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Competitive binding between 4,4′-diphenylmethane-bis(methyl) carbamate and RAGE ligand MG-H1 on human umbilical vein endothelial cell by cell membrane chromatography

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ABSTRACT

The compound 4,4′-diphenylmethane-bis(methyl) carbamate (CM1) has a protective activity on AGEs-induced endothelial dysfunction on human umbilical vein endothelial cell (HUVEC) in our previous study. It suggested that CM1 which may act as a competitive antagonist to the blockade of AGEs to receptor of AGEs (RAGE) and attenuate the HUVEC damage. In order to testify that hypothesis, the cell membrane chromatography (CMC) combined with high performance liquid chromatography (HPLC) was developed for analyzing the competitive binding properties on RAGE of HUVEC between CM1 and MG-H1, the agonist of RAGE. The results from saturation binding of CM1 and MG-H1 on cells demonstrated that dissociation equilibrium constants (K_d) of CM1 and MG-H1 were 3.653 nM and 4.12 nM, respectively; while maximum binding capacity (B_{max}) of CM1 and MG-H1 were 30.08 and 18.72 fmol/mg protein, respectively. In competition experiments, IC_{50} of CM1 with pre-incubation 10^{-10} M and 10^{-9} M MG-H1 were 1.37×10^{-9} M and 4.56×10^{-8} M, respectively. The present findings indicated that CM1 conjugated competitively to cells with RAGE ligand MG-H1. The primary study illustrated that CMC combined with HPLC analysis method could be an alternative, rapid and efficient approach for the interaction of drug molecule and receptor, and that CM1 intervene the AGEs inducing HUVEC damage may via the competitively block the AGEs–RAGE path way.

1. Introduction

Advanced glycation end products (AGEs), from non-enzymatic glycation reaction of reducing sugars and amino groups of proteins, lipids or nucleic acids, have been implicated in the pathogenesis of diabetic complications [1–3]. It is reported that AGEs initiated the pathogenesis of diabetic nephropathy via interaction with their receptors (RAGE) [4]. RAGE, a member of the immunoglobulin superfamily distributed on cell membrane surface, contains one V type domain which is responsible for its binding of extracellular ligands [5,6]. It has been shown that multiple ligands can bind to RAGE, such as high mobility group box (HMGB1/amphoterin), S100/calgranulin protein family, etc. [7,8]. Especially, some advanced glycation end products such as N(ε)-(carboxymethyl)lysine (CML) and MG-H1, are implicated in the pathogenesis of diabetic vasculopathy via binding to RAGE [9–11].

A novel compound, 4,4′-diphenylmethane-bis(methyl) carbamate (CM1, also known as DMPC) can prevent AGEs-induced endothelial dysfunction via anti-apoptosis and anti-inflammation on human umbilical vein endothelial cell (HUVEC) [12]. Previously, our results demonstrated that CM1 could attenuate AGEs, HMGB1 and S100B-induced inflammation, apoptosis and oxidative stress response on HUVEC (in press). However, how the CM1 have the multiple effects are unclear. The possibility of CM1 effects on the endothelial cell were via binding competitively to RAGE with its ligands? Therefore, it is interesting to study the role of CM1 in interaction with RAGE in order to fully characterize the mechanism of CM1 against AGEs-induced endothelium dysfunction.

Many strategies were developed to investigate the interactions between drugs and receptors, such as differential scanning calorimetry and radioligand binding assay, which is of high sensitivity and accuracy [13–15]. However, they are not the preferred methods for analyzing drugs and receptors based on its potential radioactive pollution. Recently, cell membrane chromatography

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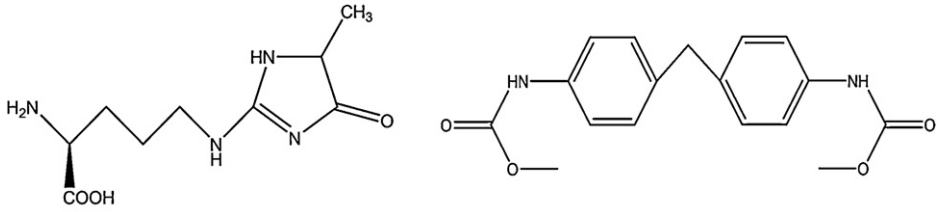

Fig. 1. The chemical structure of MG-H1(chemical formula: $C_9H_{16}N_4O_3$; molecular weight: 228.2) and CM1(chemical formula: $C_{17}H_{18}N_2O_4$; molecular weight: 314.13).

(CMC) method has been proposed to investigate the interaction of drugs and receptors [16,17]. In CMC method, high-performance liquid chromatography (HPLC) was used to analyze the binding kinetics based on its sensitivity, accuracy and convenience. In present study, a CMC method combined with HPLC was established for determining whether CM1 could block the interaction of ligand MG-H1 (a cross-link product in AGEs) and RAGE as a competitive antagonist.

2. Materials and methods

2.1. Reagents and chemicals

N_δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1, purity ≥96.6%) was obtained from PolyPeptide Laboratories (Strasbourg, France); 4,4′-diphenylmethane-bis(methyl) carbamate (CM1, purity ≥98%) was prepared by our laboratory and its

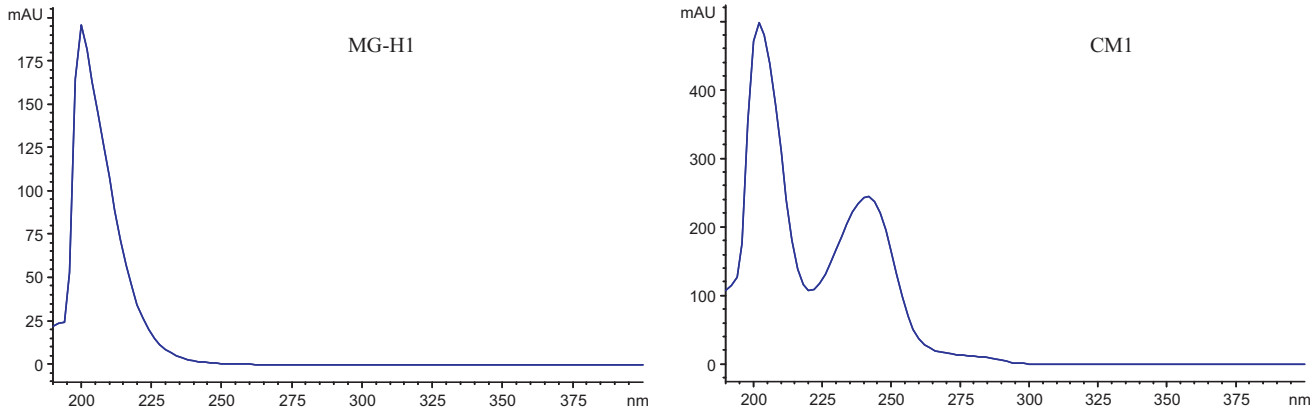

Fig. 2. The UV spectrum of MG-H1 and CM1. The scanning range was from 190 nm to 400 nm.

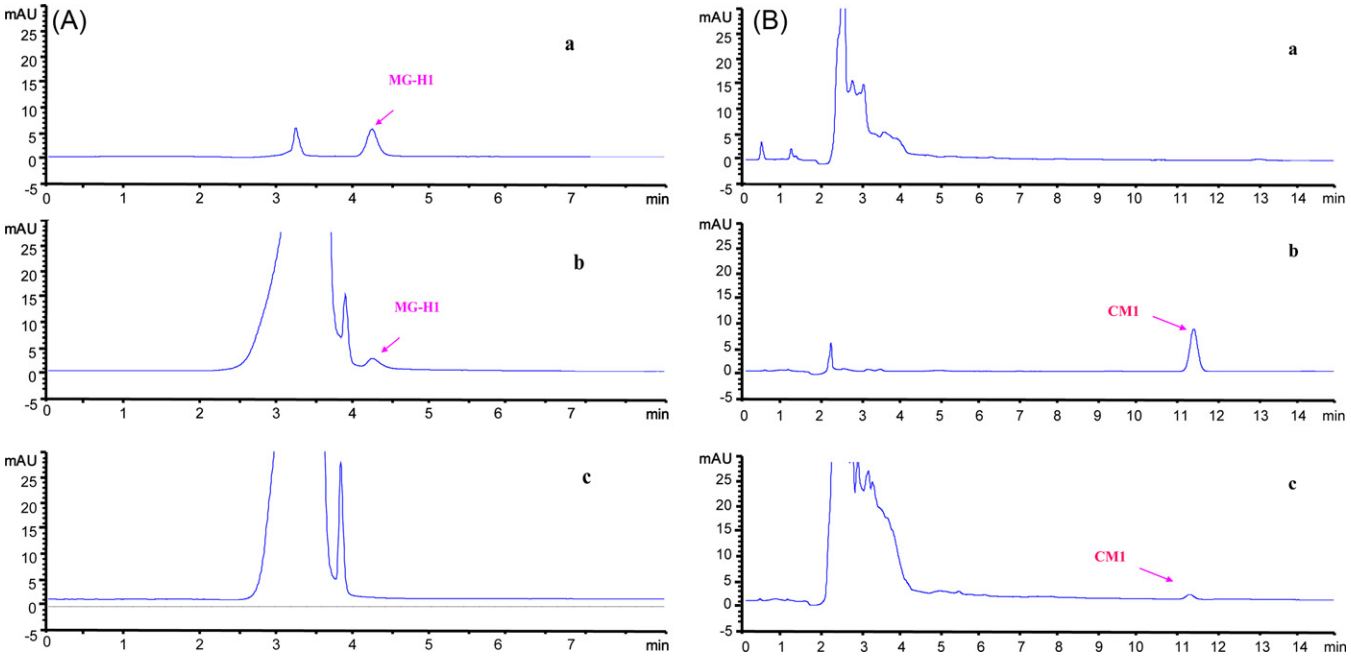

Fig. 3. Representative chromatograms of MG-H1 and CM1. (Aa) Reference substance MG-H1; (Ab and Bc) the eluates from HUVEC; (Ac and Ba) dissociation solution for blank control.

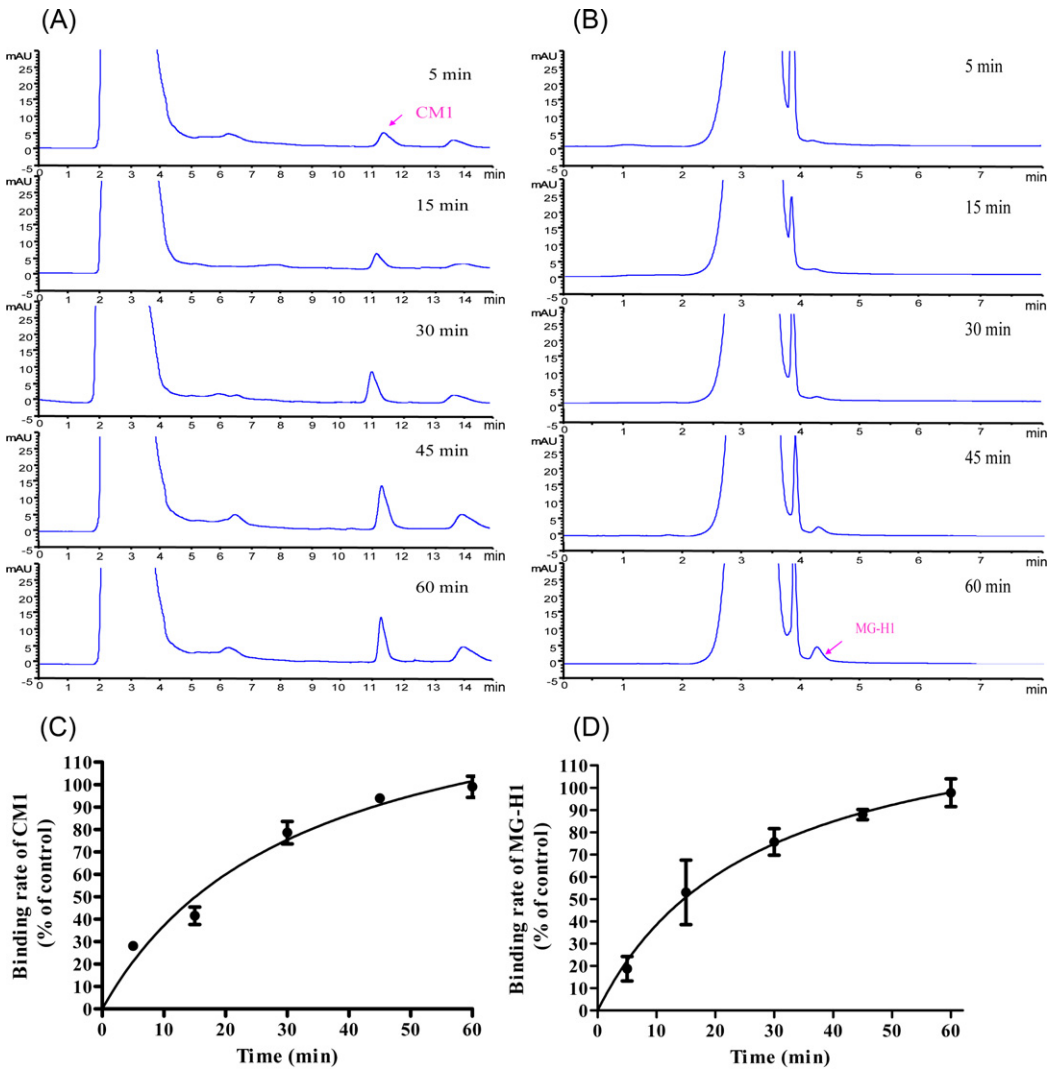

Fig. 4. The optimization of incubation time. The CM1 (10^{-6} M) or RAGE ligand MG-H1 (10^{-6} M) were maintained in 5 ml final volume PBS for 5, 15, 30, 45 and 60 min. The binding molecules were dissociated with dissociation solution from intact cells. The samples were prepared according to procedures in Section 2.4. Specific binding was expressed as % of control binding. Data are presented as means ± SD from individual experiments ($n = 3$).

chemical structure was identified with UV, LC/MS, ^{1}H NMR, and ^{13}C NMR. Trifluoroacetic acid (TFA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile was obtained from MERCK (Germany). All other reagents used were of analytical reagent grade.

2.2. Apparatus

The analysis was performed by Agilent 1200 high performance liquid chromatography (Agilent Technologies, MA, USA), equipped with an photodiode array detector (DAD), quaternary pump, autosampler, and Agilent chemstation software. The following analysis conditions were used for determination of CM1 and RAGE ligand MG-H1 in samples: For CM1, samples were analyzed on an Alltima C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm) with 50% acetonitrile and 50% water elution at 0.5 ml/min flow rate; An Agilent Hypersil ODS C_{18} (4.0 mm × 250 mm, 5 μm) was performed for RAGE ligand MG-H1 with 0.1% TFA elution at 1.0 ml/min flow rate. The detection wavelength 210 nm was set for MG-H1 and 245 nm for CM1. The column temperature of all analysis was maintained at 30 °C and injection volume was 30 μl.

2.3. Cell culture

Human umbilical vein endothelial cell (HUVEC) was obtained from ATCC (USA) and cells were cultured with Dulbecco's Modified Essential Medium (DMEM), containing low glucose, 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin and 10% fetal bovine serum (FBS). Cells were cultured in 5% CO_2 at 37 °C in 25 cm^2 flasks.

2.4. Preparations of sample and solution

The prepared HUVEC cells suspension were seeded in 25 cm^2 polystyrene flasks (Elscolab, Kruibeke, Belgium) and maintained in DMEM medium until confluence. Before this experiment, cells were starved for 12 h with serum-free medium. The medium was discarded and cells were washed with 5 ml phosphate buffered solution (PBS, 0.2 mol/l, pH 7.4) for three times. Cells were maintained in a final volume of 5 ml PBS with MG-H1 or/and CM1. For competition binding experiments, cells were incubated with RAGE ligand MG-H1 (10^{-9} and 10^{-10} M) for 60 min at 37 °C, and then increasing concentrations CM1 (from 10^{-5} to 10^{-11} M) were added

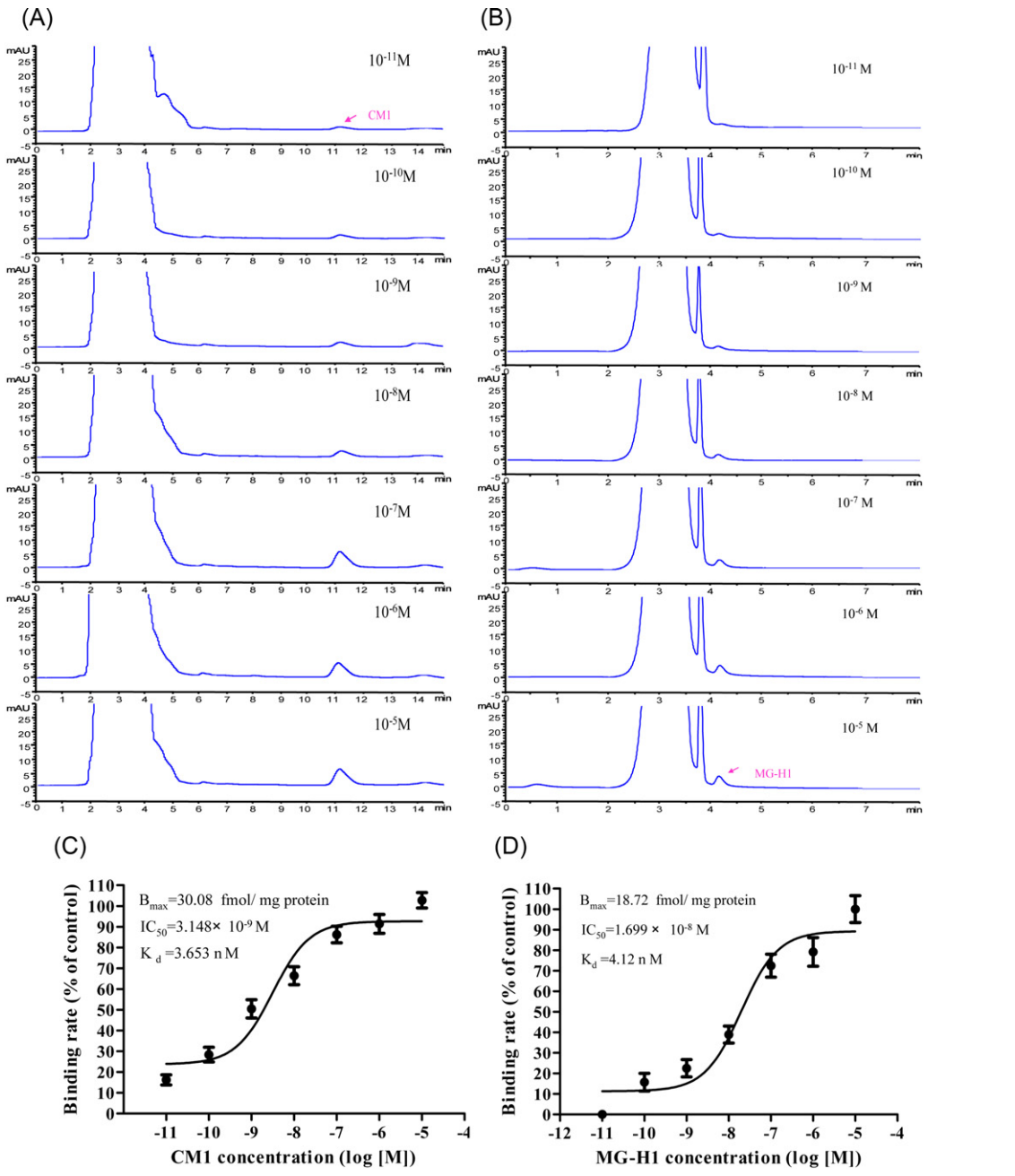

Fig. 5. The saturation binding of CM1 or MG-H1 on intact HUVEC. Cells were incubated with increasing concentration of CM1 or MG-H1 (10^{-11} M to 10^{-5} M) at 37 °C for 60 min. Specific binding was expressed as % of control binding. Data are presented as means ± SD from individual experiments ($n = 3$).

for another co-incubation of 60 min. After incubation, supernatant was transferred to 10 ml tubes and remaining cells were washed with 2 ml PBS for two times. The washing solution was combined. The remaining cells were dissociated with 5 ml dissociation solution containing citric acid (pH 4.0) at 37 °C for 60 min. The solutions were evaporated in blast oven at 60 °C until dryness and residue was dissolved with 1 ml HPLC – grade methanol. Samples were centrifuged at a speed of 12,000 × *g* for 5 min to remove insoluble substance and supernatant was taken to 1.5 ml eppendorf micro test tubes. The solvent was evaporated to dryness and residue was dissolved in 100 μl methanol. The sample solutions were filtered through a 0.45 μm microporous membrane prior to injection.

2.5. Data analysis

All data from three independent experiments was expressed as mean ± SD in this study. In saturation binding experiments, B_{max} and K_d in competitive binding curves were calculated by non-regression analysis with GraphPad Prism™ 5.0 (San Diego, CA, USA). The half maximal inhibitory concentration (IC_{50}) was examined with same software in competition binding experiments.

3. Results and discussion

3.1. Optimization of HPLC analysis conditions

In order to obtain better quantitative analysis and optimal resolution of peaks in chromatograms, Agilent Hypersil ODS C_{18} (4.0 mm × 250 mm, 5 μm) was selected for analysis of ligand MG-H1 and Alltima C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm) for CM1 based on MG-H1 is a hydrophilic compound whereas CM1 is a hydrophobic compound (Fig. 1). Considering the different maximum absorption for each compound, appropriate detection wavelengths were selected for analysis of MG-H1 and CM1,

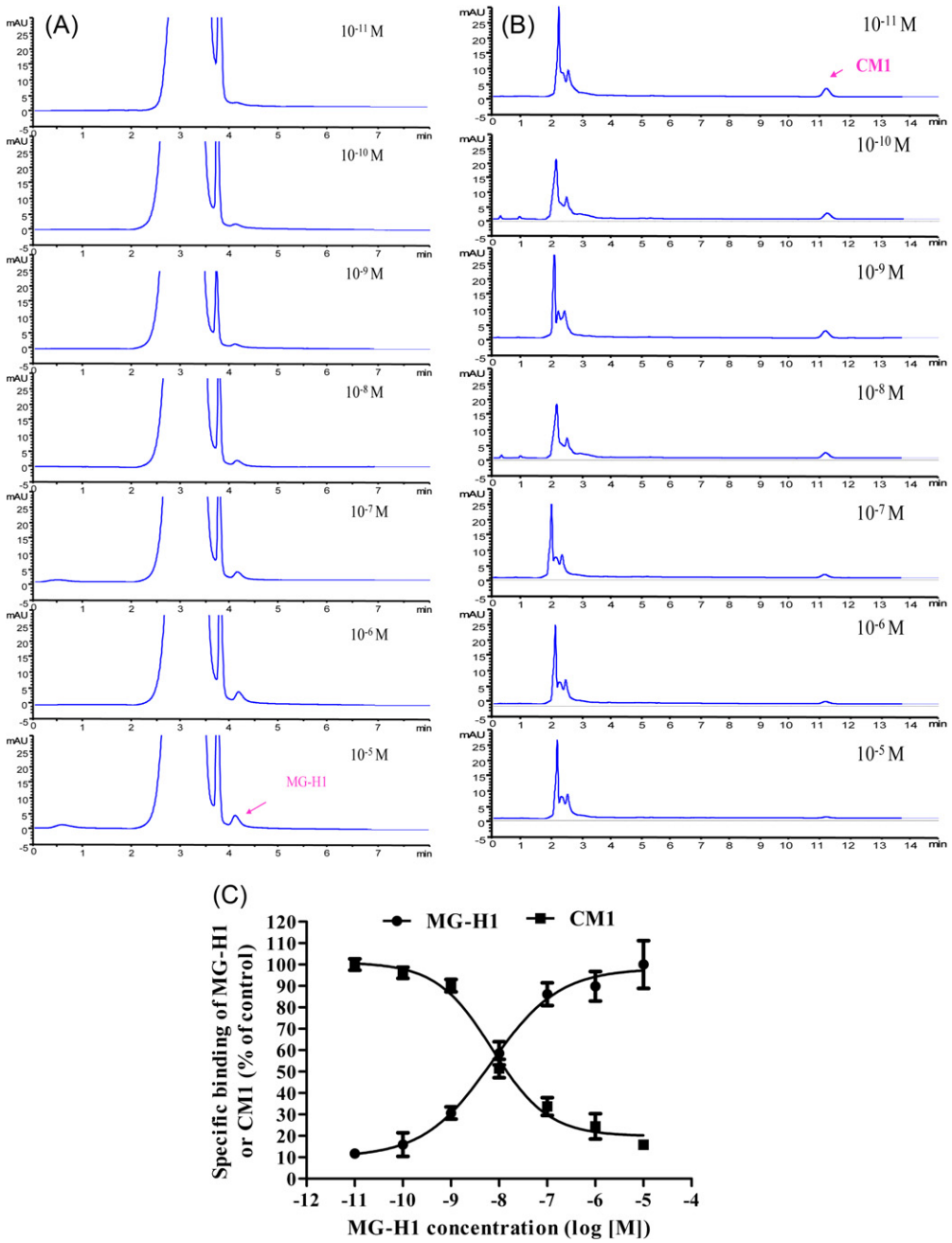

Fig. 6. Effect of MG-H1 on binding of CM1 on intact HUVEC. Cells were pre-treated with MG-H1 (from 10^{-11} to 10^{-5} M) for 60 min at 37 °C and then co-incubated for next 60 min in presence of CM1 (10^{-6} M). Date was presented as means ± SD from individual experiments (n=3).

respectively. As shown in Fig. 2, MG-H1 has wavelength maximum at 210 nm whereas CM1 at 210 and 245 nm. Therefore, 210 nm was selected for analysis of MG-H1 whereas 245 nm for CM1 based on avoiding blank interference. For mobile phase, acetonitrile was chosen as organic solvent in which its low wavelength maximum. In addition, trifluoroacetic acid (TFA) was chosen for analysis of amino acid MG-H1 in which its characteristics of dissolved protein. The representative chromatograms of sample and standard analytes were shown in Fig. 3. The retention time of ligand MG-H1 was 4.23 min and CM1 was 11.41 min.

3.2. Saturated binding of MGH1 and CM1 to intact cells

The incubation time of CM1 or RAGE ligand MG-H1 on intact HUVEC was examined for saturation experiments. Binding of CM1 or MG-H1 with intact HUVEC at different time points (5, 15, 30, 45 and 60 min) were analyzed according to the analysis conditions above. As shown in Fig. 4A and C, the binding rate of CM1 to intact cells increased gradually with the increasing of time. The experimental results demonstrated that the saturation binding of CM1 could be archived after being co-incubated with cells within 60 min. Similarly, the RAGE ligand MG-H1 can also interact saturatedly with cells within 60 min (Fig. 4 B and D).

In further saturation binding experiments, the 90% confluenced cells were treated with increasing concentrations of CM1 or MG-H1 (from 10^{-11} M to 10^{-5} M) and then maintained in PBS for 60 min. The analysis results demonstrated that the peak of CM1 or MG-H1 has an apparent increase with increasing of incubation concentration (Fig. 5A and B). The saturation binding curves were obtained and dissociation equilibrium constants (K_d) and

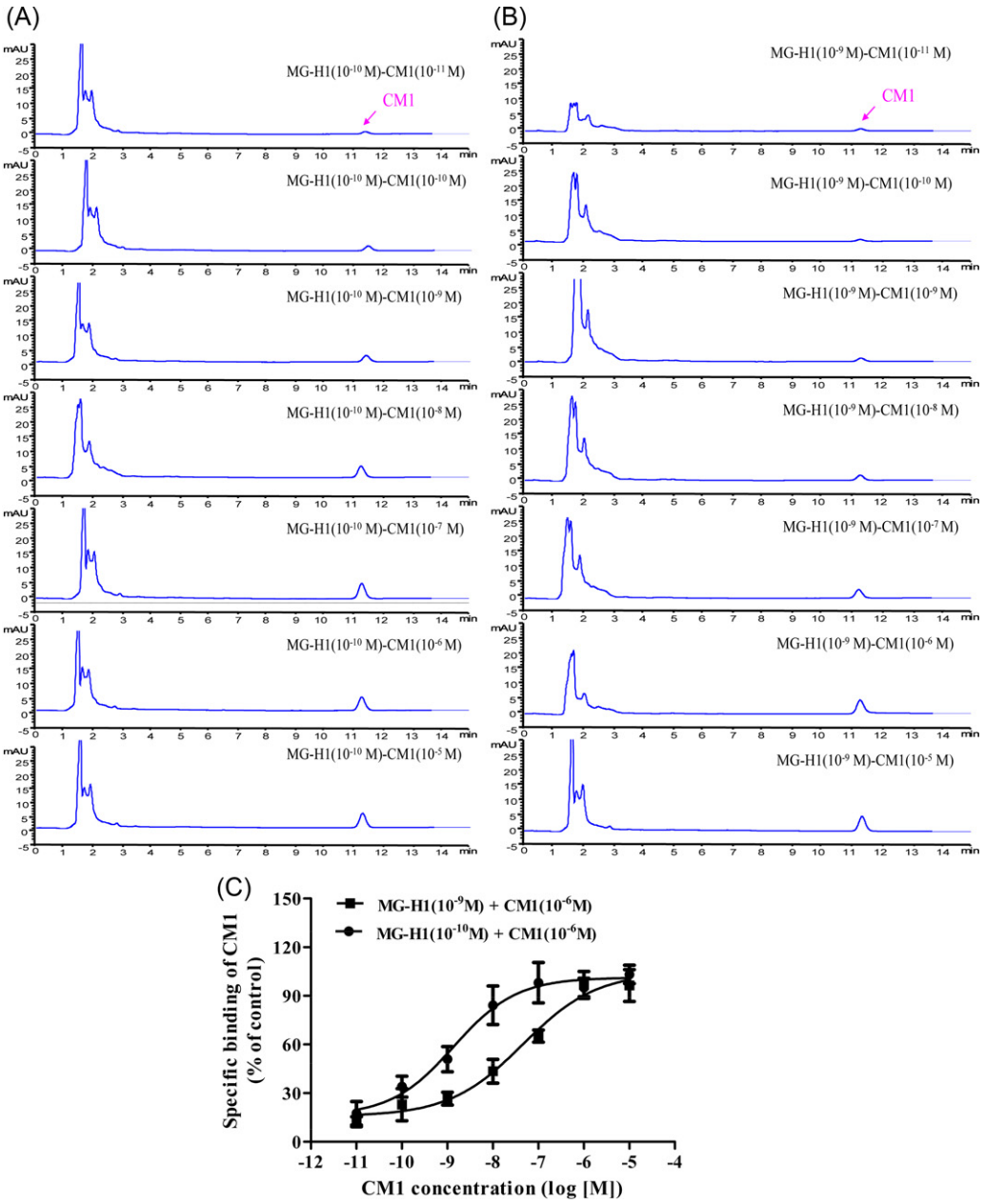

Fig. 7. Competition binding of CM1 with RAGE ligand MG-H1 on intact HUVEC. Cells were pre-treated with MG-H1 (10^{-9} or 10^{-10} M) for 60 min at 37 °C and then co-incubated for other 60 min in presence of increasing concentrations of CM1 (10^{-11} to 10^{-5} M). The binding rate was expressed as % of control binding and presented as means ± SD from individual experiments ($n = 3$).

binding sites (B_{max}) were calculated by non-liner regression analysis (Fig. 5C and D). Here, the K_D of CM1 and RAGE ligand MG-H1 were 3.65 and 4.12 nM, respectively. The results indicated that CM1 had higher affinity to cells than MG-H1. CM1 had more binding sites on cells than MG-H1 (i.e. 30.08 fmol/mg protein vs 18.72 fmol/mg protein). The half maximal inhibitory concentration (IC_{50}) of CM1 and MG-H1 were 3.148×10^{-9} M and 1.699×10^{-8} M, respectively. The results also demonstrated that CM1 had a higher affinity to intact cells under our experiment conditions. It may be associated with the two active groups of CM1 whereas one side-chain in MG-H1.

In our previous study, the compound CM1 block AGEs and HMGB1-induced endothelial dysfunction. It has been shown that CM1 attenuates the intercellular adhesion molecule-1 (ICAM-1) and transforming growth factor-β1 (TGF-β1) expressions in HUVEC CM1 also decreased AGEs-induced apoptosis and overexpression of inflammatory cytokines [12]. AGEs exert effects both directly through the formation of protein cross-links that alter the structure and function of extracellular matrix (ECM) and by interacting with specific cell surface receptors [18]. Engagement of RAGE by AGEs leads to the activation of several intracellular signaling pathways, including increasing the production of cytokines, such as transforming growth factor (TGF)-β and ICAM-1. Successful prevention and treatment of diabetic complications by blocking AGEs and RAGE activities have further indicated the pathogenic importance of AGEs [19]. Our research also shown that CM1 did not inhibit the formation of protein cross-links, it means that the effect of CM1 by AGEs–RAGE signaling pathways (in press). It strongly suggested

that the effect of CM1 on endothelial cell may be blocking the interaction of AGEs–RAGE signaling pathway. But the directive evidence is still needed.

3.3. Competitive binding of CM1 and MG-H1

In order to examine the competitive binding of CM1 with MG-H1, cells were pre-treated with MG-H1 (from 10^{-11} to 10^{-5} M) for 60 min at 37 °C and then co-incubated for next 60 min in presence of CM1 (10^{-6} M). As shown in Fig. 6A–C, the binding ratio of MG-H1 on HUVEC increased with addition of concentration. However, the binding ratio of CM1 decreased in presence of addition concentration of MG-H1. The results indicated that MG-H1 blocked the binding of CM1 with intact cells.

To further examine the competitive binding of CM1 with MG-H1, sub-saturating concentration of RAGE ligand MG-H1 (10^{-9} or 10^{-10} M) was pre-incubated with HUVEC for 60 min at 37 °C, and then the free MG-H1 was transferred to 10 ml tubes and cells were washed with PBS for three times. The cells were co-incubated with CM1 (10^{-11} to 10^{-5} M) for next 60 min 37 °C. As shown in Fig. 7A–C, the competition binding curves were steep. The IC_{50} of CM1 binding to cells under pre-incubation with different concentration MG-H1 were 1.37×10^{-9} M (10^{-10} M MG-H1) and 4.56×10^{-8} M (10^{-9} M MG-H1), respectively. Competitively binding properties of CM1 to RAGE with AGEs or other RAGE ligands on intact HUVEC is the mechanism of CM1 preventing AGEs induced endothelium dysfunction.

AGEs, nonenzymatic protein glycation reaction, has been recognized as a ligand of RAGE and plays a key role in pathophysiological damage for vascular endothelial cells [20]. In our experiment, MG-H1 rather than other RAGE ligands such as high mobility group box (HMGB-1), S100B, etc. was selected as a ligand for competition binding based on the following reasons: on one hand, HMGB-1 and S100B were chosen as ligand for competition binding owing to its endogenous characteristic. HMGB-1 was released passively from monocytes and endothelial cells whereas S100B was secreted from astrocytes in response to endotoxin [21,22]. In our previous experiment, HUVEC damage was induced by exogenous AGEs. MG-H1, a methylglyoxal-derived from imidazolones, is a typical cytotoxic compound in AGEs [15,23,24]. Hence, MG-H1 is more suitable for competition binding than HMGB-1 or S100B. On the other hand, HPLC analysis is easier for small compound MG-H1 (molecular weight: 228.2) than HMGB1 and S100B in that the detection interference in cell membrane model [25]. HMGB1 is a 35 kDa nuclear protein containing 215 amino acids and S100B protein is the homodimeric proteins [21,26]. Based on the above reasons, MG-H1 in AGEs is selected as RAGE ligand for the competition binding experiments. Here, our results also illustrated that MG-H1 was a appropriate ligand for HPLC analysis (Fig. 3A).

There is a growing body of evidences that the COOH-terminal motif of RAGE ligands such as HMGB-1 and S100B mediates the interaction with RAGE [27–29]. In chemical structure of MG-H1, arginine residues were also an COOH-terminal motif. The active group has a high affinity for the interaction of ligand and substrate recognition sites of N-terminal V domain (an important region responsible for recognition of ligands) in RAGE [30]. For the chemical structure of CM1, side chains of CM1 contain two methyl ester groups, namely, $COOCH_3$ groups. This group has higher affinity than COOH-terminal motif to RAGE on intact HUVEC. The saturated binding experiment also demonstrated that the K_D of CM1 was smaller than ligand MG-H1 (3.65 vs 4.12 nM) and IC_{50} of CM1 also was smaller than MG-H1 (3.148×10^{-9} M vs 1.699×10^{-8} M). The results support explicitly the speculation on higher affinity of CM1 than MG-H1 from chemical structure. Furthermore, the B_{max} of CM1 was higher than MG-H1 (30.08 vs 18.72 fmol/mg protein). This may be associated with two symmetrical $-COOCH_3$ groups in CM1 whereas only one –COOH in MG-H1. Of note that is our results have shown the CM1 acts as a competitive antagonist to blockade the binding of RAGE ligand MG-H1 to cells.

In classic competitive binding experiments, radioligand binding assay (RBA), which has high sensitivity and accuracy, has been used for competitive binding of ligand–receptor interaction. Compared with other methods, RBA has high specificity for receptor recognition, high sensitivity, fewer false positive and false negative features, etc. However, some disadvantages affect the use of RBA in practice, including radioactivity, environmental pollution, harmful radiation for human and long half-life [31]. In present experiments, HPLC method was performed successfully for analysis of competition binding of CM1 and MG-H1 on intact cells. However, it does not mean that HPLC method is superior to the RBA because of its limitation of UV detection and lower sensitivity than RBA. Our results demonstrated that CMC combined with HPLC was a sample, rapid, efficient and alternative methods for competitive binding of drug molecule and receptor in general laboratory without RBA research circumstance.

Taken together, our findings suggest that competitively block AGEs–RAGE signal path way is the mechanism of CM1 preventing AGEs inducing HUVEC damage. The established HPLC method could be an alternative approach for competitive binding of drug molecule to receptor owing to its rapid, reliable, and accurate advantageous features.

4. Conclusions

The most important result of this study was that competitively blocking AGEs–RAGE signal pathway is the mechanism of CM1 preventing AGEs-induced HUVEC damage. The established HPLC method could be an alternative approach for competitive binding of drug molecule to receptor, the advantage including rapid, simple, sensitive and efficient without needing radioligand and radiological research circumstance. But accumulate data still need to study its application practically.

Acknowledgements

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References

[1] H.O. Al-Mesallamy, L.N. Hammad, T.A. El-Mamoun, B.M. Khalil, J. Diabetes Complications 25 (2011) 174.
[2] B.K. Rodiño-Janeiro, M. González-Peteiro, R. Ucieda-Somoza, J. González-Juanatey, E. Alvarez, Diabetes Metab. Res. Rev. 26 (2010) 558.
[3] E. Nur, D.P. Brandjes, J.J. Schnog, H.M. Otten, K. Fijnvandraat, C.G. Schalkwijk, B.J. Biemond, CURAMA Study Group, Br. J. Haematol. 151 (2010) 69.
[4] S. Maeda, T. Matsui, M. Takeuchi, Y. Yoshida, R. Yamakawa, K. Fukami, S. Yamagishi, Pharmacol. Res. 63 (2011) 248.
[5] A. Bierhaus, P. Humpert, M. Morcos, T. Wendt, T. Chavakis, B. Arnold, D. Stern, P. Nawroth, J. Mol. Med. 83 (2005) 886.
[6] G. Srikrishna, H.J. Huttunen, L. Johansson, B. Weigle, Y. Yamaguchi, H. Rauvala, H.H. Freeze, J. Neurochem. 80 (2002) 1008.
[7] J.A. Nogueira-Machado, C.M. Volpe, C.A. Veloso, M.M. Chaves, Expert Opin. Ther. Targets 15 (2011) 1035.
[8] A. Villarreal, R.X. Aviles Reyes, M.F. Angelo, A.G. Reines, A.J. Ramos, J. Neurochem. 117 (2011) 332.
[9] T. Matsumoto, Y. Ozawa, K. Taguchi, T. Kobayashi, K. Kamata, Peptides 31 (2010) 353.
[10] T. Usui, M. Ohguchi, H. Watanabe, F. Hayase, Biosci. Biotechnol. Biochem. 72 (2008) 571.

[11] O. Brouwers, T. Teerlink, J. van Bezu, R. Barto, C.D. Stehouwer, C.G. Schalkwij, Ann. N. Y. Acad. Sci. 1126 (2008) 234.
[12] L. Feng, Y.H. Xu, S.S. Wang, W. Au-Yeung, Z.G. Zheng, R.S. Wang, Q. Zhu, P. Xiang, Phytother. Res. (2011).
[13] M. Straume, E. Freire, Anal. Biochem. 203 (1992) 268.
[14] R.A. Leppik, A. Mynett, S. Lazareno, N.J. Birdsall, Mol. Pharmacol. 57 (2000) 445.
[15] B.K. Kilhovd, A. Juutilainen, S. Lehto, T. Rönnemaa, P.A. Torjesen, K.F. Hanssen, M. Laakso, Atherosclerosis 205 (2009) 594.
[16] Y. Wang, B. Yuan, X. Deng, L. He, S. Wang, Y. Zhang, Q. Han, Anal. Bioanal. Chem. 386 (2006) 2011.
[17] W. Yu, B. Yuan, X. Deng, L. He, Z. Youyi, H. Qide, Anal. Biochem. 339 (2005) 205.
[18] S. Vasan, X. Zhang, A. Kapurniotu, J. Bernhagen, S. Teichberg, J. Basgen, D. Wagle, D. Shih, I. Terlecky, R. Bucala, A. Cerami, J. Egan, P. Ulrich, Nature 382 (1996) 278.
[19] D.J. Kelly, R.E. Gilbert, A.J. Cox, T. Soulis, G. Jerums, M.E. Cooper, J. Am. Soc. Nephrol. 12 (2001) 2107.
[20] J. Xue, V. Rai, D. Singer, S. Chabierski, J. Xie, S. Reverdatto, D.S. Burz, A.M. Schmidt, R. Hoffmann, A. Shekhtman, Structure 19 (2011) 732.
[21] G.E. Mullins, J. Sunden-Cullberg, A.S. Johansson, A. Rouhiainen, H. Erlandsson-Harris, H. Yang, K.J. Tracey, H. Rauvala, J. Palmblad, J. Andersson, C.J. Treutiger, Scand. J. Immunol. 60 (2004) 573.
[22] S. Eckert, P. Eyer, H. Mückter, F. Worek, Biochem. Pharmacol. 72 (2006) 365.
[23] T. Usui, H. Watanabe, F. Hayase, Biosci. Biotechnol. Biochem. 70 (2006) 1498.
[24] N. Rabbani, P.J. Thornalley, Amino Acids (2010).
[25] J. Li, R. Kokkola, S. Tabibzadeh, R. Yang, M. Ochani, X. Qiang, H.E. Harris, C.J. Czura, H. Wang, L. Ulloa, H. Wang, H.S. Warren, L.L. Moldawer, M.P. Fink, U. Andersson, K.J. Tracey, H. Yang, Mol. Med. 9 (2003) 45.
[26] G. Sorci, R. Bianchi, F. Riuzzi, C. Tubaro, C. Arcuri, I. Giambanco, R. Donato, Cardiovasc. Psychiatry Neurol. (2010).
[27] H.J. Huttunen, C. Fages, J. Kuja-Panula, A.J. Ridley, H. Rauvala, Cancer Res. 62 (2002) 4811.
[28] S. Banerjee, A. Friggeri, G. Liu, E. Abraham, J. Leukoc. Biol. 88 (2010) 979.
[29] M. Garbuglia, M. Verzini, G. Sorci, R. Bianchi, I. Giambanco, A.L. Agneletti, R. Dona, J. Biol. Chem. 285 (2010) 23146.
[30] Z.M. Li, C.W. Wei, Y. Zhang, D.S. Wang, Y.N. Li, J. Chromatogr. B 879 (2001) 1938.
[31] H. Du, J. Ren, S. Wang, L. He, Anal. Bioanal. Chem. 400 (2011) 3633.