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Original article

Design, synthesis and biological evaluation of novel peptide MC62 analogues as potential antihyperglycemic agents

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ABSTRACT

Two series of peptide MC62 analogues were synthesized, characterized and evaluated for their antihyperglycemic effects. Structure–activity relationship studies of the first series indicated that antihyperglycemic effects were correlated to residues 4, 5, 7 and 8. Peptide **I-6** exhibited higher antihyperglycemic activity than the MC62 parent peptide, and was chosen for further modification. Incorporation of Met at position 3 increased potency further and generated **II-3**, which was screened *in vivo* and *in vitro* using exenatide (Ex-4) and GLP-1 as positive controls. The results showed that the antihyperglycemic and antioxidative activities of **II-3** were comparable to the positive controls, suggesting **II-3** could be a candidate for use as a future diabetic treatment.

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1. Introduction

Diabetes mellitus (DM) is a major worldwide health problem associated with carbohydrate, fat and protein metabolic disorders [1]. Currently available therapies include insulin, oral drugs (including sulfonylureas, biguanides, thiazolidinediones and dipeptidyl peptidase-4 inhibitors) and glucagon-like peptide-1 (GLP-1) analogues [2–4]. Unfortunately, these drugs are associated with various adverse side effects such as hypoglycemia for the sulfonylureas and digestive tract problems with the oral drugs, and alternative treatments are much needed [5,6].

Among the many medications used in certain parts of the world, several herbs have been found to control diabetes. Of these, *Momordica charantia* (MC; bitter melon) unripe fruit, seeds and vegetation have all been used to reduce fasting blood glucose levels and to improve glucose tolerance in normal and diabetic animals as well as humans [7,8]. Nag et al. (1999) isolated a mixture of polypeptides from *M. charantia* (MC6), and the three peptides comprising MC6 (MC61, MC62 and MC63), each possessed significant antihyperglycemic activity in rats [9–11].

We previously determined that peptide MC62 exhibited significant antihyperglycemic activity at a dose of 1 μmol/kg [12]. However to date, there have been no reports on structure–activity relationships of MC62. In this study, two series of MC62 analogues were synthesized and their antihyperglycemic activities were evaluated in diabetic mice. Among these, compound **II-3** was found to possess higher antihyperglycemic activity than MC62 and subsequently screened *in vivo* and *in vitro*, with exenatide (Ex-4) and GLP-1 as positive controls. Ex-4, marketed as Byetta, is a GLP-1 agonist approved in April 2005 for the treatment of type 2 DM.

2. Results and discussion

2.1. Antihyperglycemic effects of MC62 analogues in STZ-induced diabetic mice

To identify MC62 amino acids that interacted with the active site, glycine scanning mutagenesis was carried out [13–15]. MC62 analogues with residues 1–8 replaced by Gly were synthesized on Wang resin using standard solid phase peptide synthesis (SPPS) methods employing HOBt, HBTU and DIEA as coupling reagents and Fmoc-protected amino acids [16,17].

The antihyperglycemic activities of MC62 analogues were investigated in STZ-induced diabetic mice (Table 1). After 20 days of treatment, over-night fasting blood glucose levels were measured (Table 2).

Daily treatment with MC62 or Ex-4 resulted in a small reduction in fasting blood glucose levels compared with controls.

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Table 1
Sequence and experimental data for MC62 analogues.

Name	Peptide sequence	Mass (Da)		HPLC retention time (min)
		Calculated	Observed $[M + H]^+$	
MC62	H-Lys-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-OH	1159.39	1160.2	7.876
I-1	H-Gly-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-OH	1088.27	1089.8	7.974
I-2	H-Lys-Gly-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-OH	1115.33	1116.9	7.799
I-3	H-Lys-Thr-Gly-Met-Lys-His-Met-Ala-Gly-Ala-Ala-OH	1101.34	1102.9	7.864
I-4	H-Lys-Thr-Asn-Gly-Lys-His-Met-Ala-Gly-Ala-Ala-OH	1085.24	1086.5	6.975
I-5	H-Lys-Thr-Asn-Met-Gly-His-Met-Ala-Gly-Ala-Ala-OH	1088.27	1089.5	8.098
I-6	H-Lys-Thr-Asn-Met-Lys-Gly-Met-Ala-Gly-Ala-Ala-OH	1079.30	1080.5	8.120
I-7	H-Lys-Thr-Asn-Met-Lys-His-Gly-Ala-Gly-Ala-Ala-OH	1085.24	1086.5	5.799
I-8	H-Lys-Thr-Asn-Met-Lys-His-Met-Gly-Gly-Ala-Ala-OH	1145.36	1146.8	7.746

As expected, diabetic control mice showed increased blood glucose levels. Compounds **I-1**, **I-2**, **I-3** and **I-6** were more potent than MC62. The antihyperglycemic activities of **I-5** and **I-8** were slightly decreased compared with MC62, while that of **I-4** and **I-7** were almost entirely lost. In summary, substitution by Gly at positions 1, 2, 3, 6 hardly affected antihyperglycemic activity, whereas substitution at positions 4, 5, 7 and 8 had a large effect; the residues at these positions should be retained to preserve activity.

2.2. Antihyperglycemic effects of *I-6* analogues

Interestingly, antihyperglycemic activity was almost lost completely when MC62 Met^4 or Met^7 were replaced with Gly, suggesting a key role for these residues. This could be related to oxidative damage, since Met residues in proteins can act as an endogenous antioxidant in cells [18–21].

We hypothesized that increasing the proportion of Met in the peptides may enhance antihyperglycemic and antioxidative activities, and synthesized a series of analogues with Met at positions 1, 2, 3, 9, 10 and 11 in **I-6** (Table 3). The antihyperglycemic activities of these **I-6** analogues were evaluated in STZ-induced diabetic mice,

Table 2
Levels of fasting blood glucose for mice treated with MC62 analogues.[a]

Groups	Dose (μmol/kg)	Fasting blood glucose (mmol/L)	
		Before treatment	After treatment
Normal control	Saline	6.67 ± 2.21	7.43 ± 1.53
Diabetic control	Saline	21.54 ± 1.74	26.50 ± 3.22
Ex-4	0.03	19.75 ± 1.65	10.95 ± 2.68$^{**,\#\#}$
MC62	1	21.55 ± 2.13	16.29 ± 2.10$^{**,\#}$
I-1	1	20.78 ± 1.98	15.26 ± 1.55$^{**,\#}$
I-2	1	21.22 ± 1.96	15.28 ± 2.10$^{**,\#}$
I-3	1	19.82 ± 2.18	14.36 ± 1.36$^{**,\#}$
I-4	1	19.54 ± 1.56	18.34 ± 2.27*
I-5	1	20.58 ± 2.13	18.52 ± 2.31*
I-6	1	20.81 ± 1.71	14.07 ± 2.30$^{**,\#\#}$
I-7	1	19.04 ± 1.55	21.82 ± 2.49
I-8	1	20.46 ± 2.41	18.02 ± 1.70*

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the diabetic control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ for comparison of the fasting blood glucose levels of each group on day 0 and day 20.

[a] Results are expressed as mean ± SD for eight mice per group.

Table 3
Sequence and experimental data for **I-6** analogues.

Name	Peptide sequence	Mass (Da)		HPLC retention time (min)
		Calculated	Observed	
I-6	H-Lys-Thr-Asn-Met-Lys-Gly-Met-Ala-Gly-Ala-Ala-OH	1079.36	1080.5	8.120
II-1	H-Met-Thr-Asn-Met-Lys-Gly-Met-Ala-Gly-Ala-Ala-OH	1082.36	1083.5	8.599
II-2	H-Lys-Met-Asn-Met-Lys-Gly-Met-Ala-Gly-Ala-Ala-OH	1109.39	1110.6	8.625
II-3	H-Lys-Thr-Met-Met-Lys-Gly-Met-Ala-Gly-Ala-Ala-OH	1096.39	1097.3	8.534
II-4	H-Lys-Thr-Asn-Met-Lys-Gly-Met-Ala-Met-Ala-Ala-OH	1153.44	1154.6	8.767
II-5	H-Lys-Thr-Asn-Met-Lys-Gly-Met-Ala-Gly-Met-Ala-OH	1139.42	1140.6	8.783
II-6	H-Lys-Thr-Asn-Met-Lys-Gly-Met-Ala-Gly-Ala-Met-OH	1139.42	1140.6	8.713

and over-night fasting blood levels were measured after 20 days of treatment (Table 4).

Changes in fasting blood glucose levels in diabetic control mice, and mice treated with MC62, **I-6** or Ex-4 were similar to those described above. The peptide containing a Met at position 3 (**II-3**) exerted a more potent antihyperglycemic effect than **I-6**, while the effects of **II-2** and **II-4** comparable to **I-6**. In contrast, when residues at positions 1, 10 and 11 (**II-1**, **II-5**, **II-6**) were substituted by Met, the antihyperglycemic effects were almost entirely lost. **II-3** had the highest antihyperglycemic activity and was subsequently used for *in vivo* and *in vitro* studies.

2.3. Oral glucose tolerance test of *II-3* in normal mice

To probe the underlying mechanisms of **II-3**, the effects of **II-3** at a dose of 1 μmol/kg were investigated *in vivo* and *in vitro*.

To evaluate whether **II-3** administration improved the systemic response to glucose in normal mice, the oral glucose tolerance test (OGTT) was performed. Blood glucose levels in **II-3**-treated mice were significantly lower than those of untreated control mice at 30 and 60 min after glucose administration (Fig. 1A). Thus, administration of **II-3** significantly improved glucose tolerance in normal mice.

2.4. Food and water intake, and body weight gain in *II-3*-treated STZ-induced diabetic mice

The effects of **II-3** were verified in diabetic mice that were injected intraperitoneally for 20 days. Polyphagia and polydipsia

Table 4
Levels of fasting blood glucose for mice treated with **I-6** analogues.[a]

Groups	Dose (μmol/kg)	Fasting blood glucose (mmol/L)	
		Before treatment	After treatment
Normal control	Saline	6.08 ± 2.82	7.50 ± 1.54
Diabetic control	Saline	19.28 ± 3.10	26.02 ± 3.36
Ex-4	0.03	19.14 ± 3.16	10.84 ± 2.98$^{*,\#\#}$
MC62	1	20.95 ± 2.12	16.16 ± 1.80$^{*,\#}$
I-6	1	19.69 ± 2.63	13.29 ± 2.23$^{***,\#\#}$
II-1	1	21.23 ± 1.57	20.83 ± 3.28*
II-2	1	19.42 ± 1.33	13.44 ± 2.76$^{***,\#}$
II-3	1	20.87 ± 1.62	11.80 ± 2.42$^{***,\#\#}$
II-4	1	20.97 ± 1.43	14.03 ± 2.88$^{**,\#}$
II-5	1	19.41 ± 1.97	17.38 ± 3.57*
II-6	1	20.17 ± 2.47	20.18 ± 4.15*

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the diabetic control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ for comparison of the fasting blood glucose levels of each group on day 0 and day 20.

[a] Results are expressed as mean ± SD for eight mice per group.

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