



Carnosine inhibits high glucose-induced mesangial cell proliferation through mediating cell cycle progression

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ABSTRACT

Increased mesangial cell proliferation is one of the major pathologic features in the early stage of diabetic nephropathy (DN). Carnosine is an endogenously synthesized dipeptide that has been reported as a protective factor in diabetic nephropathy. However, the underlying mechanism involved in this effect remains to be elucidated. In this study, the effect of carnosine on cell proliferation and its underlying mechanisms were investigated in cultured rat mesangial cells by the methylthiazolotetrazolium (MTT) assay, the 5-bromo-2-deoxy-uridine (BrdU) cell proliferation assay, flow cytometry and western blotting. The results showed that pretreatment of mesangial cells with carnosine significantly inhibited cell proliferation and DNA synthesis in a dose-dependent manner by increasing the cell population in G1 and reducing that in S-phase. In addition, carnosine could reverse high glucose-induced down-regulation of cyclin-dependent kinase inhibitor p21 but not that of p27. Furthermore, carnosine could reduce the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK). Taken together, these results suggest that carnosine can inhibit mesangial cell proliferation by modulating cell cycle progress, indicating that carnosine could be a potential therapeutic agent for the prevention of DN in the early stage.

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1. Introduction

Diabetic nephropathy (DN), one of the most severe microvascular complications of type 1 and type 2 diabetes, is a major cause of end-stage renal disease [1]. Though the precise mechanisms of DN have not been elucidated, several *in vivo* studies have recently unraveled the significance of mesangial cell proliferation in the early stages of DN. It seems that mesangial hypercellularity precedes an increase in the extracellular matrix proteins and glomerular sclerosis, hallmarks of diabetic nephropathy [2]. Mesangial cell proliferation has been shown to be correlated with the degree of glycemic control, indicating that abnormally high blood glucose may be a crucial risk factor for DN [3]. There are few data on the effects of pharmacological intervention on mesangial cell proliferation in DN. Therefore, a search for agents

potentially capable of inhibiting mesangial cell proliferation could be of significant importance.

The mammalian cell cycle is a tightly controlled nuclear event regulated positively by cyclin-dependent kinases (CDK) and their cyclin-regulatory subunits, and negatively by cyclin-dependent kinase inhibitors (CDKI). Progression of the cell cycle is therefore regulated by the balance between the levels and activities of cyclin-CDK complexes and CDKI [4]. Some studies have shown a role for CDKI p21 and p27 in the proliferation of rat mesangial cells [5–7]. Terada et al. reported that over-expression of p21 using adenovirus vectors ameliorated serum- and PDGF-induced cell proliferation of rat mesangial cells [5,8].

Carnosine (β -alanyl-L-histidine) is an endogenously synthesized dipeptide, widely distributed in brain, muscle, kidney, stomach tissues and in large amounts in skeletal muscle [9–13]. Carnosine has been demonstrated to possess a number of biological roles such as an anti-inflammatory agent, free radical scavenger and protein glycosylation inhibitor [14,15]. More recently, it has been suggested that carnosine may confer renoprotection on ischemia/reperfusion (I/R)-induced acute renal failure in rats [16]. In addition, carnosine can delay diabetic deterioration in diabetic Balb/cA mice and protect human low density lipoprotein against oxidation and glycation [10]. Recently, Van der Woude studied DNA polymorphisms in patients with diabetic nephropathy and found that carnosine was an important protective factor in the pathogenesis of DN; further investigation suggested that

Abbreviations: DN, diabetic nephropathy; RMC, rat mesangial cell; CDKI, cyclin-dependent kinase inhibitor; CDK, cyclin-dependent kinase; ERK1/2, extracellular signal-regulated kinase 1/2; p38 MAPK, p38 mitogen-activated protein kinase; p-ERK1/2, phospho-extracellular signal-regulated kinase 1/2; p-p38 MAPK, phospho-p38 mitogen-activated protein kinase; AGEs, advanced glycation end products; ROS, reactive oxygen species.

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carnosine inhibited the production of transforming growth factor beta and extracellular matrix when the renal cells were exposed to 25 mM high glucose [17]. However, no information is available on the effect of carnosine on the proliferation of rat mesangial cells induced by high glucose.

Thus, the aim of this study was to investigate whether or not carnosine could inhibit high glucose-stimulated cell proliferation and the possible underlying mechanisms in a rat mesangial cell line.

2. Materials and methods

2.1. Cell culture and reagents

The established rat mesangial cell line (RMC) HBZY-1 was obtained from The Chinese Center for Type Culture Collection (Wuhan, China). The cells were cultured in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in an atmosphere containing 5% CO₂. RMC cells between passages 3 and 10 were used for experiments. After pre-incubation in DMEM supplemented with 0.1% fetal calf serum for 24 h, cells were then treated with various concentrations of glucose and/or carnosine as indicated: normal glucose group (NG, 5.6 mM glucose); high glucose group (HG, 25 mM glucose); high glucose and carnosine group (HG + CAR, 25 mM glucose and 20 mM carnosine); normal glucose and carnosine group (NG + CAR, 5.6 mM glucose and 20 mM carnosine); mannitol group (MN, 5.6 mM glucose and 19.4 mM mannitol). Cells were incubated for another 12 h, 24 h or 48 h before further investigation. DMEM (5.6 mM or 25 mM glucose) was purchased from GIBCO BRL (GIBCO/Invitrogen Corp, Carlsbad, CA, USA). Mannitol and carnosine were from Sigma (St. Louis, MO, USA).

2.2. Cell proliferation assay

The MTT reduction assay was used as a qualitative index of cell viability. After 12 h, 24 h or 48 h incubation with different compounds as described above, 20 µl MTT (5 mg/ml, Invitrogen Corp, Carlsbad, CA, USA) was added and cells were cultured for additional 4 h. Subsequently, cells were lysed using dimethylsulfoxide (150 µl/well, Pierce Biotechnology, USA). When the formazan crystals were completely dissolved, the optical density (OD) was measured at 490 nm using a Microplate Reader Model 3550-UV Spectrophotometer (Bio-Rad Laboratories, Marnes La Coquette, France).

2.3. The LDH toxicity assay

The cytotoxicity was estimated by measuring lactate dehydrogenase (LDH) release into culture medium. Cultured cells were treated with or without 20 mM carnosine for 24 h and LDH activity was assayed by absorbance change at a wavelength of 440 nm with an LDH assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. DNA synthesis assay

A colorimetric immunoassay kit (Cell proliferation ELISA, BrdU (colorimetric), Boehringer Mannheim GmbH, Mannheim, Germany) was used for quantification of cell proliferation. This assay is based on the measurement of BrdU incorporation during DNA synthesis. Briefly, the cells were seeded in 96-well plates; after pre-incubation in DMEM supplemented with 0.1% fetal calf serum for 24 h, the cells were treated with various concentrations of glucose and/or carnosine for 24 h. They were then labeled with BrdU for 3 h at 37 °C, washed, and fixed and stained with anti-BrdU antibody for 90 min at 37 °C. After three washes, the substrate, tetramethylbenzidine, was added, followed by incubation for 30 min. A blocking solution (1 M H₂SO₄) was then added, and the absorbance of the samples was measured at 450 nm with a reference wavelength of 690 nm using an ELx800 multiwell plate reader (Bio-Tek Instruments; Winooski, VT).

2.5. Flow cytometry

Cell cycle analysis was performed using flow cytometry. After 24 h treatment with different compounds, cells were harvested and fixed in 70% ethanol and stored at –20 °C. They were then washed twice with phosphate buffered saline (PBS), harvested by trypsinization, centrifuged, and suspended with 1 ml cold PBS and then fixed in methanol for 30 min on ice. Following two washes with PBS, fixed cells were incubated in RNase (25 µg/ml) at 37 °C for 30 min, followed by staining of the DNA with propidium iodide (50 µg/ml) at 4 °C for 30 min in the dark. Then each sample was analyzed using a Coulter Epics XL Flow Cytometer (Miami, FL) and the proportion (percentage) of cells within the G1, S, and G2/M phases of the cell cycle were determined.

2.6. Western blotting

After being treated with different compounds for 24 h, cells were harvested and washed with ice-cold phosphate buffer. The whole-cell extracts were obtained by lysing cells with buffer containing 150 mM NaCl, 0.1% Triton X-100, 0.5% Deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 7.0) and 1 mM EDTA. Protein concentrations were determined using the BCA method (Beyotime Institute of Biotechnology, China). Equal amounts of protein were loaded, separated by SDS-PAGE and transferred to nitrocellulose membranes. After being blocked with 5% skimmed milk in Tris-buffered saline (pH 7.6) (TBS) at room temperature, the membranes were incubated overnight at 4 °C with primary antibodies for p21 (1:500; Santa Cruz, CA, USA), p27 (1:1000; Thermo Fisher Scientific Anatomical Pathology, CA, USA), β-actin (1:500; Santa Cruz), phosphor-ERK1/2 (1:500; Santa Cruz), ERK1/2 (1:500; Santa Cruz), p38

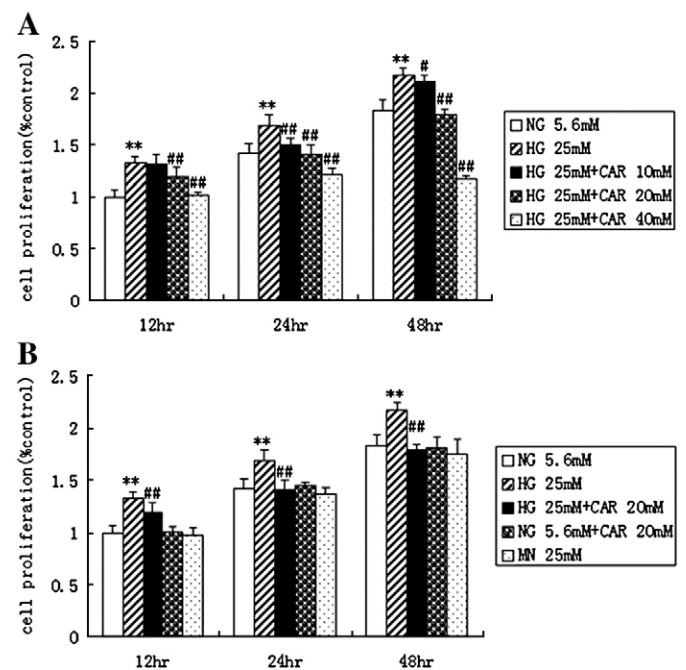


Fig. 1. Effect of carnosine on mesangial cell proliferation stimulated by high glucose using the MTT assay. (A) Carnosine inhibits mesangial cell proliferation induced by high glucose at a final concentration 10, 20, 40 mM, respectively, in culture media. RMC were preincubated for 2 h with various concentrations of carnosine, and then stimulated with high glucose for different time intervals. (B) Effect of 20 mM carnosine on mesangial cell proliferation under normal or high glucose condition. NG: normal glucose 5.6 mM, HG: high glucose 25 mM, HG + CAR: high glucose 25 mM and carnosine 20 mM, NG + CAR: normal glucose 5.6 mM and carnosine 20 mM, MN: 25 mM, glucose 5.6 mM and mannitol 19.4 mM. Values are given as mean \pm S.D. from 3 independent experiments in triplicate and $p < 0.05$ is considered statistically significant. ** $p < 0.01$ vs NG; # $p < 0.05$ vs HG, ## $p < 0.01$ vs HG.

MAPK (1:1000; Cell Signaling, Beverly, MA, USA) and phosphor-p38 MAPK (1:1000; Cell Signaling). After being incubated with the respective second antibody, immune complexes were detected using ECL western blotting reagents. Immunoreactive bands were quantified using the Tanon GIS Gelatum imaging system (Shanghai, China). Values were corrected with the absorbance of the internal control (β -actin). The activation of ERK1/2 was expressed as the ratio of phosphor-ERK1/2 versus ERK1/2 and the activation of total p38 MAPK was expressed as the ratio of phosphor-p38 versus total p38.

2.7. Statistical analysis

The differences were tested using ANOVA. All values are expressed as mean \pm S.D., and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Effect of carnosine on cell proliferation induced by high glucose in rat mesangial cells

Mesangial cell proliferation was evaluated using MTT analysis. The results showed that compared with the NG group, 25 mM glucose (HG) alone increased RMC proliferation after treatment for 12 h, 24 h and 48 h, respectively ($p < 0.05$). However, addition of carnosine inhibited high glucose-induced RMC proliferation in a dose-dependent manner at concentrations ranging from 10 to 40 mM (Fig. 1A). As expected, 25 mM mannitol, as an osmotic control, did not alter mesangial cell growth, suggesting that the HG-triggered RMC proliferation was not due to high osmolarity. In addition, 20 mM carnosine had no effect on RMC growth with normal glucose exposure (Fig. 1B).

24 h treatment with 20 mM carnosine did not affect the concentration of lactate dehydrogenase (LDH) in the supernatant (NG, 0.688 ± 0.0313

versus NG + CAR, 0.678 ± 0.0249 , $p > 0.05$); Moreover, the detached cells in the supernatant were less than 5% and did not differ from the NG group, suggesting that the antiproliferative effect of carnosine upon cultured rat mesangial cells was not due to carnosine's cytotoxicity. Therefore, 20 mM carnosine was used in all subsequent studies as it achieved the desired inhibition without any obvious toxicity.

3.2. Effects of carnosine on DNA synthesis in rat mesangial cells measured by the BrdU cell proliferation assay

To assess the effect of carnosine on DNA synthesis in RMC proliferation, a BrdU cell proliferation assay was carried out. As shown in Fig. 2, high glucose (25 mM) caused an increase in DNA synthesis by 40% ($p < 0.05$), in agreement with the findings of Kim et al. [18]. Similarly, carnosine decreased DNA synthesis in a dose-dependent fashion in RMC cell proliferation stimulated by high glucose. In contrast, neither 20 mM carnosine nor 25 mM mannitol affected DNA synthesis in mesangial cells under normal glucose conditions. Such a result indicated that carnosine treatment inhibited BrdU incorporation into DNA synthesis stimulated by high glucose within mesangial cells.

3.3. Effect of carnosine on cell cycle distribution induced by high glucose in rat mesangial cells

In order to further evaluate the effect of carnosine treatment upon cell cycle profiles, we then performed flow cytometry. Fig. 3 shows that high-glucose treatment decreased the proportion of cells in G1 (from $80.7 \pm 1.6\%$ to $64.8 \pm 2.9\%$, $p < 0.01$), and increased that in S phase (from $11.9 \pm 0.9\%$ to $27.0 \pm 1.4\%$, $p < 0.01$), indicating that high glucose could promote cell cycle progression. In contrast, the addition of carnosine increased the number of cells in G1 (from $64.8 \pm 2.9\%$ to $69.1 \pm 0.8\%$, $p < 0.01$) and decreased that in S phase (from $27.0 \pm 1.4\%$ to $21.3 \pm 0.8\%$, $p < 0.05$). These results showed that carnosine could block high-glucose induced cell cycle progression by inhibiting the G1–S phase transition and arresting cells in G1.

3.4. Effect of carnosine on protein expression of p21 and p27 induced by high glucose in rat mesangial cells

For mammalian cells, cell proliferation requires the coordinated interaction of cyclins and cyclin-dependent kinases to drive cells through the cell cycle. Cyclin-dependent kinase inhibitors can bind cyclin-CDK complexes and cause G1 arrest [19,20]. To determine the mechanisms of carnosine's inhibition of cell cycle progression, western blot analyses were performed for p21 and p27. As shown in Fig. 4A and B, compared with cells treated with a normal glucose concentration, the expression level of p21 was significantly reduced by a single high glucose treatment for 24 h; interestingly, in the presence of carnosine, high-glucose-induced reduction of the p21 protein level was diminished, bringing it to a level comparable to the NG group. Carnosine alone did not show any effect on p21 expression ($p > 0.05$). In contrast, the p27 expression level was unchanged in the presence of glucose and/or carnosine, suggesting that p27 did not participate in the modulation of mesangial cell proliferation.

3.5. Effects of carnosine on the phosphorylation levels of ERK1/2 and p38 MAPK induced by high glucose in rat mesangial cells

Since the activation of ERK1/2 and p38 MAPK signaling is a key step in the proliferation process of a variety of cell types [21], western blot analysis was performed to evaluate the role of the active form (phosphorylated) of these signaling proteins in high-glucose-induced proliferation. As shown in Fig. 5A, treatment of rat mesangial cells with high glucose for 24 h alone led to a prominent increase in the phosphorylation level of ERK1/2. Co-incubation with a MEK inhibitor (50 μ M PD-98059) partly reversed the high-glucose-induced increase

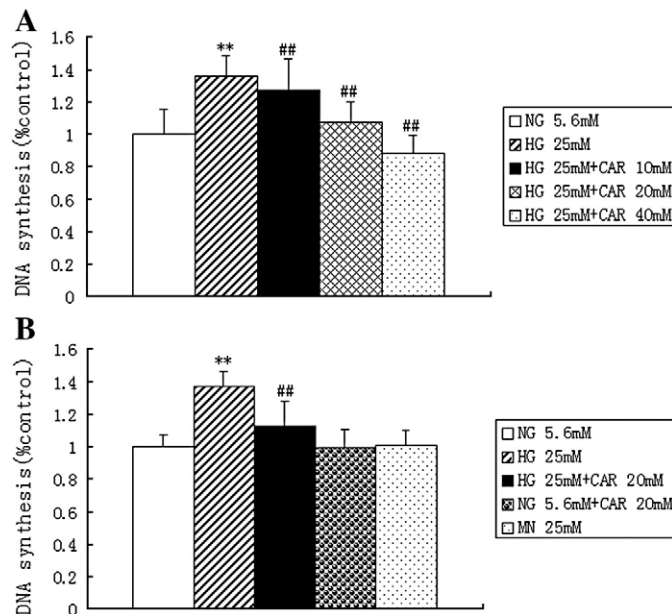


Fig. 2. Carnosine inhibits high-glucose-induced DNA synthesis in mesangial cells. DNA synthesis was measured by ELISA according to the manufacturer's instructions. (A) Carnosine inhibits high-glucose-induced DNA synthesis in a dose-dependent manner in mesangial cells. (B) Effect of 20 mM carnosine on DNA synthesis under normal or high glucose condition. NG: normal glucose 5.6 mM, HG: high glucose 25 mM, HG + CAR: high glucose 25 mM and carnosine 20 mM, NG + CAR: normal glucose 5.6 mM and carnosine 20 mM, MN: 25 mM, glucose 5.6 mM and mannitol 19.4 mM. Results are expressed as percentage of NG. Values are given as mean \pm S.D. from 3 independent experiments in triplicate and $p < 0.05$ is considered statistically significant. ** $p < 0.01$ vs NG, ## $p < 0.01$ vs HG.

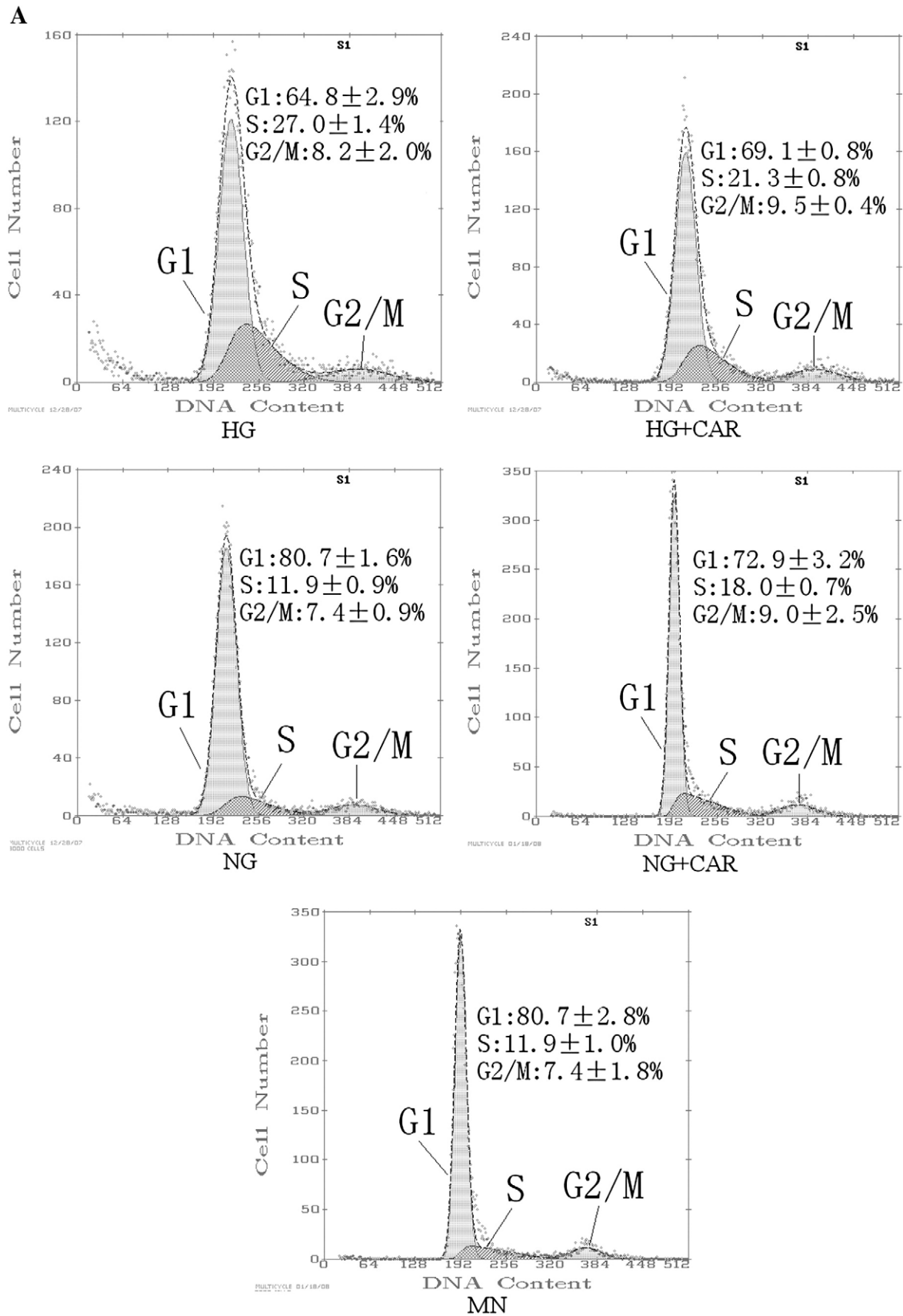


Fig. 3. Effect of carnosine on cell cycle distribution induced by high glucose in mesangial cells. Synchronized cells were treated with different concentrations of glucose and carnosine for 24 h, DNA was stained with propidium iodide and assayed by flow cytometry. NG: normal glucose 5.6 mM, HG: high glucose 25 mM, HG + CAR: high glucose 25 mM and carnosine 20 mM, NG + CAR: normal glucose 5.6 mM and carnosine 20 mM, MN: 25 mM, glucose 5.6 mM and mannitol 19.4 mM. Data are expressed as a percentage of total cells. Values are given as mean ± S.D. from 3 independent experiments and $p < 0.05$ is considered statistically significant. ** $p < 0.01$ vs NG, ## $p < 0.01$ vs HG. The gray area represents G1 (left) and G2 (right) and hatched area represents the number of cells in S phase.

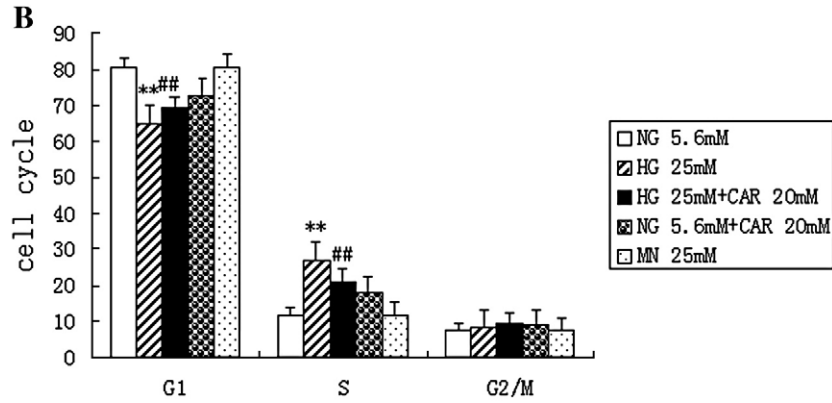


Fig. 3 (continued).

in ERK1/2 phosphorylation ($p < 0.05$). In Fig. 5B and C, 20 mM carnosine alone was found to decrease the phosphorylation level of ERK1/2 and p38 MAPK significantly under high glucose conditions ($p < 0.05$). In Fig. 5D, both the MEK inhibitor PD-98059 and the p38 inhibitor SB-

203580 inhibited mesangial cell proliferation stimulated by high glucose, and this result reveals that both the ERK1/2 and p38 MAPK pathways take part in the regulation of RMC proliferation. Together, these results show that the inhibition of carnosine on RMC proliferation stimulated by high glucose is mediated by the signaling pathways of both ERK and p38 MAPK.

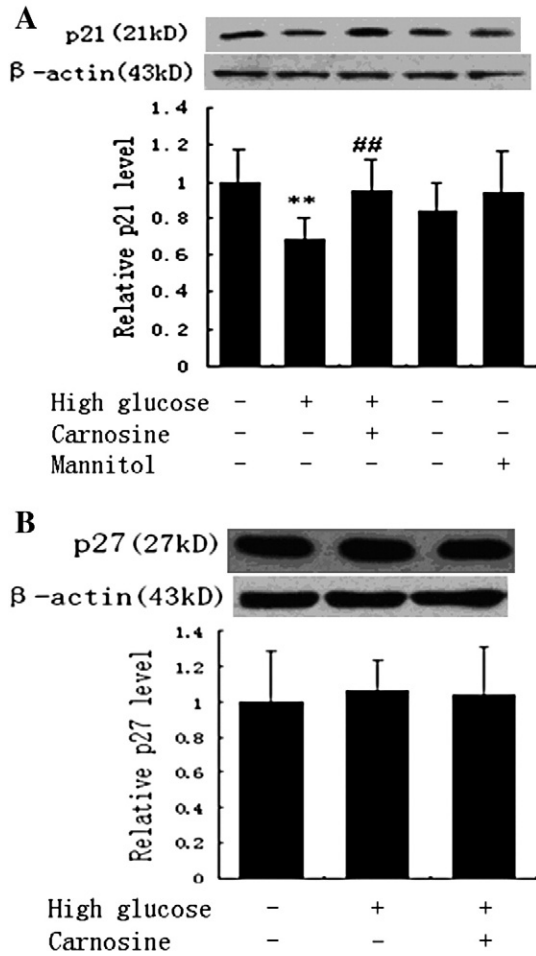


Fig. 4. Effect of carnosine on the expression level of cell cycle protein p21 and p27 under high glucose conditions in mesangial cells. Representative blots for p21 and p27 protein were chosen from 3 independent experiments and quantitative data for p21 protein and p27 were measured by western blotting. Actin was detected to confirm equal loading. NG: normal glucose 5.6 mM, HG: high glucose 25 mM, HG + CAR: high glucose 25 mM and carnosine 20 mM, NG + CAR: normal glucose 5.6 mM and carnosine 20 mM, MN: 25 mM, glucose 5.6 mM and mannitol 19.4 mM. Values are given as mean \pm S.D. and $p < 0.05$ is considered statistically significant. ** $p < 0.01$ vs NG, ## $p < 0.01$ vs HG.

4. Discussion

In the current study, we show an inhibitory effect of carnosine on high-glucose-induced RMC proliferation. To elucidate the underlying mechanism, we demonstrated that the inhibitory effect of carnosine acts via inhibiting DNA synthesis, resulting in arrest of the cell-cycle transition from G1 to S phase. The up-regulation of p21 protein expression was responsible for this cell cycle arrest. Additionally, the signaling pathways of p38 and ERK1/2 were shown to participate in mesangial cell proliferation stimulated by high-glucose exposure, and carnosine-induced inhibition was correlated with the alteration in phosphorylation level of p38 and ERK1/2.

Mesangial cell proliferative responses to a variety of stimuli are associated with matrix accumulation and the development of glomerulosclerosis, which finally leads to progressive renal disease. A high glucose concentration has been shown to contribute mainly to uncontrolled cell proliferation in mesangial cells, distal tubular epithelial cells and vascular smooth muscle cells in diabetes [22]. In this study, rat mesangial cells were used as an *in vitro* model that has been widely used in studying cell proliferation in the early stage of diabetic nephropathy. Because clinical trials have demonstrated that high glucose is the principal cause of renal damage in both type 1 and type 2 diabetes [23], high glucose was employed to stimulate RMC proliferation in this study. In the proliferation assay, we demonstrated that carnosine potently inhibited the cell proliferation and DNA synthesis induced by high glucose in a dose-dependent manner at 12 h, 24 h and 48 h. However, the antiproliferative effect of carnosine was not due to cytotoxicity, as demonstrated by the LDH assay. Our results also agreed with those of Janssen et al. in that carnosine exhibited antiproliferative activity at millimolar concentrations [17].

In cell cycle progression, traversing the G1–S phase boundary is coupled to DNA synthesis, followed by entry into G2 and finally mitosis occurs in M phase. Here, compared with normal glucose treatment, high-glucose significantly increased the cell population at S-phase (from $11.9 \pm 0.9\%$ to $27.0 \pm 1.4\%$, $p < 0.01$) and decreased that at G1-phase (from $80.7 \pm 1.6\%$ to $64.8 \pm 2.9\%$, $p < 0.01$), whereas 20 mM carnosine could block the G1–S phase transition by high glucose.

It has been reported that CDK inhibitors, in particular those of the Cip/Kip family, contribute to cell-cycle regulatory functions [24–26]. The Cip/Kip family includes the CDK inhibitors p21/WAF1/CIP1 (p21),

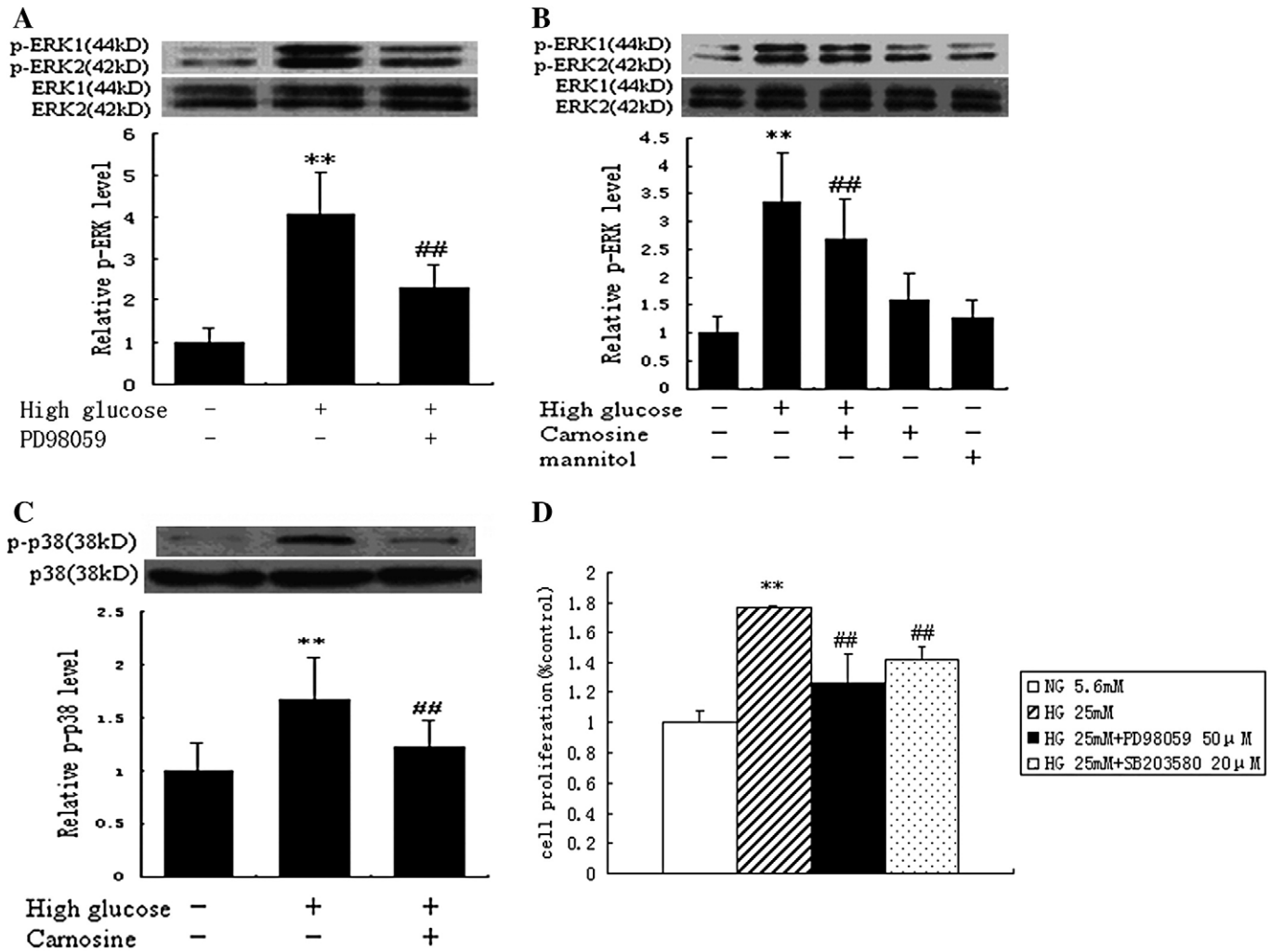


Fig. 5. Effect of carnosine on the phosphorylation level of ERK1/2 and p38 in mesangial cells measured by western blot. Mesangial cells were maintained in normal glucose or high glucose conditions for 24 h. (A) MEK inhibitor PD-98059 reverses the increase of the phosphorylation level of ERK1/2 induced by high glucose. (B) Carnosine decreases the increase of the phosphorylation level of ERK1/2 stimulated by high glucose in mesangial cells. (C) Carnosine reduces the increase of the phosphorylation level of p38 stimulated by high glucose in mesangial cells. (D) Either the MEK inhibitor PD-98059 or the p38 inhibitor SB-203580 inhibits mesangial cell proliferation stimulated by high glucose as measured by the MTT assay. Results are expressed as percentage of NG. NG: normal glucose 5.6 mM, HG: high glucose 25 mM, HG + CAR: high glucose 25 mM and carnosine 20 mM, NG + CAR: normal glucose 5.6 mM and carnosine 20 mM, MN: 25 mM, glucose 5.6 mM and mannitol 19.4 mM. p-ERK1/2 and p-p38 represent the phosphorylated forms of ERK1/2 and p38 respectively. ERK1/2 and p38 represent the total (phosphorylated and unphosphorylated) ERK1/2 and p38. Values are given as mean \pm S.D. from 3 separate experiments and $p < 0.05$ is considered statistically significant. ** $p < 0.01$ vs NG, ## $p < 0.01$ vs HG.

p27/kip1(p27) and p57/kip2(p57), which share homology in the amino-terminal domain, the region that mediates inhibition of CDK activity. Recent reports demonstrated that p27 and p21 were critically involved in the G1-phase cell cycle arrest in mesangial cells when the cells were exposed to high-glucose in experimental type 1 and type 2 diabetic mice [27]. In addition, Kim and Han reported that high-glucose-induced signaling down-regulated the expression level of p21 and p27 in mouse embryonic stem cells [28]. In our present study, the high-glucose-induced proliferation of RMC cells was associated with a decrease in p21 protein expression, but not with that of P27; this result is consistent with a report by Danesh et al. [2] that carnosine reversed the down-regulation of p21 at the cell-cycle level. Our result indicates that p21 may play a major role in carnosine-induced G1 arrest in mesangial cells.

Recently, three mitogen-activated protein kinase (MAPK) families have been identified and characterized [29]: extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 MAP kinase. These kinases can be activated by various extracellular stimuli, such as growth factors and environmental stresses, and they play an important role in signal transduction cascades that lead to

alterations in cell growth and other key functions [29,30]. Although the precise mechanism underlying RMC proliferation is not fully elucidated, the ERK1/2 MAPK pathway was found to be more responsive to high glucose stimuli [31]. Moreover, it has been reported that ERK1/2 is activated in glomeruli of diabetic rats as well as in mesangial cells cultured under high-glucose conditions [32,33]. On the other hand, ERK1/2 activation in some cells is believed to occur through a PKC-dependent mechanism [32]. Interestingly, the role of p38 activation in the pathogenesis of mesangial cell proliferation and extracellular matrix accumulation has been demonstrated by extensive evidence from several types of renal lesion, including inflammatory renal disease, ischemic or toxic renal injury and diabetic nephropathy [2,26,34]. The inhibition of p38 by specific blockers such as SB-239063 has proved effective in preventing or ameliorating mesangial cell proliferation, extracellular matrix accumulation and progressive glomerulosclerosis [35]. Also, several agents reportedly could exert their renal protective effects by inhibiting p38, such as aminoguanidine [36], mycophenolic acid [37] and adrenomedullin [38]. In contrast to these two kinases, JNK is not activated in the glomeruli

of diabetic rats or stimulated in mesangial cells upon exposure to high glucose [39]. These observations led us to suggest the participation of p38 MAP kinase and ERK1/2 MAP kinase in mesangial cell proliferation. Our results showed that high concentrations of extracellular glucose stimulated the phosphorylation level of p38 MAP kinase and ERK1/2 MAP kinase in mesangial cells. However, carnosine treatment significantly decreased p-ERK and p-p38 activities. This result is consistent with that observed in cultured human umbilical vein endothelial cells (HUVECs) in which the suppressive effects of emodin on VEGF-induced HUVEC proliferation is accompanied by decreasing p38 phosphorylation [40]. Thus, we demonstrate that carnosine could ameliorate RMC proliferation via inhibiting the activities of p-ERK and p-p38 for the first time.

Although the mechanisms of RMC proliferation induced by high glucose are still poorly defined, there are growing evidences showing that several molecules may play roles in the etiology. Recently, reactive oxygen species (ROS) and advanced glycation end products (AGEs) are well documented in diabetes as well as in model systems treated with high glucose. In addition, these species have been frequently shown to augment cell proliferation and DNA synthesis, and suggested as the main contributor to diabetic nephropathy in affected subjects [41]. As a potent hydrophilic antioxidant, carnosine has been reported to reduce the intracellular ROS production in neuronal cells and attenuate AGEs formation in dialysis fluids. Therefore, apart from its inhibitory effect on cell cycle, it is also possible that carnosine may exert its effect via its powerful antiglycative/antioxidative pathway [14,42,43].

Thus, in the present study, we have demonstrated that carnosine can inhibit high-glucose-induced mesangial cell proliferation. The mechanism of growth suppression induced by carnosine may be due to cell cycle arrest at G1. Further investigation of the cell cycle arrest revealed that carnosine increased p21 protein level through the inhibition of the phosphorylation level of ERK1/2 and p38. Taken together, these data strongly suggest a potential role of carnosine in the treatment of diabetic nephropathy in the future.

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