Carbohydrate Polymers 89 (2012) 302–304

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Antioxidant and renoprotective activity of chitosan in nephrectomized rats

Makoto Anraku[a,c,*,1], Hisao Tomida[c,1], Akihiro Michihara[c], Daiju Tsuchiya[c], Daisuke Iohara[a], Yuji Maezaki[d], Kaneto Uekama[a], Toru Maruyama[b], Masaki Otagiri[a,b], Fumitoshi Hirayama[a]

[a] Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan
[b] Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan
[c] Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, 1 Sanzo, Gakuen-cho, Fukuyama 729-0292, Japan
[d] Nippon Kayaku Food Techno Co., Ltd., Gunma 370-1208, Japan

ARTICLE INFO

Article history:
Received 27 December 2011
Received in revised form 2 March 2012
Accepted 3 March 2012
Available online 12 March 2012

Keywords:
Chitosan
Antioxidant
Oxidative stress
Chronic renal failure
Indoxyl sulfate

ABSTRACT

The effect of chitosan on oxidative stress and chronic renal failure was investigated using 5/6 nephrectomized rats. The ingestion of chitosan over a 4-week period resulted in a significant decrease in total body weight, glucose, serum creatinine and indoxyl sulfate levels ($P = 0.0011$, $P = 0.0006$, $P = 0.0012$, and $P = 0.0005$, respectively), compared with the non-treated nephrectomized group. The ingestion of chitosan also resulted in a lowered ratio of oxidized to reduced albumin ($P = 0.003$) and an increase in biological antioxidant potential ($P = 0.023$). Interestingly, the oxidized albumin ratio was correlated with serum indoxyl sulfate levels *in vivo*. These results suggest that the ingestion of chitosan results in a significant reduction in the levels of pro-oxidants, such as uremic toxins, in the gastrointestinal tract, thereby inhibiting the subsequent development of oxidative stress in the systemic circulation.

1. Introduction

Oxidative stress, which involves the production of excessive levels of reactive oxygen species, is a pathogenic condition of great importance in chronic renal failure patients, and has a great impact on their survival (Himmelfarb, 2004). Furthermore, the management of cardiovascular disease is an important issue in cases of patients with chronic renal failure, and oxidative stress has been speculated to greatly contribute to such onset (Fort, 2005). Thus, the development of effective anti-oxidant therapy for treating chronic renal failure would be highly desirable. One proposed mechanism of oxidative stress in chronic renal failure is the accelerated production of oxidants, such as uremic toxins and their reduced renal clearance. Therefore, the removal of such substances from the systemic circulation may lead to a reduction in oxidative stress in chronic renal failure.

Chitosan, a linear polymer comprised of β(1–4) linked D-glucosamine units, has been recommended as a suitable functional material for accomplishing this, because of its biocompatibility, biodegradability, absence of toxicity, adsorption properties and free radical scavenging activities (Anraku et al., 2009; Park, Je, Byun, Moon, & Kim, 2004; Porporatto, Bianco, Riera, & Correa, 2003). In recent world-wide studies, chitosans were tested as a dietary supplement for the possible inhibition of the absorption of certain lipids and bile acids (Gades & Stern, 2005; Muzzarelli et al., 2006). Regarding the mechanism of lipid absorption, when ingested, chitosan develops an HCl-layer in the stomach. As capsulated particles of chitosan move into the duodenum, the HCl-layer becomes diluted and the chitosan particles form agglomerates with fatty acids and cholesterol, thus reducing the absorption of lipids in the gastrointestinal tract (Muzzarelli & Muzzarelli, 2006). In general, a high molecular weight chitosan preparation would be expected to inhibit the absorption of certain lipids and bile acids. On the other hand, a low molecular weight chitosan preparation would be predicted to absorb such substances, but would also be expected to show increased antioxidant effects. In fact, we showed that medium molecular weight chitosan has a high antioxidant activity as well as antilipidemic effects in metabolic syndrome model rats (Anraku et al., 2010). Although several studies have been reported concerning the antioxidant activities of chitosan in several diseases, including the metabolic syndrome and acute renal failure (Anraku et al., 2011; Yoon et al., 2011), relationships between the progression of renal failure and its antioxidant activity have not been extensively reported in chronic renal failure.

In this study, we report on the effects of chitosan on renal function as well as oxidative stress in chronic renal failure using 5/6 nephrectomized rats.

* Corresponding author at: Department of Physical Pharmaceutics, Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan. Tel.: +81 96 326 4163; fax: +81 96 326 5048.
E-mail address: anraku@ph.sojo-u.ac.jp (M. Anraku).
[1] These authors contributed equally to this work.

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2012.03.014

2. Materials and methods

2.1. Materials

The chitosan (Chitosamin®; weight-average molecular weight (Mw = 1000 kDa), degree of deacetylation 90%) used in these studies was a generous gift from Nippon Kayaku Food Techno Co., Ltd. (Gunma, Japan). Other chemicals were also of the highest grade commercially available, and all solutions were prepared using deionized, distilled water.

2.2. Animals and treatment

Six-week-old male rats weighting 140–150 g that had been subjected to a 5/6 nephrectomy as a chronic renal failure model were obtained from the Disease Model Co-operative Research Association, Japan. The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Fukuyama University and the Japanese government was fully informed prior to the commencement of the study. These rats were divided into two groups as follows: (a) untreated nephrectomized group ($N = 5$). These rats received only standard rat chow. (b) Chitosan treated nephrectomized group. These rats received standard rat chow and chitosan at 1.5 g/kg of body weight for 4 weeks ($N = 5$). All of the rats received standard rat chow. Untreated nephrectomized rats were pair-fed with the same amount of chow as the chitosan treated nephrectomized rats. Chitosan was administered with the chow.

2.3. Blood and urine analyses

Blood and urine sample were collected at 0 and 4 weeks after the administration of chitosan. Biochemical parameters including serum indoxyl sulfate levels were measured according to previously described methods (Anraku et al., 2010; Kadowaki et al., 2007; Shimoishi et al., 2007).

2.4. Effects of chitosan on oxidative stress using oxidized albumin ratio and biological antioxidant potential

Oxidized albumin ratios were determined by high-performance liquid chromatography as described previously (Anraku et al., 2004; Shimoishi et al., 2007). The biological antioxidant potential test is a photometric test that measures the plasma biological antioxidant potential as the capacity of the plasma sample to reduce iron from the ferric (Fe_3^+) to the ferrous (Fe_2^+) state (Benzie & Strain, 1996).

2.5. Statistical analysis

Statistical significance was evaluated by the 2-tailed paired Student's t-test for comparison between 2 mean values. For all analyses, values of $P < 0.05$ were regarded as statistically significant. Results are reported as the mean ± SEM.

3. Results and discussion

In the present study, we observed a reduction in several important biological parameters as the result of chitosan administration (Table 1). In particular, compared to the corresponding results for the untreated nephrectomized group, a significant decrease in the levels of serum indoxyl sulfate was found ($P = 0.0005$). Indoxyl sulfate, which is the most widely studied uremic toxin, has been shown by us and other research groups to cause an increase in free radical production and to induce the production of inflammatory cytokines in the kidneys and blood circulation (Motojima et al., 1991; Shimoishi et al., 2007). Therefore, low indoxyl sulfate levels might lead to renal function being maintained and low oxidative stress in the systemic circulation. In fact, as shown in Table 1, chitosan treatment caused a significant decrease in the oxidized albumin ratio after 4 weeks ($P = 0.003$ vs. ratio of untreated group at 4th week). Since the extent of oxidation of this prominent protein can be taken as an index of oxidative stress, these results demonstrate the potential of chitosan for reducing the effects of stress in chronic renal failure rats. This conclusion is supported by an increase in biological antioxidant potential as the result of the chitosan treatment after 4 weeks (Table 1). These results suggest that chitosan itself is a powerful *in vivo* antioxidant. Further, the results shown in Fig. 1 show the existence of a significant relationship between oxidized albumin ratios and serum indoxyl sulfate levels ($r = 0.854$, $P = 0.001$). These results also suggest that the oxidized albumin ratio is a reliable index of the effectiveness of chitosan treatment on chronic renal failure. Since chitosan itself is not absorbed from the digestive tract, it is unlikely that the mechanism of the antioxidant activity for chitosan involves the direct scavenging of radicals in the blood. In general, indoxyl sulfate in the blood arises exclusively from metabolism by intestinal bacteria. Intestinal bacteria metabolize L-tryptophan to indole, which is absorbed into the blood and is then metabolized to indoxyl sulfate in the liver. Thus, we hypothesize that the antioxidant activity of chitosan is indirect in nature, in which substances such as indole or precursors are adsorbed in the gastrointestinal tract, thereby suppressing indoxyl sulfate levels in the blood.

Table 1
Effects of chitosan ingestion on some selected biochemical and antioxidative properties of chronic renal failure rats.

		Without chitosan	With chitosan
Weight (g)	0 week	204.8 ± 4.7	198.8 ± 5.6
	4 week	357.5 ± 7.6	304 ± 5.2
Glucose (mg/dL)	0 week	81.3 ± 8.5	85.0 ± 2.5
	4 week	201.8 ± 15.6	115.0 ± 2.8[a]
Triglyceride (mg/dL)	0 week	99.4 ± 4.4	99.1 ± 11.9
	4 week	110.8 ± 14.8	88.9 ± 14.9
Serum albumin (mg/dL)	0 week	3.58 ± 0.1	3.68 ± 0.2
	4 week	3.10 ± 0.1	3.10 ± 0.1
Urine albumin (mg/day)	0 week	85.3 ± 8.3	88.8 ± 11.2
	4 week	159.7 ± 14.2	91.8 ± 15.4[a]
Serum creatinine (mg/dL)	0 week	1.20 ± 0.42	1.31 ± 0.42
	4 week	2.22 ± 0.22	1.68 ± 0.32[a]
Serum indoxyl sulfate (μM)	0 week	13.8 ± 3.82	14.5 ± 3.52
	4 week	41.5 ± 5.61	19.5 ± 5.33[a]
Lowering of oxidized albumin ratio	0 week	1.14 ± 0.06	1.09 ± 0.13
	4 week	1.81 ± 0.06	1.21 ± 0.12[a]
Increase in biological antioxidant potential	0 week	2867 ± 66.7	2741 ± 40.1
	4 week	2754 ± 65.6	3032 ± 59.1[a]

[a] $p < 0.05$ vs. at non-treated ($N = 5$).

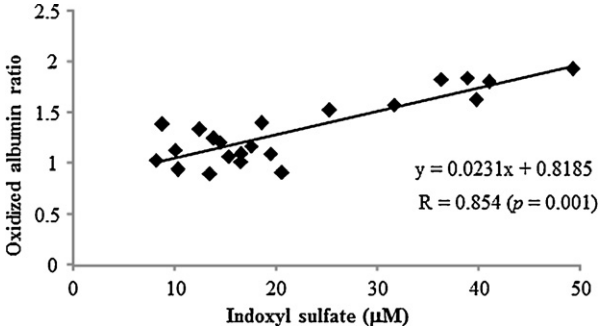

Fig. 1. Relationship between serum indoxyl sulfate levels and oxidized albumin ratio. The plot shows the linear regression of the two sets of results ($N = 20$, $r = 0.854$, $P = 0.001$).

This conclusion was also supported by a correlation between the serum concentration of indoxyl sulfate and the oxidized albumin ratio in chronic renal failure rats (Fig. 1).

4. Conclusion

The findings reported herein serve to demonstrate the antioxidative and renoprotective potential of chitosan in chronic renal failure. To date, the oral carbonaceous adsorbent, AST-120 (Kremezin®), has been used in pre-dialysis, uremic stage renal failure patients to adsorb biologically active substances, so-called uremic toxins, in the circulation that accumulate during chronic renal failure, thereby prolonging the progression of chronic renal failure and the interval to the inception of dialysis (Owada et al., 1997). However, the present results suggest a new potential use for chitosan as an antioxidant in chronic renal failure. We therefore propose that, from the perspective of antioxidant therapy, the initiation of chitosan administration as a healthy food, would be preferable at a stage earlier than the conventional state of pre-dialysis uremia with or without the administration of drugs. Thus, the antioxidative effect of chitosan is unique and differs from that of typical, conventional antioxidants such as antioxidant vitamins and N-acetyl cysteine. This fact suggests that chitosan can be co-administered with such agents and represents a new strategy for antioxidative treatment in chronic renal failure.

Acknowledgement

We wish to thank the Nippon Kayaku Food Techno Co., Ltd (Gunma, Japan) for the generous gift of Chitosamin®.

References

Anraku, M., Michihara, A., Yasufuku, T., Akasaki, K., Tsuchiya, D., Nishio, H., et al. (2010). The antioxidative and antilipidemic effects of different molecular weight chitosans in metabolic syndrome model rats. *Biological and Pharmaceutical Bulletin*, *33*, 1994–1998.

Anraku, M., Fujii, T., Furutani, N., Kadowaki, D., Maruyama, T., Otagiri, M., et al. (2009). Antioxidant effects of a dietary supplement: Reduction of indices of oxidative stress in normal subjects by water-soluble chitosan. *Food and Chemical Toxicology*, *47*, 104–109.

Anraku, M., Kitamura, K., Shinohara, A., Adachi, M., Suenaga, A., Maruyama, T., et al. (2004). Intravenous iron administration induces oxidation of serum albumin in hemodialysis patients. *Kidney International*, *66*, 841–848.

Anraku, M., Fujii, T., Kondo, Y., Kojima, E., Hata, T., Tabuchi, N., et al. (2011). Antioxidant properties of high molecular weight dietary chitosan in vitro and in vivo. *Carbohydrate Polymers*, *83*, 501–505.

Benzie, F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Fort, J. (2005). Chronic renal failure: A cardiovascular risk factor. *Kidney International, Supplement*, *99*, S25–S29.

Gades, M. D., & Stern, J. S. (2005). Chitosan supplementation and fat absorption in men and women. *Journal of the American Dietetic Association*, *105*, 72–77.

Himmelfarb, J. (2004). Linking oxidative stress and inflammation in kidney disease: Which is the chicken and which is the egg? *Seminars in Dialysis*, *17*, 449–454.

Kadowaki, D., Anraku, M., Tasaki, Y., Kitamura, K., Wakamatsu, S., Tomita, K., et al. (2007). Effect of olmesartan on oxidative stress in hemodialysis patients. *Hypertension Research*, *30*, 395–402.

Motojima, M., Nishijima, F., Ikoma, M., Kawamura, T., Yoshioka, T., Fogo, A. B., et al. (1991). Role for Buremic toxin in the progressive loss of intact nephrons in chronic renal failure. *Kidney International*, *40*, 461–469.

Muzzarelli, R. A. A., Orlandini, F., Pacetti, D., Boselli, E., Frega, N. G., Tosi, G., et al. (2006). Chitosan taurocholate capacity to bind lipids and to undergo enzymatic hydrolysis: An in vitro model. *Carbohydrate Polymers*, *66*, 363–371.

Muzzarelli, R. A. A., & Muzzarelli, C. (2006). Chitosan, a dietary supplement and a food technology commodity. In C. G. Biliaderis, & M. S. Izydorczyk (Eds.), *Functional food carbohydrates* (pp. 215–248). Orlando, USA: Francis and Taylor.

Owada, A., Nakao, M., Koike, J., Ujiie, K., Tomita, K., & Shiigai, T. (1997). Effects of oral adsorbent AST-120 on the progression of chronic renal failure: A randomized controlled study. *Kidney International, Supplement*, *63*, S188–S190.

Park, P. J., Je, J. Y., Byun, H. G., Moon, S. H., & Kim, S. K. (2004). Antimicrobial activity of hetero-chitosans and their oligosaccharides with different molecular weights. *Journal of Molecular Microbiology and Biotechnology*, *14*, 317–323.

Porporatto, C., Bianco, I. D., Riera, C. M., & Correa, S. G. (2003). Chitosan induces different L-arginine metabolic pathways in resting and inflammatory macrophages. *Biochemical and Biophysical Research Communications*, *304*, 266–272.

Shimoishi, K., Anraku, M., Kitamura, K., Tasaki, Y., Taguchi, K., Hashimoto, M., et al. (2007). An oral adsorbent, AST-120 protects against the progression of oxidative stress by reducing the accumulation of indoxyl sulfate in the systemic circulation in renal failure. *Pharmaceutical Research*, *24*, 1283–1289.

Yoon, S. P., Han, M. S., Kim, J. W., Chang, I. Y., Kim, H. L., Chung, J. H., et al. (2011). Protective effects of chitosan oligosaccharide on paraquat-induced nephrotoxicity in rats. *Food and Chemical Toxicology*, *49*, 1828–1833.