Short Communications

Effect of zinc on hepatoprotectivity of α1-acid glycoprotein

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Abstract

α1-Acid glycoprotein (AGP) prevented primary hepatocytes from undergoing cell death induced by the chemical toxin, bromobenzene at range from 0.1 to 0.2 mg/ml. Addition of zinc (0.2 mM) to medium containing AGP (0.02 mg/ml) was found to prevent the cell death by bromobenzene. Zinc is shown to enhance the hepatoprotective effect of AGP. Circular dichroism and thermal analysis were done to investigate the mechanism of the effect of zinc mentioned above. The results suggest that zinc stabilizes the conformation of AGP.

Keywords : α1-acid glycoprotein, zinc, hepatoprotection, thermal property

Introduction

Primary cultures of hepatocytes are widely used as an important component for studying xenobiotic metabolism and toxicity [1-4]. To use hepatocytes in such studies, it is crucial that isolated primary hepatocytes survive and maintain their functions in vitro over a long period. After isolation, it is well known that primary hepatocytes soon exhibit a decreased survival ratio and lose liver-specific functions. Therefore, hepatoprotective agents, which minimize the death of hepatocytes, are required. To date, we have studied several hepatoprotective agents [5-8]. For the application of hepatocytes in studies of xenobiotic metabolism and toxicity, hepatoprotective agents, which do not influence the metabolism of xenobiotics, are required. Therefore, we performed another screening of hepatoprotective agents.

α1-Acid glycoprotein (AGP) is classed as one of the positive acute-phase proteins, and possesses five N-glycan chains including sialic acids as terminal groups [9]. It was also reported that AGP prevented activation of caspases in lethal experimental hepatitis models [10]. As AGP is included in normal serum, it is considered to be one candidate for a hepatoprotective agent. In previous paper [11], it was reported that AGP prevented primary hepatocytes from chemically induced cell death. Moreover, zinc was found to enhance the hepatoprotectivity of AGP. In this paper, we will report the obtained results.

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Materials and Methods

The basal medium A consisted of William's E medium (WE; ICN Biochemical, Costa Mesa, CA, USA), 100 U/ml penicillin G, 100 μg/ml streptomycin, 50 ng/ml amphotericin B, 100 ng/ml aprotinin (Nacalai Tesque, Kyoto), and 10% (v/v) fetal bovine serum (ICN Biochemicals). Medium B consisted of medium A supplemented with 1 nM insulin and 1 nM dexamethasone.

Hepatocytes were isolated from male Sprague-Dawley rats weighting 150-200 g by perfusing the liver with collagenase (from *Clostridium histolyticum*; Sigma-Aldrich, St. Louis, MO, USA) using the method of Seglen [12]. A hepatocyte preparation with more than 90% viability at the time of isolation was used for the experiments. Cells were seeded at a density of 1×10^5 cells/cm^2 on polystyrene culture plates (Nippon Becton and Dickinson, Tokyo), and incubated for 6 h

in medium B under the conditions of humidified air with 5% CO_2 at 37°C. After 6 h, medium B was exchanged for medium A.

One day after the isolation of hepatocytes, the medium was replaced with fresh medium A containing 0.8% (v/v) bromobenzene (BB) dissolved in dimethyl sulfoxide (DMSO). The final concentration of BB was adjusted to 1 mM. In the experiments investigating the effect of zinc on the hepatoprotective activities of AGP (from bovine serum; Sigma-Aldrich), zinc chloride (0.2 mM) was added to medium A with BB and AGP (0.01 mg/ml). Hepatocytes were incubated under each condition for another 24 h, and then the number of viable cells was determined by the Trypan Blue exclusion assay. Briefly, 0.2% (w/v) Trypan Blue solution was added to a trypsinized cell suspension and cells that were not stained were counted as viable. A control (−BB) run was performed in the medium containing only 0.8% (v/v) DMSO. Also, a control (+BB) run, where hepatocytes were treated with only BB, was performed.

Circular dichroism (CD) spectra were measured by JASCO J-720W spectropolarimeter (JASCO, Tokyo, Japan). Differential scanning calorimetry (DSC) was measured by DSC 6100 (SII, Tokyo, Japan).

Results & Discussion

In previous paper [11], we reported that AGP can act as a hepatoprotective agent in vitro, and that, in the expression of the hepatoprotective effect of AGP, it was also shown that the terminal sialic acids in the N-glycan chain of AGP play an important role. Sialic acid is expected to be a good chelator to metal ion. As it has been shown that zinc complexes show the hepatoprotectivity in pervious studies [6,7], the effects of zinc ion and other metal ions on hepatoprotectivity of AGP were examined.

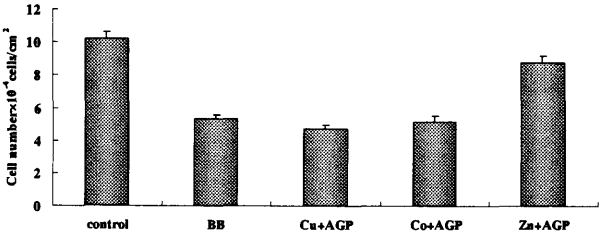

Fig. 1 Effect of the addition of Zn^{2+} and other metals on hepatoprotectivity of AGP. Control and BB represent hepatocytes treated without and with BB, respectively. In experiments, 0.01 mg/ml of AGP and 0.2 mM of metal ions were used. Results shown are means±SD of three independent measurements. *$p<0.05$ compared with BB.

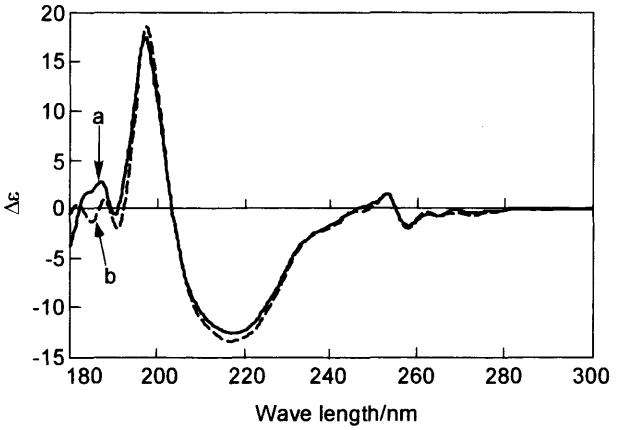

Fig. 2 CD spectra of native and Zn^{2+}-added AGP. Solid line (a) shows the spectrum of native AGP, and dashed line (b) shows that of AGP (1.0 mg/ml) with Zn^{2+} (20 mM). CD spectra were measured by JASCO J-720W spectropolarimeter (JASCO, Tokyo, Japan).

0.01 mg/ml of AGP used in experiment was confirmed not to show the hepatoprotectivity. As shown in Fig.1, by addition of zinc ion (Zn^{2+}) 0.01 mg/ml AGP showed the hepatoprotectivity. Control and BB represent hepatocytes treated without and with BB, respectively. Thus, it was suggested that Zn^{2+} enhanced the hepatoprotectivity of AGP. However, other ions (Cu^{2+}, Co^{2+}) did not affect the hepatoprotectivity of AGP. Other metal ions, such as Fe^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+} etc, did not also affect the hepatoprotectivity of AGP (data not shown).

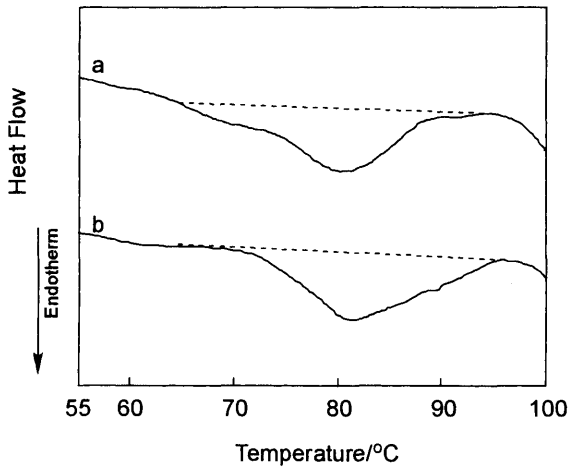

Fig. 3 DSC curves of native and Zn^{2+}-added AGP. Line a and b are DSC curves of native and Zn^{2+}-added AGP, respectively. DSC was measured by DSC 6100 (SII, Tokyo, Japan). In measurement, 1.3 % of AGP and 20 mM $ZnCl_2$ were used.

In order to investigate the mechanism of the enhancing effect of Zn^{2+} in the hepatoprotectivity of AGP, conformation of AGP was examined by circular dichroism (CD) spectroscopy. CD spectra were measured by JASCO J-720W spectropolarimeter (JASCO, Tokyo, Japan) and were shown in Fig.2 In Fig.2, solid line (a) shows the spectrum of native AGP, and dashed line (b) shows that of AGP with Zn^{2+}. There is little difference between these spectra. These results show that the conformation of AGP is not affected by the addition of Zn^{2+}. Thereafter, the stability of AGP was examined by differential scanning calorimetry (DSC). DSC was measured by DSC 6100 (SII, Tokyo, Japan), and the results were shown in Fig.3 Figures 3(a) and 3(b) are DSC curves of native and Zn^{2+}-added AGP, respectively. These results show that the start temperature of denature of AGP becomes higher by the addition of Zn^{2+}, and that total energy for the denature of AGP also increase about 18 % by the addition of Zn^{2+}. These results suggest that the stabilization of AGP conformation by Zn^{2+} is one of the important mechanisms in the expression of the enhancing effect of zinc to the hepatoprotectivity of AGP. The mechanism of hepatoprotectivity of AGP has been unclear, yet. The information obtained here is also considered to be important in clarifying the mechanism of hepatoprotectivity of AGP.

In future study, we will clarify the complete mechanism of the enhancing effect of zinc to the hepatoprotectivity of AGP, and develop the zinc chemistry in biomedical field.

References

1) Canaple L, Nurdin N, Angelova N, Saugy D, Hunkeler D, Desvergne B: Maintenance of primary murine hepatocyte functions in multicomponent polymer capsules – in vitro cryopreservation studies, J Hepatol, 34: 11-18, 2001.

2) McKay GC, Henderson C, Goldie E, Connel G, Westmoreland C, Grant MH: Cryopreservation of rat hepatocyte monolayers: cell viability and cytochrome P450 content I post-thaw cultures, Toxicol In Vitro: 16: 71-79, 2002.

3) Li AP, Gorycki PD, Hengstler JG, Kedderis GL, Koebe HG, Rahmani R: Present status of the application of cryopreserved hepatocytes in the evaluation of xenobiotics: consensus of an international expert panel, Chem Biol Interact, 121: 117-123, 1999.

4) De Sousa G, Nicolas F, Placidi M, Rahmani R, Benicourt M, Vannier B, Lorenzon G, Mertens K, Coecke S, Callaerts A, Rogiers V, Khan S, Roberts P, Skett P, Fautrel A, Chesne C, Guillouzo A: A multi-laboratory evaluation of cryopreserved monkey hepatocyte functions for use in pharmacotoxicology, Chem Biol Interact, 121: 77-97, 1999.

5) Kagaya N, Tagawa Y, Nagashima H, Saijo R, Kawase M, Yagi K: Suppression of cytotoxin-induced cell death in isolated hepatocytes by tea catechins, Eur J Pharmacol, 450: 231-236, 2002.

6) Kagaya N, Kawase M, Maeda H, Tagawa Y, Nagashima H, Ohmori H, Yagi K: Enhancing effect of zinc on hepatoprotectivity of epigallocatechin gallate in isolated hepatocytes, Biol Pharm Bull, 25: 1156-1160, 2002.

7) Kawase M, Kagaya N, Akamatsu S, Kamiyoshi A, Muto S, Tagawa Y, Yagi K: Liver protection by bis(maltolato)zinc (II) complex, Exp Anim, 53: 1-9, 2004.

8) Kagaya N, Hara Y, Saijo R, Kamiyoshi A, Tagawa Y, Kawase M, Yagi K: Novel function of rare catechin, epigallocatechin-3-(3"-O-methyl)gallate, against cold injury in primary rat hepatocytes, J Biosci Bioeng, 96: 559-563, 2003.

9) Fournier T, Medjoubi-N N, Porquet D: Alpha-1-acid glycoprotein, Biochim Biophys Acta, 1482: 157-171, 2000.

10) Molle WV, Denecker G, Rodriguez I, Brouckaert P, Vandenabeele P, Libert C: Activation of caspases in lethal experimental hepatitis and prevention by acute phase proteins, J Immunol, 163: 5235-5241, 1999.

11) Kagaya N, Kamiyoshi A, Tagawa Y, Akamatsu S, Isoda K, Kawase M, Yagi K: Suppression of cell death in primary rat hepatocytes by ?1-acid glycoprotein. J Biosci Bioeng, 99: 81-83, 2005.

12) Seglen. PO: Preparation of isolated rat liver cells, Methods Cell Biol, 13: 29-83, 1976.