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Immunohistochemical localization and dynamics of paraquat in the stomach and esophagus of rats

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Summary. The dynamics of paraquat in the stomach and esophagus of rats were demonstrated using immunohistochemical techniques. The Rats were killed 3 h, 12 h, 24 h, 3 days, 7 days and 10 days after intravenous administration of paraquat. In the stomach, paraquat was localized in the epithelial cells between 24 h and 10 days after injection, whereas in the esophagus, paraquat was localized in epithelial cells and the lamina propria mucosa between 12 h and 10 days after administration. Although these findings were similar to those observed in the intestine of rats, no clear changes in the distribution of paraquat with time were observed, suggesting that the stomach and esophagus are important reservoirs for the redistribution of paraquat.

Key words: Paraquat – Immunohistochemistry – Rat – Stomach – Esophagus

Zusammenfassung. Die Verteilung von Paraquat im Gewebe des Magens und des Ösophagus von Ratten wurde mittels immunhistochemischer Verfahren bestimmt. Nach intravenöser Applikation von Paraquat wurden Ratten nach Zeitspannen von 3 h, 12 h, 24 h, 3 Tagen und 10 Tagen getötet. Im Magen wurde Paraquat in den Epithelzellen in einem Zeitraum von 24 h bis 10 Tagen nach Applikation gemessen, wohingegen im Ösophagus sowohl in den Epithelzellen als auch in der Lamina propria mucosa Paraquat 12 h bis 10 Tage nach Injektion gemessen werden konnte. Obwohl diese Ergebnisse mit Paraquat-Befunden im Intestinum von Ratten korrelieren, konnte keine eindeutige Zeitabhängigkeit der Verteilung beobachtet werden. Dieses Ergebnis führt zu der Annahme, daß Magen und Ösophagus als wichtige Speichergewebe an einer Redistribution von Paraquat beteiligt sind.

Schlüsselwörter: Paraquat – Immunhistochemie – Ratte – Magen – Ösophagus

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Introduction

Paraquat (1.1′-dimethyl-4,4′-bipyridinium) is a widely used herbicide which has been the cause of fatal self-poisoning in humans [1, 2]. Pulmonary fibrosis is one of the most severe complications of paraquat poisoning. Therefore, the mechanism of paraquat-induced pulmonary fibrosis has attracted the interests of many researches and several studies have been performed [2–5]. But reports of studies on the dynamics of paraquat in vivo are very few [6,7]. Several drugs have been reported to be secreted from the gastric wall [8], and the importance of the stomach wall as a reservoir of drugs has also been pointed out [9]. On the other hand, we could not find any reports in the literature concerning the dynamics of drugs in the esophagus. Specific reports concerning the detailed dynamics of paraquat in these organs are also lacking. The purpose of this study is to report the immunohistochemical localization and dynamics of paraquat in the stomach and esophagus of rats, as we have already conducted the immunohistochemical demonstration of paraquat in several other organs [10, 11].

Materials and methods

Chemicals. Paraquat dichloride (1,1′-dimethyl-4,4′-bipyridinium) was purchased from Wako Pure Chemicals Industries Co. Ltd., Osaka, Japan. A biotin-streptavidin kit amplified system (peroxidase) was purchased from Seikagaku Co., Tokyo, Japan. 3,3′-Diaminobenzidine was obtained from Sigma Chemical Co., St. Louis, MO, USA. All other reagents were high grade commercially available substances.

Animal experiments. Male Sprague-Dawley rats (200–250 g) were intravenously injected with paraquat dichloride (5 mg/kg) dissolved in saline, and sacrificed 3 h, 12 h, 24 h, 3 days, 7 days and 10 days after administration. The tissues (stomach and esophagus) were removed and fixed in 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde for 6 h. After fixation 2 mm thick sections were obtained and fixed again in paraformaldehyde fixative for 3 days. The sections were dehydrated in upgrading series

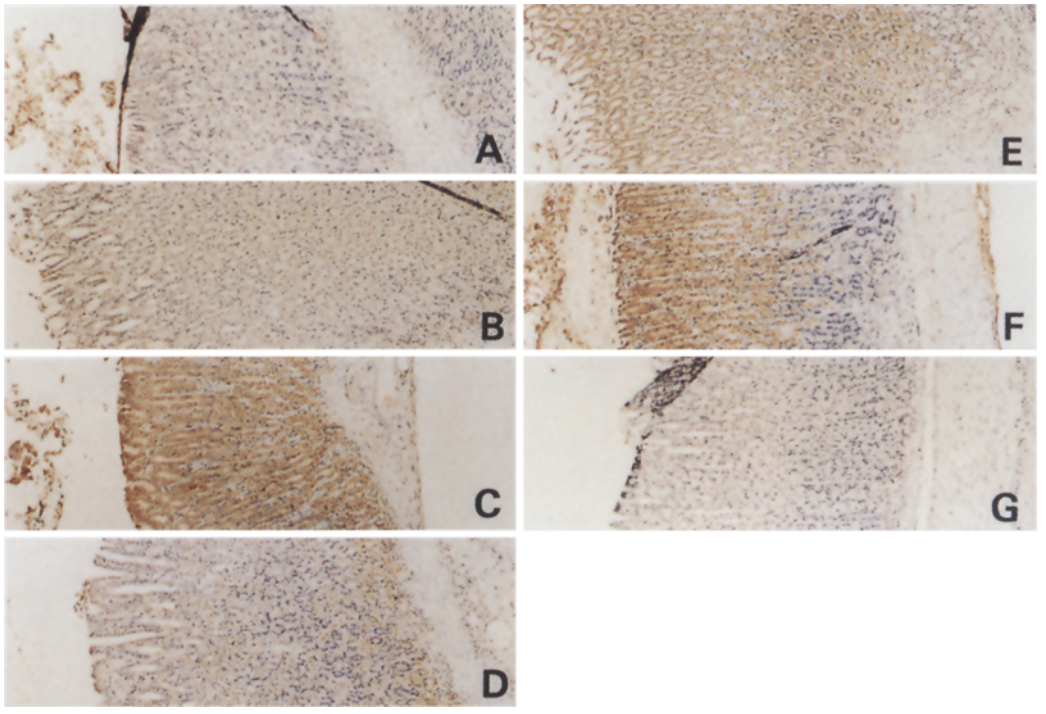

Fig. 1A–G. Immunohistochemical localization of paraquat in stomach (× 100). **A–G** were 3 h, 12 h, 24 h, 3 days, 7 days, 10 days after the administration of paraquat, and control, respectively

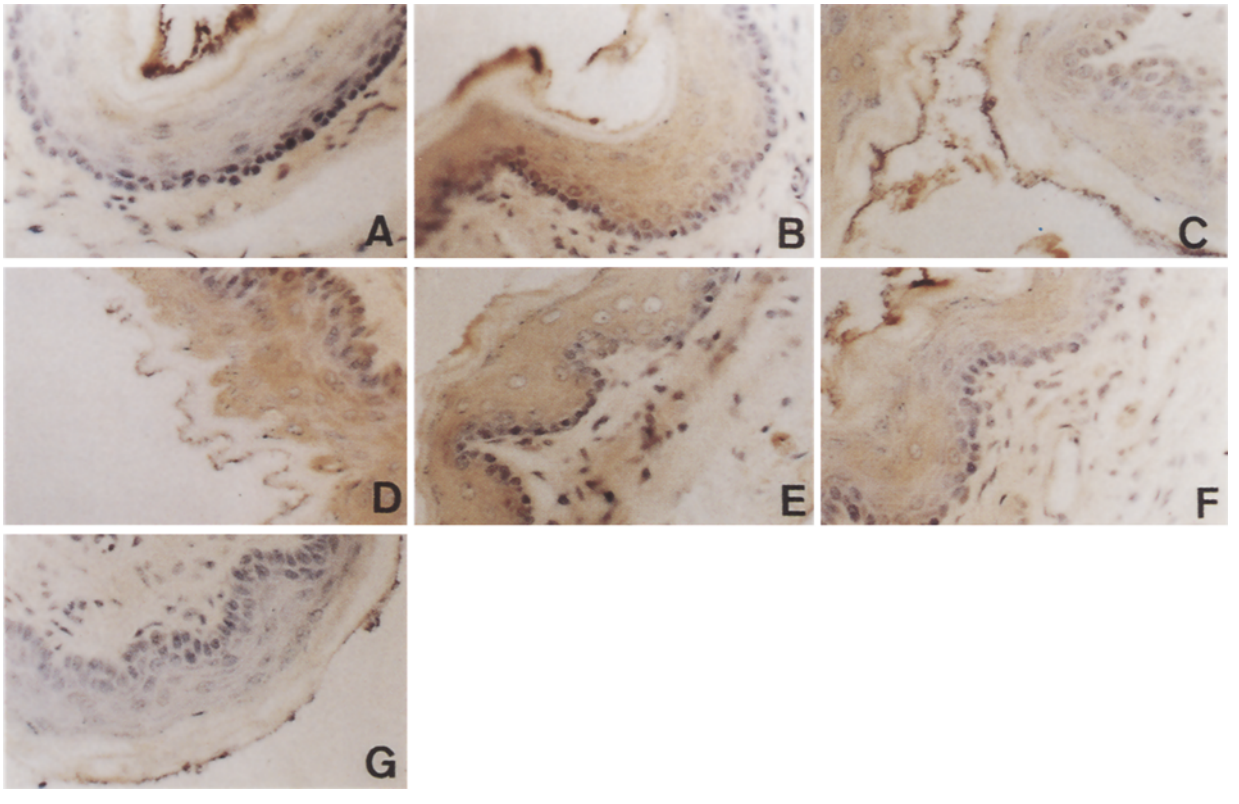

Fig. 2A–G. Immunohistochemical localization of paraquat in esophagus (× 400). **A–G** were 3 h, 12 h, 24 h, 3 days, 7 days, 10 days after the administration of paraquat, and control, respectively

of ethanol, cleaned in xylene, and embedded in paraffin. Sections 4 μm thick were used for the immunohistochemical demonstration of paraquat. Immunohistochemical staining was performed according to the method as described previously [10]. These experiments were approved by the Animal Care and Use Committee, Hokkaido University School of Medicine, Sapporo, Japan.

Results and discussion

The localization of paraquat in the stomach and esophagus of rats was demonstrated immunohistochemically. Since paraquat is highly water soluble, it would be removed by the fixation and staining processs except for the intracellularly localized paraquat. It is not clear whether paraquat is localized intracellularly as a free form, or bound to cellular components. However, the antiserum used in this study selectively recognizes both the methyl group and bipyridyl ring of paraquat, and does not bind to analogs of paraquat [12], suggesting that the antiserum specifically binds to the intracellularly localized paraquat which retains its original structure.

In the stomach, paraquat was localized in the epithelial cells between 24 h and 10 days after administration (Fig. 1). In the esophagus, paraquat was localized in epithelial cells and lamina propria mucosa between 12 h and 10 days after administration (Fig. 2).

Although the distribution pattern of paraquat in both organs was similar to that found in the intestine of rats as reported previously [10], clear changes in the distribution pattern were not observed with time. The reason of this difference was thought to be that reabsorption of paraquat from bile to mucosa does not occur in stomach and esophagus. The mechanisms of accumulation of paraquat in both organs are still unclear, but is interesting that paraquat is retained for such a long time. A previous report pointed out the importance of the gastric wall as a reservoir of fentanyl in clinical pharmacology [9]. The stomach and esophagus also seem to be an important reservoir for the redistribution of paraquat.

In this paper, we demonstrated the histological localization and dynamics of paraquat in stomach and esophagus of rats using the immunohistochemical technique.

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