***In vitro* Adsorption of Paraquat onto Substituted Chitosan Beads**

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Abstract Paraquat removal by chitosan with different substituents was investigated *in vitro* for evaluation of chitosan as a paraquat adsorbent in emergency treatment for acute paraquat poisoning.

Chitosan CM and SU, in which the hydroxyl group at the C₆ position is replaced by a carboxymethyl and sulfonic group, respectively, removed paraquat abundantly in purified water. However an inhibitory effect of sodium chloride on paraquat removal by CM and SU was recognized.

On the other hand, paraquat removal by cross-linked chitosan (BCW) was accelerated in saline solutions. It was recognized that more paraquat was removed by BCW chitosan when the sodium chloride concentration was less than 1.8 %.

It was confirmed that some chitosan beads (CM and SU) could function as effective paraquat adsorbents through *in vitro* experiments.

Key words : Paraquat poisoning, Chitosan, Adsorbent, Sodium chloride addition

Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium chloride) is widely used as a non-hormone, non-selective herbicide, and its amount of utilization has increased year by year because of its superior herbicidal effect¹⁾. Paraquat is unlikely to produce serious problems when properly used; however, many people have died through accidental, suicidal or homicidal ingestion of it^{2, 3)}. The mortality from paraquat poisoning is higher than that from any other type of poisoning associated with agricultural chemicals¹⁾.

Emergency treatment for paraquat poisoning involves methods which minimize the internal absorption by excretion of paraquat through the digestive tract and methods which remove paraquat in the blood. The former method involves

gastrointestinal lavage and the administration of a purgative⁴⁾. The latter utilizes activated carbon hemoperfusion, with reported survival cases⁴⁾. On the other hand, a method which hinders the absorption of paraquat by adsorbents has been proposed. As adsorbents, the effectiveness of aluminium silicate⁵⁾, bentonite⁶⁾, activated carbon⁴⁾ and cation-exchange resin⁷⁻⁹⁾, have been reported. However, no specific and effective antidote for paraquat poisoning is available at present.

Recently, chitin and chitosan have drawn great interest from the standpoint of utilization of natural resources. Chitin, β -(1-4)-poly-N-acetyl-D-glucosamine, as well as its deacetylated derivative chitosan, is widely and abundantly distributed in shells of crustaceans and insects, organs of molluscs, and cell walls of fungi and

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bacteria. Chitin and chitosan have extremely low toxicity, and are biocompatible. Furthermore, chemical modification of chitin and chitosan is easy¹⁰⁾.

Most studies carried out using these biopolymers have dealt with water treatment^{11~13)}, or medical and pharmaceutical fields^{14~16)}, and little work on the application of chitin and chitosan as antidotes for paraquat poisoning has been done so far.

In this investigation, we use substituted chitosan beads and discuss paraquat removal for the purpose of emergency treatment for acute paraquat poisoning *in vitro*.

Materials and Methods

Paraquat was purchased from Wako Pure Chemical Industry Ltd., and its grade was for the testing of agricultural chemical residues. Four kinds of substituted chitosan beads (CM, SU, CS and BCW) made by Fuji Spinning Co.

Ltd. were obtained from Wako Pure Chemical Industry Ltd., and their particle sizes were 42-70 mesh. They have different substituted groups in their chemical structures as shown in Figure 1. Activated carbon (BAC-MUL) was obtained from Kureha Chemical Ltd., and its particle sizes were 32-48 mesh.

The time courses of amounts of paraquat adsorbed onto chitosan were measured with a paraquat solution/chitosan system at 37°C. One gram of chitosan or activated carbon was added to 100ml of a stirred paraquat solution (300rpm, *ca.* 800mg/l) and the paraquat concentrations in the suspension were measured at regular intervals. The paraquat concentration was measured by a colorimetric method with an alkaline sodium dithionite solution¹⁷⁾. Paraquat solution was diluted with purified water, artificial gastric juice¹⁸⁾, which contained 2.4g of sodium chloride and 24.0ml of diluted hydrochloride (10%) in 1000 ml or normal saline solution (0.9% sodium chlo-

Fig. 1 Chemical structures of chitosan beads

ride). Furthermore, the time courses of amounts of paraquat adsorbed onto chitosan beads in various saline concentrations were measured.

The removal ratio of paraquat was calculated by the following formula; $[(C_0 - C_t)/C_0] \times 100$, where C_0 is the initial concentration of paraquat and C_t is the paraquat concentration at an elapsed time of 60 min.

Results

The removal ratio for paraquat after 60 min is indicated in Table 1. The removal ratios with CM and SU in purified water and artificial gastric juice were higher than those with activated carbon. In normal saline solution, however, the removal ratios of chitosan beads were lower than

that of activated carbon.

Figure 2 shows the time courses of amounts of paraquat adsorbed onto chitosan beads and activated carbon in purified water, artificial gastric juice and normal saline solution. In purified water, the amounts adsorbed onto CM and SU were abruptly increased at an elapsed time of 30 min and then increased slowly. The increases of the amounts of paraquat adsorbed onto CS and BCW were small, and the values at each elapsed time were smaller than that onto activated carbon. In artificial gastric juice, the amount of paraquat adsorbed onto CM was greatly increased at 40 min and then increased slowly. The amount adsorbed onto chitosan beads was lower in artificial gastric juice than in purified water except in the case of BCW. In normal saline solution, the amounts adsorbed onto CM and SU were smaller than in purified water and in artificial gastric juice. In normal saline solution, the amount of paraquat adsorbed onto activated carbon was larger than that adsorbed onto chitosan beads. The paraquat removal by CM and SU was more effective in purified water than in artificial gastric juice and/or in normal saline solution. In contrast, the degrees of increases in the amounts of paraquat adsorbed onto BCW and

Table 1 Paraquat removal ratios of chitosan beads and activated carbon

adsorbents	removal ratio (%)		
	H ₂ O*	HCl**	NaCl***
chitosan bead CM	97.0	80.9	26.1
chitosan bead SU	74.1	43.9	21.0
chitosan bead CS	9.9	5.3	9.3
chitosan bead BCW	3.6	11.1	13.0
activated carbon	16.9	28.5	45.1

*: in purified water, **: in artificial gastric juice, ***: in normal saline solution

Fig. 2 Time courses of amounts of paraquat adsorbed onto chitosan beads and activated carbon
●: CM, ▲: SU, ■: CS, □: BCW, ○: activated carbon

activated carbon were successively larger in the order of normal saline solution > in artificial gastric juice > in purified water.

In addition, the effect of the sodium chloride concentration in the solution on the removal of paraquat by chitosan was investigated. The time courses of the amounts of paraquat adsorbed in various saline solutions are shown in Figure 3. The concentrations of sodium chloride in the paraquat solution were 0, 0.45, 0.9 and 1.8%. Addition of sodium chloride showed no effect on the absorbance at 600nm as measured by colorimetric determination of the paraquat concentration.

With the increase of sodium chloride concentration, the amounts of paraquat adsorbed onto CM and SU were decreased. No effect of sodium chloride concentration on the removal of paraquat by CS was recognized. However, with the increase of sodium chloride concentration, the amount adsorbed onto BCW was increased.

It was hence concluded that paraquat was easily adsorbed onto CM and SU in purified water, but that paraquat removal was inhibited by sodium chloride addition. The paraquat removal by BCW and activated carbon was accelerated with the increase of the sodium chloride concen-

Fig. 3 Time courses of amounts of paraquat adsorbed in solutions of different sodium chloride concentrations
NaCl : ○ : 0 %, ● : 0.45%, ▲ : 0.9%, ■ : 1.8%

tration in the solution.

Discussion

For the primary treatment of acute drug poisoning, activated carbon has been used, and its efficacy has been reported^{4, 19, 20)}. The efficacy of some adsorbents for paraquat poisoning has been also reported^{4~9)}. However, little study on the evaluation of chitosan, which is a safe biopolymer for the organism and environment, for paraquat poisoning has been done so far. Although activated carbon has been evaluated as a safe and effective gastrointestinal adsorbent, the removal capacity of CM and SU chitosan in purified water for paraquat is inferior to that of activated carbon²¹⁾. Paraquat removal by activated carbon is mostly based on physical adsorption, whereas that by CM and SU chitosan appeared to occur via ion exchange and/or chemisorption because the paraquat removal by these chitosan beads was inhibited in saline solutions. Kayexalate and kalimate are medical cation exchange resins for the treatment of hyperkalemia, and these resins are noted as a new antidote for paraquat poisoning^{8, 9)}. However, the capacity to remove paraquat of these resins is easily inhibited by the presence of saline solution²²⁾. That of chitosan is also inhibited by the presence of sodium chloride in the solution. Therefore, the inhibitory effect of saline solution on the paraquat removal by CM and SU chitosan must be considered. On the other hand, the paraquat removal by BCW chitosan was accelerated in the presence of sodium chloride. The enhancing effect of saline on the paraquat adsorption capacity of activated carbon has been reported. Honda *et al*²³⁾ reported that the amount of paraquat adsorbed onto activated carbon was larger in normal saline solution than in artificial gastric juice or in purified water. We reported that the amount of paraquat adsorbed onto activated carbon was increased with the increase of the sodium chloride concentration in the solution²¹⁾. It is not clear why the acceleration of paraquat adsorption

onto activated carbon occurs with the addition of saline. The dissociation of paraquat in solution was inhibited by the addition of saline, that is, salting out takes place in the solution. Therefore, it is considered that paraquat is adsorbed more abundantly onto activated carbon. The change in surface charge caused by the presence of sodium chloride can not be neglected. In saline adsorption onto activated carbon, anions are specifically adsorbed. Then an electrical double layer is formed on the surface of the adsorbent, and attracts cations in solution²⁴⁾. It is assumed that paraquat cations are more attracted to the electrical double layer than sodium cations in adsorption onto chitosan beads. Therefore, it is considered that paraquat removal is accelerated by the addition of sodium chloride.

Chitosan has three functional groups, amino-, primary hydroxyl- and secondary hydroxyl groups, in its structure. The C6 hydroxyl group in CM and SU is substituted for by carboxymethyl and sulfonic groups, respectively. These substituted groups are negatively charged, and paraquat is dissociated into cations in solution. Therefore, it is assumed that the high removal capacity of CM and SU for paraquat in purified water is due to the electrostatic interaction between the negatively charged substituted group of chitosan beads and paraquat cations.

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(Received Mar. 25, 1993/Accepted Sep. 27, 1993)