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ORIGINAL RESEARCH ARTICLE

Bioavailability of Orally Administered Des-Aspartate-Angiotensin I in Human Subjects

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Abstract In an earlier single-dose escalation study to evaluate the safety and pharmacokinetics of orally administered des-aspartate-angiotensin I (DAA-I) in healthy subjects, the plasma level of DAA-I could not be determined because DAA-I is rapidly degraded in the circulation. The present study investigated the oral bioavailability of DAA-I by measuring the prostaglandin E_2 metabolite (PGEM) in the plasma samples of the same trial. PGEM is a stable derivative of PGE_2, which has been shown to be a biomarker of DAA-I. The data show that plasma from two of the three subjects who were orally administered the efficacious preclinical dose of 0.70 mg/kg DAA-I exhibited a significant PGEM peak at 5–6 h postdose. Plasma of subjects who were administered 0.08 and 1.5 mg/kg DAA-I, the subefficacious and two-times efficacious dose, respectively, did not exhibit a similar PGEM

peak. This observation is concordant with the known in vivo actions of DAA-I, especially its hypoglycemic action where maximum efficacy occurred at a dose of 0.7 mg/kg, and decreased to nil at the two-times efficacious dose. The onset of the PGEM peak at 5–6 h postdose was closed to the 4-h onset of absorption of $[C^{14}]$DAA-I seen in preclinical rat studies, albeit the absorption kinetics between rodents and humans are not identical. The occurrence of polymorphism of enzymes involved in the formation and degradation of PGE_2 is common, and this has been attributed to contributing to the variation in response, onset and peak PGEM observed among the three subjects who were administered the efficacious dose.

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Key Points

The bioavailability of orally administered des-aspartate-angiotensin I (DAA-I) was investigated in human subjects.

Prostaglandin E_2 metabolite (PGEM), a stable metabolite derivative of PGE_2 was measured as a biomarker of DAA-I in plasma samples obtained from 15 subjects in a single-dose phase I trial on DAA-I.

Plasma of two of the three subjects who were administered a preclinical efficacious dose of DAA-I (0.7 mg/kg) showed a significant PGEM peak concentration at 5–6 h postdose. No similar significant PGEM peak was present in the plasma of the other subjects who were administered a subefficacious and a two-time efficacious dose. This observation was in concordance with the known in vivo actions of DAA-I.

1 Introduction

Des-aspartate-angiotensin I (DAA-I) is an endogenous angiotensin peptide that has been shown to exhibit efficacy in animal models of 11 human pathologies [1–3]. DAA-I acts as an agonist on the angiotensin AT_1 receptor and releases prostaglandins, which mediate its actions [1, 4]. In cultured human umbilical vein endothelial cells (HUVECs), DAA-I specifically increased the production of prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) via cyclooxygenase (COX)-1 at sub-nanomolar concentrations, but did not stimulate production of these two pros-taglandins via COX-2 [4]. DAA-I was rapidly degraded by circulating enzymes when infused into the systemic cir-culation of the dog [5, 6] and human [7]. Despite being an endogenous peptide and having a rapid systemic metabo-lism, DAA-I has been shown to be active when adminis-tered orally [1–3]. This is probably attributed to its ability to cross Caco-2 cells via passive diffusion [8], indicating that it would cross intestinal epithelial cells, and its activity at concentrations lower than the Km of enzymes [1]. However in a single-dose phase I study, where subjects were orally administered a subefficacious, efficacious, and twice efficacious dose in terms of its preclinical hypogly-caemic activity, we found no significant increase in sub-jects' plasma DAA-I concentration over a period of 12 h post-administration [9]. We have advanced various possi-bilities to explain the absence of DAA-I bioavailability in the phase I study. In a further attempt to obtain physical evidence to show that DAA-I is orally bioavailable, and support its oral efficacy, we measured PGEM, a stable derivative of PGE_2 metabolites, as a biomarker in the plasma samples of the single-dose phase I study. The assay was carried out using a commercial ELISA kit, as preliminary experiments showed that the limit of detection for PGE_2 and its metabolites, as determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), was higher than their basal levels in the plasma samples. The results show that two of the three subjects adminis-tered the efficacious dose exhibited a plasma PGEM pro-file, with a maximum at 5–6 h post-administration. No similar profile was seen in the other plasma samples of subjects administered the other two doses of DAA-I.

2 Materials and Methods

2.1 Plasma Samples

Plasma samples were from subjects of a single-dose phase I trial on DAA-I [9]. The samples had been stored frozen at −80 °C for a period of 18 months, prior to analysis. Three cohorts of six subjects were recruited for the phase I trial and the collected plasma samples were labelled A1–A6, B1–B6, and C1–C6 for Cohorts A, B, and C, respectively. Two subjects in each cohort were administered placebo (sterile water), and the other four subjects from Cohorts A, B and C were administered 0.08, 0.7 and 1.5 mg/kg DAA-I in an aqueous solution, respectively. Volunteers were randomly selected into treatment cohorts. The placebo group plasma samples are denoted with A3, A5, B2, B6, C2 and C5. Due to the use of a plasma sample from each cohort for preliminary analysis in earlier studies, only plasma samples from five subjects in each cohort were available for the present study.

2.2 Plasma Sampling Time

For each subject, plasma samples were taken at 10 different time points, i.e. at time 0 (predosing), and 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 12 h postdosing.

2.3 Assay of Prostaglandin E_2

PGE_2 in the plasma samples was assayed as its stable end metabolite, PGEM, using a Cayman Prostaglandin E Metabolite ELISA Kit (Cat No. 514531; Cayman Chemi-cal, Ann Arbor, MI, USA). The analysis was performed according to the manufacturer's instructions, as described previously [3]. The Cayman's assay is based on the com-petition between a stable PGE metabolite derivative, PGEM, and a PGEM-acetylcholinesterase conjugate (PGEM tracer) for a limited number of PGEM-specific rabbit anti-serum binding sites. PGE_2 metabolites in plasma were first converted to PGEM. Briefly, each 100 μL plasma sample was incubated overnight at 37°C with 30 μL of 1 M carbonate buffer. 40 μL of 1 M phosphate buffer and 30 μL of ELISA buffer were added to the plasma mixture. With this treatment, the plasma sample was diluted twice. Eight 100 μL aliquots of PGEM ELISA standard, each aliquot containing an increasing concentra-tion of PGEM, were similarly treated. A 50 μL aliquot of each treated plasma sample was incubated in a well of an assay plate precoated with goat anti-rabbit immunoglobu-lin G. 50 μL PGEM tracer and 50 μL PGEM ELISA anti-serum were added into each well. After an 18-h incubation at ambient temperature, the plate was rinsed five times with wash buffer. An aliquot of 200 μL freshly prepared Ell-man's reagent was added to each well and the plate was immediately wrapped in aluminum foil and incubated for 90 min in an orbital shaker. Absorbance at 412 nm was read after incubation. The content of PGEM in the eight standard solutions was similarly assayed. Preliminary experiments showed that readings obtained from twice-diluted plasma samples fell within the 20–80% range of the

standard curves, and provided the rationale for a two times dilution of the plasma samples.

2.4 Statistical Analysis

One-way analysis of variance was used to test for significance of the increase in PGEM concentrations that occurred between 4.5 and 5.5 h post-administration in plasma samples of subjects B4 and B5 of cohort B. Post hoc analysis was corrected using Tukey's honest significant difference (HSD) test to correct for the type I error on the pairwise comparisons. Statistical significance was set at $p < 0.05$.

3 Results

Figure 1 displays the PGEM concentration profile in the 15 plasma samples obtained from subjects who participated in the single-dose phase I trial on DAA-I. The plasma PGEM concentration profile in two of the three samples in cohort B (B4 and B5) had a peak at 5 and 6 h post-administration, respectively. The mean concentration between 4.5 and 5.5 h of the two peak concentrations was significantly higher than the corresponding concentrations in the placebo samples. Additionally, the mean concentration for sample B5 was significantly higher than the corresponding concentrations of the other two samples from DAA-I-treated subjects. The period between 4.5 and 5.5 h post-administration covers the half hour before and half hour after the peak PGEM concentration of sample B4, and the mean plasma PGEM concentrations of cohort B during this period were used for statistical comparison. This period was selected because the peak PGEM concentration of sample B4 was lower than that of sample B5. The PGEM concentration profiles in the plasma samples of cohorts A and C did not exhibit a similar peak concentration.

4 Discussion

As therapeutics, peptides are recognized for being highly selective, efficacious, and well-tolerated [10]; however, most endogenous peptides are not suitable for use as therapeutics per se because they have a short circulating half-life. Glucagon-like peptide 1, amylin, and somatostatin are examples, and their respective analogues such as exenatide, pramlinitide and octreotide have considerable longer half-life and are used as parental drugs in lieu of the parent peptides. DAA-I, although an endogenous peptide with a similar short circulating half-life, has been shown to be active when administered orally [1–3]. Its bioavailability was estimated to be 0.06 in a study where both intravenous and oral DAA-I were shown to produce similar
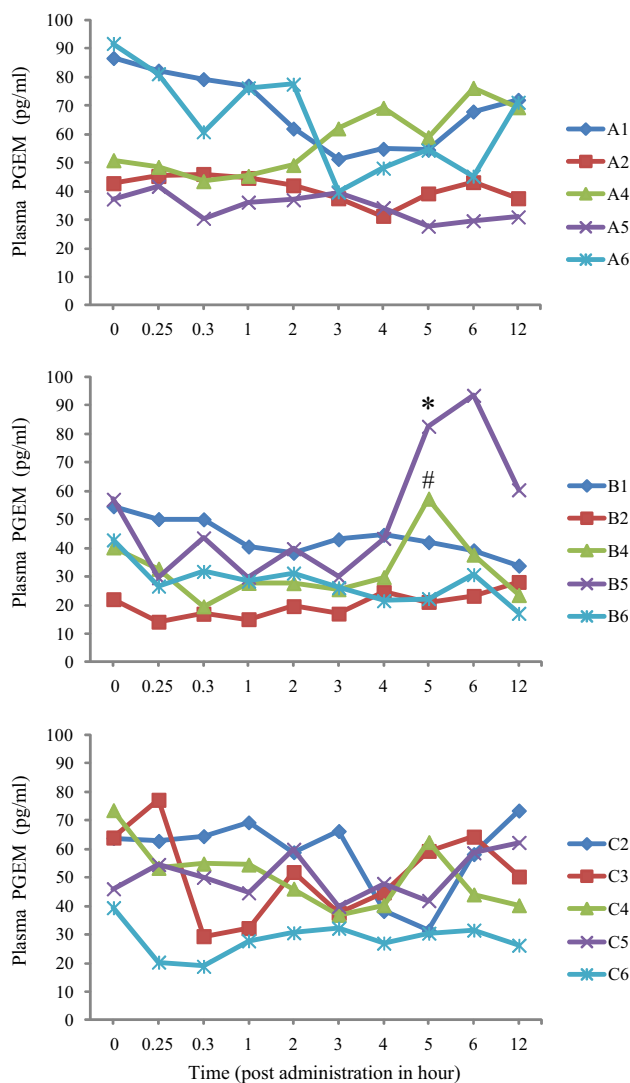

Fig. 1 Plasma PGEM concentrations. A5, B2, B6, C2 and C5 were placebo samples, and samples in the upper, middle and lower plots were from subjects who were administered 0.08, 0.70, and 1.5 mg/kg DAA-I, respectively. * Indicates significantly greater than the corresponding values of B1, B2, B4 and B6 ($p < 0.01$), # indicates significantly greater than the corresponding values of B2 and B6 ($p < 0.01$)

magnitudes of attenuation of cardiac hypertrophy [1]. In preclinical toxicology studies, a [^{14}C]-DAA-I-like peak was detected at 4 h after [^{14}C]-DAA-I was orally administered to rats, indicating that the onset of DAA-I absorption was 4 h post-administration (unpublished preclinical toxicological data). These findings, together with the ability of DAA-I to passage Caco-2 cells via passive diffusion [8], are indications of its oral bioavailability.

The data in Fig. 1 lend further credence to the oral bioavailability of DAA-I. The bioavailability was denoted by the presence of a peak concentration of PGEM, a biomarker of DAA-I, appearing at 5–6 h post-administration in two of the three plasma samples of subjects who were

administered the preclinical efficacious dose of 0.7 mg/kg. This observation is in concordance with several known actions of DAA-I. First, DAA-I has been shown to specifically release PGE_2 from cultured HUVECs [4]. This enables PGE_2 to be a candidate biomarker of DAA-I as the endothelium is the likely tissue of first contact with absorbed DAA-I. HUVECs lack the enzymes that degrade PGE_2, but, in systemic circulation, PGE_2 is rapidly degraded to its 13,14-dihydro-15-keto metabolites, and in this study was measured as PGEM, the stable derivative of these metabolites [11]. The second commonality is the dose-dependent action of DAA-I, where doses higher than its maximum efficacious dose caused an absence of effect, as shown by the absence of peak plasma concentrations of PGEM in the plasma samples of subjects who were administered a subefficacious (cohort A) and two-times efficacious (cohort C) dose. This dose-related action is also seen in the hypoglycaemic action of DAA-I in the KKAy mouse model of type 2 diabetes, where an efficacy occurred at a dose of 0.7 mg/kg DAA-I; doubling of this dose caused a total loss of the hypoglycaemic effects [12].

The PGEM peak occurred at 5–6 h post-administration in subjects B4 and B5, respectively. This is 1–2 h later than the 4 h absorption onset seen in preclinical studies, and could be due to a variation in absorption kinetics between rodents and humans, or the time taken for the released PGE_2 to degrade to its 13,14-dihydro-15-keto metabolites. Variation in response to DAA-I could also have occurred among the DAA-I-treated subjects of cohort B, where subject B1 did not respond, while subject B5 had a more marked response but a later onset than subject B4. The formation of PGE_2 involves multiple enzymes [13] and its breakdown to 13,14-dihydro-15-keto metabolites involves both enzymatic and non-enzymatic degradation [11]. The key enzymes involved in the multistep pathway of PGE_2 formation and degradation are polymorphic [14, 15], and this could be responsible for the variable responses seen in the three DAA-I-treated subjects of cohort B.

5 Conclusion

The data in the present study are further evidence of the oral bioavailability of DAA-I. Although the bioavailability was determined by measuring PGEM, a stable metabolite derivative of the biomarker PGE_2, the observed plasma PGEM profiles were in concordance with the known in vivo actions of DAA-I. This singular matching of plasma kinetics and in vivo actions is a reflection of the specific and unique properties of DAA-I.

Compliance with Ethical Standards

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