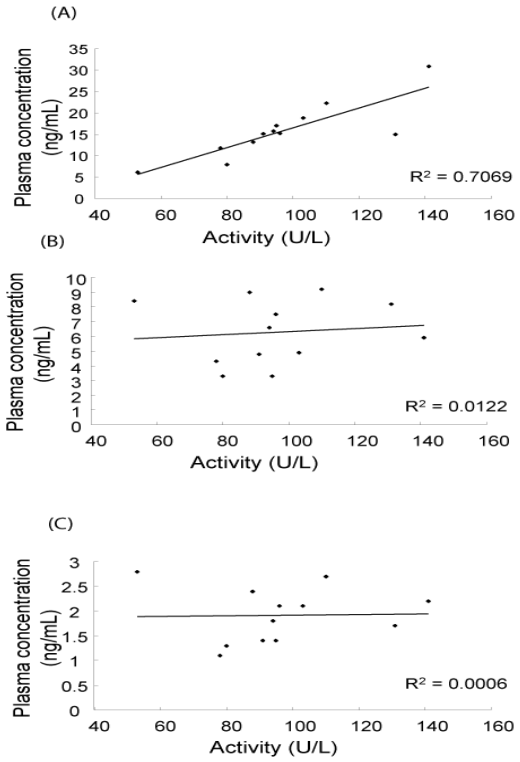

Figure 8: The correlation analysis between the total α-amylase activity and the concentrations of AMY1A (A), AMY2A (B), and AMY2B (C) on Day 1-pre in plasma.

Conflict of Interest

All authors are employees of Daiichi Sankyo Group, which developed CS-1036.

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References

1. Brayer GD, Luo Y, Withers SG (1995) The structure of human pancreatic alpha-amylase at 1.8 A resolution and comparisons with related enzymes. Protein Science: A Publication of the Protein Society 4: 1730-1742.
2. D'Souza R, Pandeya DR, Rahman M, Seo Lee H, Jung JK, et al. (2012) Genetic engineering of Lactococcus lactis to produce an amylase inhibitor for development of an anti-diabetes biodrug. The New Microbiologica 35: 35-42.
3. Breuer HW (2003) Review of a carbose therapeutic strategies in the long-term treatment and in the prevention of type 2 diabetes. Int J Clin Pharmacol Ther 41: 421-440.
4. Ponnusamy S, Zinjarde S, Bhargava S, Rajamohanan PR, Ravikumar A (2012) Discovering Bisdemethoxycurcumin from Curcuma longa rhizome as a potent small molecule inhibitor of human pancreatic alpha-amylase, a target for type-2 diabetes. Food Chem 135: 2638-2642.
5. Honda T, Kaneno-Urasaki Y, Murai T, Kakuta M, Nasu H, et al. (2013) Absorption, elimination, and metabolism of CS-1036, a novel alpha-amylase inhibitor in rats and monkeys, and the relationship between gastrointestinal distribution and suppression of glucose absorption. Drug Metab Dispos 41: 878-887.
6. Honda T, Kaneno-Urasaki Y, Ito T, Kimura T, Matsushima N, et al. (2014) Alpha-amylase inhibitor, CS-1036 binds to serum amylase in a concentration-dependent and saturable manner. Drug Metab Dispos 42: 326-333.
7. Winn-Deen ES, David H, Sigler G, Chavez R (1988) Development of a direct assay for alpha-amylase. Clin Chem 34: 2005-2008.
8. Okabe H, Uji Y, Netsu K, Noma A (1984) Automated measurement of amylase isoenzymes with 4-nitrophenyl-maltoheptaoside as substrate and use of a selective amylase inhibitor. Clin Chem 30: 1219-1222.
9. Abe A, Nishimura T, Noma A, Hamano K (1991) Automated measurement of amylase isoenzymes by a double kinetic assay with "blocked" beta-2-chloro-4-nitrophenyl maltopentaoside as substrate and with wheat germ inhibitor. Clin Chem 37: 1345-1349.
10. Leclerc P, Forest JC (1982) Electrophoretic determination of isoamylases in serum with commercially available reagents. Clin Chem 28: 37-40.
11. Morishita Y, Iinuma Y, Nakashima N, Majima K, Mizuguchi K, et al. (2000) Total and pancreatic amylase measured with 2-chloro-4-nitrophenyl-4-O-beta-D-galactopyranosylmaltoside. Clin Chem 46: 928-933.
12. Uchida R, Tokutake S, Motoyama Y, Hosoi K, Yamaji N (1995) Automated measurement of alpha-amylase isoenzymes with 6(3)-deoxymaltotriose as selective amylase inhibitor. Clin Chem 41: 519-522.
13. Lorentz K (2000) Routine alpha-amylase assay using protected 4-nitrophenyl-1, 4-alpha-D-maltoheptaoside and a novel alpha-glucosidase. Clin Chem 46: 644-649.
14. Omichi K, Hase S (1992) Detection of human urinary alpha-amylase encoded by the AMY2B gene using a fluorogenic substrate, FG5P. J Biochem 112: 303-305.
15. Omichi K, Hase S (1993) Identification of the characteristic amino-acid sequence for human alpha-amylase encoded by the AMY2B gene. Biochim Biophys Acta 1203: 224-229.
16. Picotti P, Aebersold R (2012) Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 9: 555-566.
17. Grebe SKG, Singh RJ (2016) Clinical peptide and protein quantification by mass spectrometry (MS). Trends Anal Chem 84: 131-143.
18. Gillette MA, Carr SA (2013) Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. Nat Methods 10: 28-34.
19. Anderson L, Hunter CL (2006) Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins. Mol Cell Proteomics 5: 573-588.
20. Anderson NL, Anderson NG, Haines LR, Hardie DB, Olafson RW, et al. (2004) Mass spectrometric quantitation of peptides and proteins using Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA). J Proteome Res 3: 235-244.
21. Sakurai H, Kubota K, Inaba S, Takanaka K, Shinagawa A (2013) Identification of a metabolizing enzyme in human kidney by proteomic correlation profiling. Mol Cell Proteomics 12: 2313-2323.
22. Tachida Y, Sakurai H, Okutsu J (2015) Proteomic Comparison of the Secreted Factors of Mesenchymal Stem Cells from Bone Marrow, Adipose Tissue and Dental Pulp. J Proteomics Bioinform 8:266-273.
23. Simeoni F, Tasselli L, Tanaka S, Villanova L, Hayashi M, et al. (2013) Proteomic analysis of the SIRT6 interactome: novel links to genome maintenance and cellular stress signaling. Sci Rep 3: 3085.
24. Rappsilber J, Ishihama Y, Mann M (2003) Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. Anal Chem 75: 663-670.
25. Okuda S, Watanabe Y, Moriya Y, Kawano S, Yamamoto T, et al. (2017) jPOSTrepo: an international standard data repository for proteomes. Nucleic Acids Res 45: 1107-1111.
26. Deutsch O, Fleissig Y, Zaks B, Krief G, Aframian DJ, et al. (2008) An approach to remove alpha amylase for proteomic analysis of low abundance biomarkers in human saliva. Electrophoresis 29: 4150-4157.

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