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Application of Chitosan / Cyclodextrin Nanoparticles for Tissue Glutathione Delivery

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The aim of this study was to investigate an ability of chitosan nanoparticles for tissue delivery of the peptide glutathione. Formulations composed of chitosan or chitosan plus cyclodextrin-beta comlex were prepared. Reduced glutathione was loaded and delivered to mucosal layer of small intestine after ischemia-reperfusion injury more efficiently in chitosan/cyclodextrin-beta nanoparticles. From the data obtained, we believe that chitosan / cyclodextrin nanoparticles can be used for the oral administration of glutathione and other small peptides.

Keywords: Glutathion, Nanoparticles, Cyclodextrin, Chitosan.

PACS number: 87.85.Rs

1. INTRODUCTION

Glutathione (GSH) is the predominant intracellular non-protein thiol in a wide range of cells. It has important functions in the detoxification system of almost every cell type. The reversible oxidation of GSH provides electrons for the GSH-peroxidase catalyzed reduction of hydrogen peroxide or organic peroxides [1]. However, the application of GSH as a functional food ingredient has been limited because of its low bioavailability due to its poor cellular uptake and its instability. Under conditions of oxidative stress, the GSH thiol group can be easily oxidized to glutathione disulfide, which loses its antioxidant activity based on its radical scavenging activity. Nanoparticle (NP) prototypes from biodegradable polymers have been proposed for local drug delivery [2]. Among them, NPs based on the polysaccharide chitosan (CS) have shown particularly promising results due to their intrinsic properties including biocompatibility, mucoadhesion and ability to transiently open the tight junctions of the intestinal barrier [3].

2. MATERIAL AND METHODS

2.1 Materials

Chitosan (CS, Mw 600 kDa, degree of deacetylation 90 %) is a commercial product of Bioprogress (Russian Federation). Sodium tripolyphosphate (TPP) was purchased from Sigma (USA). Reduced glutathione and cyclodextrin-beta (CD) were purchased from Applichem (Germany). All other chemicals were of analytical grade and used as received. The water used in all experiments was double distilled.

2.2 Preparation of Nps

Preparation of NPs was based on the ionic gelation of CS with TPP anions [4] in 1st group (CS NPs) and CS with TPP and CD (CS / CD NPs) in 2nd group [5]. In all groups CS nanoparticles were prepared with CS concentration of 1 mg/ml and CS : TPP ratio 3 : 1. In both groups, CS was first dissolved in acetic acid aqueous solution. The concentration of acetic acid in the aqueous media was 1.75 times that of CS. To 9 ml of CS 2 ml aqueous solution of 0,5 % w/v of GSH or 2 ml of mixture of GSH and CD in ratio 1 : 1 as 1.0 % w/v (2nd group) were added and mixed 45 min under magnetic stirring. Than TPP aqueous solution was added drop wise under magnetic stirring onto the corresponding amount of the CS solution at room temperature.

2.3 Encapsulation Efficiency of Nps

The prepared Nps suspension containing the GSH was centrifugated at 9,000 rpm for 90 min and free GSH in the supernatant was quantified by spectrophotometric method [6].

2.4 In Vivo Determination of Ability of NPs for Tissue GSH Delivery

To determine the ability of NPs deliver GSH to tissue intestinal ishemia-reperfusion injury were applied on 60 Wistar rats by clamping superior mesenteric artery for 60 min with 4 hours of reestablishing of blood circulation under ketamine anastesia. CS or CS / CD NP loaded with 3 mg of GSH were infused into jejunum just before ischemia period in two groups of animals and appropriate volume of normal saline in control animals ($n = 15$ in every group). After reperfusion period animals were killed by sodium thiopental overdosing and concentration of GSH, malone dialdehyde (MDA), diene conjugates (DC) and catalase (CAT) in mucosal layer of small intestine were determined. Morphological examination of the NP was performed by transmission electron microscopy (TEM).

3. RESULTS

Regular and spherical morphology was revealed by TEM both for CS and CS / CD NPs with similar diameters. The capacity of the different NP systems to load GSH was determined: CS NPs presented encapsulation efficiency near 10 % and CS / CD NPs – 25 %. GSH was delivered to mucosal tissue in rat model of intestinal ischemia-reperfusion injury by CS / CD NPs on 34,9 % ($p < 0,05$) higher level than CS NPs (Table 1), which was followed by decreasing of concentration of oxidant injury markers.

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Table 2 – Concentration Of Markers of Oxidant Injury and Antioxidants In Mucosal Layer Of Small Intestine

	Groups			
	CS NPs, (n = 15)	CS / CD NPs, (n = 15)	Control, (n = 15)	Sham-operated, (n = 15)
GSH, μmol/g	1,22 ± 0,16[c]	2,32 ± 0,14[a,b]	0,92 ± 0,17[c]	2,62 ± 0,08
MDA, nmol/g	7,61 ± 0,44	6,11 ± 0,31[a]	8,88 ± 0,29[c]	5,61 ± 0,21
DC, nmol/g	10,14 ± 0,31[c]	9,04 ± 0,31[a]	11,43 ± 0,41[c]	8,13 ± 0,21
CAT, μmol/min/g	375 ± 21,4[c]	445 ± 21,4	305 ± 31,4[c]	542 ± 11,4

a) $p < 0,05$ with control group; b) $p < 0,05$ between CS and CS / CD NPs; c) $p < 0,05$ with control group

4. DISCUSSION

CS and CS / CD NPs were prepared by ionic gelation in the presence of TPP, as described in the methodology section. The mechanism of formation of the nanosystems combines the electrostatic interaction between CS and CDs, which are oppositely charged, with the ability of CS to undergo a liquid-gel transition due to its ionic interaction with TPP. The low affinity of GSH to CS NPs matrix might be explained by the low molecular weight of the peptide and to the presence of only one net negative charge in its chemical structure. Formation of CD GSH complex before it incorporation in CS matrix increased encapsulation effeiciency of NPs system thus enhancing GSH delivery to tissue.

CS has mucoadhesive properties so its nanosystems may be used for sustained drug delivery to mucosal layer of intestine [4]. CS / CD NPs were only 25 % loaded with GTH but their oral administration diminished ischemia-reperfusion injury of small inteatinal mucosa: concentration of MDA decreased on 31,2 % ($p < 0,05$), DC – on 20,9 % ($p < 0,05$), but CAT increased on 45,9 ($p > 0,05$).

5. CONCLUSION

CS NPs prepared by TPP gelation alone have low GSH capacity. Formation of CS / CD NPs increased affinity to GSH, enhanced its delivery to tissue and diminished intestinal mucosa ischemia-reperfusion injury.

REFERENCES

1. E. Valencia, A. Marin, G. Hardy, *Nutrition* 783 (2001).
2. G. Sandri, M.C. Bonferoni, S. Rossi, et al., *Eur. J. Pharm. Biopharm.* **68**, 65 (2007).
3. S. Chopra, S. Mahdi, J. Kaur, et al., *J. Pharmacy Pharmacology* **58**, 1021 (2006).
4. P. Calvo, C. Remunan-Lopez, J.L. Vila-Jato, M.J. Alonso, *Pharmacol. Res.* **14**, 1431 (1997).
5. S.K.H. Khalil, G.S. El-Fekyb, S.T. El-Bannaa, W.A. Khalil, *Carbohydrate Polymers* **90**, 1244 (2012).
6. G. Ellman, *Arch. Biochem. Biophysics*, **32**, 7130 (1959).