

Research Article

Research on Inhibitory Effect of *Momordica charantia* L. Polyphenol on Vascular Endothelial Intercellular Adhesion Molecular-1 (ICAM-1) mRNA Abnormal Expression

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Abstract: The study held a research on the anti-oxidative damage effect and mechanism of *Momordica charantia* L. Polyphenol from gene level. Method: it tested the influence of *Momordica charantia* L. Polyphenol to human umbilical veins blood vessel endothelial cell strain (CRL-1730) ICAM-1 mRNA cellular expression caused by oxidative damage with RT-PCR method. Result: the experimental result indicated that the luminance and area values of electrophoretic band of 4, 5, and 6 with different doses of *Momordica charantia* L. Polyphenol for protection were all lower than positive control group, while higher than normal control group, furthermore, it presented a state that be increasing with the augment of *Momordica charantia* L. Polyphenol dose. The degree of protection of the three dose groups, high, medium and low, were separately 91.1, 82.7 and 37.3%, respectively after making a comparison in each group. Conclusion: *Momordica charantia* L. Polyphenol can inhibit cell adhesion molecular-1 expression and make a protective effect to vascular endothelial cell damage.

Keywords: *Momordica charantia* L. Polyphenol (MCLP), oxidative damage, vascular endothelial cell

INTRODUCTION

Momordica charantia L.. Also known as Chinese bitter melon, this herbaceous tropical vine is a tender perennial. The fruit is edible when harvested green and cooked (Yuan *et al.*, 2012). The taste is bitter. Bitter melon has twice the potassium of bananas and is also rich in vitamin A and C. It is a monoecious climber with dark green, deeply lobed leaves with hairs on it. The dioeciously flowers are yellow and the fruits oblong and lumpy with a light green to greenish-white, waxy skin (Jine-Shang *et al.*, 2012).

MCLP is the natural plant polyphenols active material extracted from balsam pear, which widely exists in its peel and flesh. In recent years, plenty of researches on development and utilization, physicochemical property, and biological function of MCLP have been made and achieved a breakthrough in abroad. It has been proved that MCLP possesses biological function in multiple aspects of scavenging free radical, anti-oxidation, antineoplastic, antiviral, as well as cardiovascular activity. MCLP can protect lipids and interdict radical chain reaction by competitively integrating with radical and oxidizing material, due to that it is with multiple phenol hydroxide radicals and easy to be oxidized. Pietta (Facino *et al.*, 1998a) discovered that the antioxidant activity of MCLP was

relatively stronger, and there was a positive correlation between TAA and polyphenol content, after making a study on the antioxidant activity of the chosen 11 kinds of vegetable drug and having a comparison of its Total Antioxidant capacity (TAA). Bagchi *et al.* (2001) found that the scavenging activity of MCLP was far higher than that of vitamin E after making a research on the scavenging activity of MCLP, VitC, VitE to oxygen radical (Chao *et al.*, 2011).

This thesis detected the in impact of MCLP on human umbilical veins blood vessel endothelial cell strain (CRL-1730) ICAM-1 mRNA cellular expression caused by oxidative damage with RT-PCR method, and made an investigation to anti-oxidative damage effect and mechanism of MCLP from gene level. Protective effect of MCLP on human endothelial cells apoptosis was studied through this method.

MATERIALS AND METHODS

Materials:

Cell strain and pretreatment: CRL-1730 was provided by Shanghai X-Y Biotechnology Co., Ltd, which was placed in DMEM culture medium containing 10% fetal calf serum, and fostered it into single-cell suspension under condition of 5% CO₂

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Table 1: Experimental classification situation

Group	Name	Added reagent 1	Added reagent 2	Concentration of the added MCLP (ug/mL)	Incubation time (h)
1	Normal control group	Nutrient solution 2mL	--	0	14
2	MCLP control group	Nutrient solution 2mL	--	100	14
3	Positive control group	Nutrient solution 2mL	Fenton reagent	0	14
4	Injure protection group 1	Nutrient solution 2mL	Fenton reagent	10	14
5	Injure protection group 2	Nutrient solution 2mL	Fenton reagent	100	14
6	Injure protection group 3	Nutrient solution 2mL	Fenton reagent	500	14

37°C, then prepared for use after digesting and regulating the cell density to be 1×10^5 when cell was overspreading the bottom of the bottle (Peilong *et al.*, 2013).

Momordica charantia L. polyphenol: It can be obtained by having ethanol digestion and purification to the peel and flesh of fresh balsam pear being degreased with petroleum ether. Its purity was >98.3% after liquid phase method determination.

Other reagents: Trizol reagent and Access RT-PCR kit were provided respectively by American Gibco Company and Promega Company.

Key instruments: DTC-3E gene amplification thermal cyclers were manufactured by Xian Tianlong Science and Technology Co., Ltd, Vidas 21 Image Analyzer was the product of Carl Zeiss AG.

Experimental method: Cell Adhesion Molecular (CAMS) is a large class of membrane protein mediating the identification and combination between cell and cell, cell and extracellular matrix, as well as between some plasma proteins. As one of many adhesion molecules, ICAM-1 participated in the process of the adhesion of leukocytes and endothelial cells, as well as moving outward the blood vessel, besides, it was in low level under normal circumstances, while its expression sharply rose with the stimulation of interferon- γ and other cell factors and cellular damage elements (Maffei *et al.*, 1994). Moreover, other researches reported that mRNA expression increased correspondingly when ICAM-1 level rose. Some protective factors, such as antioxidant, high-density lipoprotein, had function of restraining ICAM-1 mRNA expression (Bagchi *et al.*, 2001). This research made use of a new kind of molecular biological technique, reverse transcription PCR, to observe the change situation of cell ICAM-1 mRNA expression when vascular endothelial cell was injured by OH under condition of culture in vitro (Wei-Guo *et al.*, 2012), and the influence of MCLP to its expression, as well as to discuss the protective effect of MCLP to endothelial injury.

Experimental classification: Inoculate vascular endothelial cell under digestion to 6 pore plates with the density of 1×10^5 /mL, and each pole was with 4mL. Discard the original nutrient solution and add different reagents on the basis of the grouping after being

incubated for 8 h in accordance with the above condition. There were six groups shown in Table 1.

Among them, the added Fenton reagent was FeSO_4 and H_2O_2 , their final concentrations were respectively 12umol/L and 3umol/L.

RNA extraction: Take the treated six pore plates, and add 1ml of Trizol reagent in each pore after discarding nutrient solution. Then, remove detached and broken cells to centrifuge tube of 1ml after blow and beat. Inject 0.2 mL chloroform into each tube, then still it for three minutes, after that, centrifuge 15 min under 4°C in the speed of 10000 r/min. Absorb the colorless and transparent phase on upper layer to move it to another centrifuge tube, furthermore, add 0.5 mL isopropanol into each tube, and shake the tube severely for 15 sec, then still it for ten min, and centrifuge 10 min under the same condition. Discard the supernatant, add 1 mL ethanol configured by no-RNA enzyme water with the concentration of 75%, blend it with vortex mixing machine, then centrifuge for five minutes, obtain extraction by discarding the supernatant, and dissolve the obtained total RNA with 20 uL no-RNA enzyme water.

Primer design and synthesis: Design a pair of primers at the two ends based on the known ICAM-1 gene coding sequence, as well as the two-stage primer of internal reference housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (Brett *et al.*, 2012), the following was the base sequence:

ICAM-1:

Sense primer: 5'-GGCTGGAGCTGTTTGAGAAC-3'
Antisense primer: 5'-ACTGTGGGGTTCAACCTCTG-3';

GAPDH:

Sense primer: 5'-ATGGCACCGTCAAGGCTGAG-3';
Antisense primer: 5'-CGCCTGCTTACCACCTCTT-3'.

Inverse transcription compounding cDNA and PCR reaction: Inverse transcription and PCR reaction can be accomplished in the same buffer solution by making use of Access RT-PCR kit [30]. Primers of ICAM-1 and GAPDH were used for conducting RT-PCR reaction to

each RNA sample, and the main steps included: add 4 uL total RNA aqueous solution and 5 uL 10×buffer solution in each centrifuge tube successively, then, 1 uL primer was used respectively for upstream and downstream of dNTP 1 uL, ICAM-1 and GAPCH, furthermore, add 1ul AMV reverse transcriptase, and 1ul TfiDNA synthetase into no-RNA enzyme water until the reaction system be 50 uL. After that, have reverse transcription by placing into 42°C thermostatic water-bath for 60 min (through which to compound first chain of cDNA). Again, compound the second chain of cDNA and augment the gene segment by shifting to PCR instrument: inactivate AMV enzyme by staying in thermostatic water-bath of 95°C for two minutes, and make RNA/cDNA primer degenerate, then, stay in 95°C for thirty seconds, 60°C for 45 sec, and 72°C for 1 min and 30 sec. Circulate in this way for 35 times, and then finish the amplification after staying in 72°C for 7 min.

Agarose gel electrophoresis: Have electrophoresis in 1.5% agarose gel containing 0.5uL/mL ethidium bromide by taking 10 uL amplification product of ICAM-1and GAPDH in each experimental group, moreover, scan with gel and print to form images.

Electrophoresis image analysis: Analyze the optical density and electrophoretic bands area by applying Vidas 21 image analyzer, and the mean value of the two experiments was the result.

EXPERIMENTAL RESULTS

Electrophoretic bands can be seen at 625 bp in GAPDH contrast. Bands with different brightness emerged at 202 bp after having gel electrophoresis to the obtained cDNA amplification products of the six groups, which indicated that each group of ICAM-1 mRNA was expressed. It illustrated that the third group (positive control group) was brightest, the first group (normal control group) was obviously less brighter than that of the third group, stating that the expression level of the third group was higher than that of the first group, after making an observation to the brightness of the bands in each group. The brightness of the fourth, fifth, and sixth experimental groups was between the above two groups, and weakened successively (Fig. 1).

The determination results of image analyzer showed that the electrophoretic band area of the third group was the largest, groups of the fourth, fifth and sixth were lesser than the third group, moreover, their areas reducing successively, and their expression inhibition rates in sequence were 14.7, 49.8 and 56.9%, besides, the differences examined by statistics u among the three groups were with significance ($p < 0.05$). ICAM-1 mRAN expression inhibition rates, optical density, and electrophoretic band areas of each group were shown in Table 2. Expression inhibition rate (%) can be calculated by formula: (estimated value of

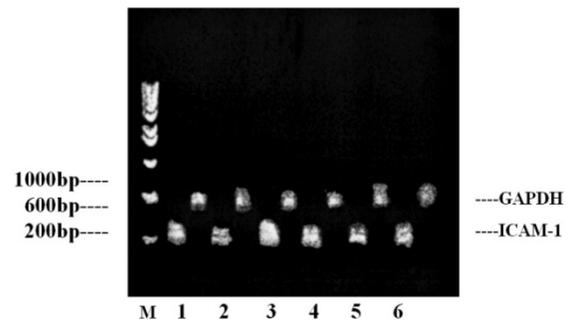


Fig. 1: RT-PCR amplification endothelial cells ICAM-1 cDNA electrophoretic image. M, molecular weight reference substance (DL2000 Marker); 1---6, separately are the electrophoretic bands of the six experimental groups

positive control group-estimated value of determination group)/estimated value of positive control group×100%; degree of protection (%) can be calculated by formula: (numerical value of positive control group-numerical value of determination group)/(numerical value of positive control group-numerical value of negative control group)×100%.

This experimental result showed that the electrophoretic band of GADPH emerged at 635 bp, besides, the gel electrophoresis image of PCR amplification product turned up bands also at 200 bp, which corresponded with the fragment length between upstream and downstream, which was analyzed and determined by DNA star software, and obtained arrays of ICAM-1and GADPH mRNA by Genbank search. It indicated that PCR amplification product showing on the band was ICAM-1 cDNA fragment, RNA extracted through reaction was complete without degradation (Facino *et al.*, 1998b). The electrophoresis images and data analysis results of each different treatment group all can be seen. Positive control group was higher than normal control group when compared their brightness and area value of electrophoresis bands, which indicated that free radical injury can cause the augment of endothelial cells mRNA expression. The results of groups of 4th, 5th, and 6th, protected by adding different doses of MCLP, were all lower than positive control group, and higher than normal control group, moreover, ICAM-1 mRAN expression presented the trend of reducing in sequence with the increase of MCLP dose, which illustrated that MCLP can inhibit ICAM-1 expression caused by oxidative damage. It can be shown that the degree of protection of the three groups with high, medium, low doses separately was 91.1, 82.7, and 37.3% ($p < 0.05$), which revealed their significant dose-effect relationship, and proposed that MCLP possessed good function in defending the oxidative damage of endothelial cells, and inhibiting the ICAM-1 expression of endothelial cells. In addition,

Table 2: Analysis result of AGE image

Group	MCLP dose (µg/mL)	Area			Optical density		
		Estimated value	Suppression degree (%)	Degree of protection (%)	Estimated value	Suppression degree (%)	Degree of protection (%)
Normal control group	0	545	--	--	0.170	--	--
MCLP control group	100	282	82.1a	--	0.183	27.4a	--
Positive control group	0	1683	0.0	0.0	0.144	0.00	0.00
MCLP(10)group	10	1269	14.7a	37.3a	0.153	4.80a	42.7a
MCLP(100) group	100	748	49.8ab	82.7a	0.157	7.50a	68.1ab
MCLP(500) group	500	686	56.9ab	91.1a	0.168	11.7ab	95.2ab

a: compared with positive control group p<0.05; b: compared with group four p<0.05

this experimental result also revealed that ICAM-1 mRNA expression inhibition rate of MCLP control group distinctly decreased compared with normal control group, and their difference had significance (p<0.05) which indicating that MCLP also possessed the function of preventing self-oxidative damage of normal tissues.

CONCLUSION

Researchers in domestic and in abroad in recent years showed that MCLP can effectively eliminate multiple free radicals, interdict lipid per-oxidation reaction, and lessen oxidative damage. It can be observed from this experiment that ICAM-1 mRNA expression increased after vascular endothelial cell was injured by .OH free radical, while, MCLP possessed inhibition effect on the increase of such class of expression, which illustrated that MCLP can inhibit ICAM-1 expression of the cell, and was with protective effect on vascular endothelial cell damage. It still needed a further study to the increase of ICAM-1 mRNA expression after vascular endothelial cell was injured by .OH free radical, as well as the mechanism of the function of MCLP in inhibiting its expression.

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