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Application of Microwave Irradiation for Histochemical Analysis of *Griffonia simplicifolia* Agglutinin-1 (GSA-1) Binding Sites in Sigmo-rectal Polyps.

Michiko Tanaka and Makoto Watanabe

Ten specimens each of carcinoma, adenoma and non-neoplastic mucosa which were obtained by endoscopic biopsy from sigmo-rectal polyps were treated with microwave irradiation and then examined by lectin histochemistry. The results were compared with those obtained by the conventional method. With the conventional method *Griffonia simplicifolia* Agglutinin-1 (GSA-1) binding sites on the apical surface were positively detected in 4/10 carcinomas but not in adenomas or non-neoplastic mucosa. In the Golgi area, the GSA-1 binding sites were revealed in 2/10 adenomas, but not in carcinomas or non-neoplastic mucosa. When microwave irradiation was applied, the number of GSA-1 binding sites detected on the apical surface increased to 7/10 carcinomas but remained negative in adenomas and non-neoplastic mucosa. In the Golgi area, detectable GSA-1 binding sites increased to 3/10 carcinomas, 9/10 adenomas and 9/10 non-neoplastic mucosa. Comparing the conventional method with the microwave irradiation method, the incidences of the detectable GSA-1 binding sites increased through the microwave irradiation in both apical surface and Golgi area.

Key Words

INTRODUCTION

The differentiation-dependent alteration of carbohydrates was reported in mouse tissues\(^1\) and in many human cancers as well\(^2-10\). Lectin and anti-carbohydrate antibodies were used to study the histological distribution of specific carbohydrate moieties\(^1-10\). However, the staining patterns were variable and controversial\(^8-10\).

On the other hand, microwave irradiation (MWI) has been applied for tissue fixation, histochemical staining and the retrieval of antigenicity for immunohistochemical staining\(^11-17\). In this study, we applied MWI to enhance and improve lectin binding in conventionally fixed tissues.

MATERIALS AND METHODS

Specimens

Among the specimens of sigmo-rectal polyps in our laboratory which were obtained by endoscopic biopsy and conventionally fixed with 10% buffered formalin for 1 to 3 days, 10
cases each of carcinoma, adenoma and non-neoplastic mucosa were selected and used for the study. Paraffin-embedded specimens were sectioned at 4 µm and placed on glass slides coated with 3-aminopropyltriethoxysilane (APS). The tissue sections were then stained with hematoxylin and eosin as usual and used for lectin histochemistry.

**Lectins**

Biotinated GSA-1 was purchased from E-Y Laboratory, San Mateo, CA. Lectin histochemistry was performed according to the avidin biotin peroxidase complex (ABC) method using Vectastain Elite ABC Kit (Vector Laboratories Inc., Burlingame, CA).

**Staining method**

Lectin histochemistry was performed according to the previously reported conventional method (3).

1. Tissue sections were deparaffinized and washed in water.
2. The activity of endogenous peroxidase was blocked by 3% hydrogen peroxide for 10 min.
3. Washing with distilled water.
3'. Pretreatment of sections with MWI. (see detail in text)
4. Immersion in Dulbecoo's phosphate buffered saline (PBS).
5. Incubation for 20 min with 1% bovine serum albumin (BSA).
6. Incubation for 30 min with 0.01 mg/ml of biotinated lectin in PBS.
7. Washing 2 times in PBS.
8. Incubation for 30 min with ABC.
9. Washing 2 times in PBS.
10. Incubation with diaminobenzidine.
11. Washing in running water.
12. Counterstaining with hematoxylin or methyl green.
13. Dehydration in alcohol, clear in xylene, and mount.

**Microwave irradiation (MWI)**

A microwave oven, MR-A330 (Hitachi, Tokyo) was used for MWI. The level of the irradiation power was continuous at 500W. Heat absorption water was used during irradiation. MWI was performed after blocking the activity of endogenous peroxidase as described above in 3' step. Sections were immersed in 60 ml of the distilled water within a heat-resistant coplin jar. After putting lid on and fastening with a rubber band, the coplin jar was placed in heat-resistant glass vat with 900 ml of absorption water in it. Then the glass vat with the coplin jar in it was placed on the center of the turn table in the microwave oven. MWI was performed while referring to previous reports (12–17). In most cases, the sections had been boiled for 5 to 30 min by a domestic microwave oven at around 700 to 900 W. The boiling time was divided into a few cycles with an interval between cycles to check the fluid level in the jar (12,14,14–17). In this study, at first, MWI time was set at 15 min to boil the distilled water. Then, 5 min of MWI was repeated 3 times as written by Cuevas et al. (14). After each 5 min period, the fluid level in the jar was checked. After MWI was completed, the coplin jar was removed from the oven and allowed to cool down to room temperature for about a half hour. Other steps were performed according to the conventional method.

By the conventional method, the lectin binding sites were recognized mainly on the apical surface and in the Golgi area of the cytoplasm of epithelial cell as reported previously.
When one positive gland was recognized among other negative glands in tissue section, such specimen was classified as positive. The staining results were classified as follows. Positive glands were diagnosed as carcinomas, adenomas or non-neoplastic mucosa based on their morphology.

RESULTS

All of 10 carcinomas were identified as adenocarcinoma, and further histologically classified as tubular adenoma with mild dysplasia (8 cases), moderate dysplasia (1 case), and villous adenoma with moderate dysplasia (1 case).

By the conventional method the positive rates of the lectin binding sites in polyps are shown on Table 1. GSA-1 binding sites on the apical surface were detected in 4/10 carcinomas, while completely negative in adenomas and non-neoplastic mucosa. In the Golgi area, the binding sites were detected in 2/10 adenomas but not in carcinomas or non-neoplastic mucosa. After MWI, however, GSA-1 binding sites on the apical surface increased to 7/10 positive carcinomas but still remained negative in adenomas and non-neoplastic mucosa. A case of carcinomas which improved their staining pattern by MWI is shown on Figs. 1 and 2. In the Golgi area, GSA-1 binding sites were found in 3/10 carcinomas, 9/10 adenomas and 9/10 non-neoplastic mucosa after MWI. Thus, positive rates were increased by MWI. Though GSA-1 binding sites were not found on goblet cells and mucin appearing to be almost negative by the conventional method, MWI allowed detection of binding sites in goblet cells and mucin (data not shown).

DISCUSSION

Lectin histochemistry is performed to detect the carbohydrate moieties on the cell surface, which are well known to alter during differentiation1-10. These alterations are also studied in cases of cancer2-10. Among sigmoid-rectal carcinoma, GSA-1 binding sites were reported by several authors8-10. But discrepancies in their results were as follows: Lee reported that the distribution of the GSA-1 binding sites in rectal polyps was not specific to carcinoma or cancerous lesion8. McGarrity et al. reported that GSA-1 stained all colonic mucosa9. On the other hand, Caldero et al. reported that GSA-1 binding sites appeared on cancer cells

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<th>Golgi area</th>
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<td>conventional</td>
<td>MWI</td>
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<tr>
<td>carcinoma</td>
<td>4/10</td>
<td>7/10</td>
</tr>
<tr>
<td>adenoma</td>
<td>0/10</td>
<td>0/10</td>
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<td>non-neoplastic mucosa</td>
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with a high incidence in distal colon\textsuperscript{10}. In our previous study, the GSA-1 binding sites appeared on carcinoma more frequently than on adenoma and non-neoplastic mucosa\textsuperscript{3}. These discrepancies could be due to the difference in the histochemical procedures, though the precise reason for these discrepancies remains unclear.

Although formalin is the most popular fixative, it is not always the optimal fixative to preserve antigenicity of tissues for immunohistochemistry.

The mechanism underlying tissue fixation by formalin has not yet been adequately explained\textsuperscript{18}. To retrieve the antigenicity, pretreatment by enzymatic digestion is often recommended. However, Shi achieved improved staining by applying MWI to the formalin-fixed tissues instead of the enzymatic digestion in 1991\textsuperscript{12}. Many authors then observed enhanced immunohistochemical staining after MWI\textsuperscript{13–17}. Thus these methods were applied in this study. The MWI time varies among previous

\textbf{Figure 1.} A: Conventional method. A part of gland showing GSA-1 binding sites on the apical surface (arrows).

B: MWI method. Most of the apical surface was intensively positive for GSA-1 binding sites.
Application of Microwave Irradiation for Histochemical Analysis of *Grijjonia simplicifolia* Agglutinin-1 (GSA-1) Binding Sites in Sigmo-rectal Polyps.

Following each report, it was fundamental that the solution in the jar was first boiled and irradiated for a while. Shi first explained that immunohistochemical staining intensity could be increased by heating slides up to high temperatures and that heating the slides with microwave oven was clearly superior to heating the slides with the conventional oven in the case of tissues fixed in formalin for 24 hr or longer. The irradiation time also varied on account of the respective differences among microwave oven. In this study, the authors set the time as written in Methods. Although Cuevas et al. reported that several irradiations of 5 min were effective, it would appear that less than 3 times are sufficient, for we once successively tried 2 times of 5 min MWI. Hewicker-Trautwein et al. reported that MWI, whether for 10 or 25 min, did not make any difference. Thus, the optimal time for MWI needs more detailed investigation.

Concerning the solution in the co-

Figure 2. High power field of Fig 1.
plin jar during MWI, Shi recommended metal solutions such as saturated lead thiocyanate and zinc sulfate\textsuperscript{12).} On the other hand, Mori recommended phosphate buffer, zinc sulfate or distilled water\textsuperscript{17).} Since our preliminary study indicated that distilled water as well as zinc sulfate were employable, distilled water was used in this study (data not shown). A large volume of absorption water was used so that the solution in the jar heated slowly. During repeated MWI for 5 min at boiling, the loss of water in the coplin jar was insignificant.

Comparing the MWI method with the conventional method, detectable GSA-1 binding sites increased in both apical surface and Golgi area by MWI, although the effect of MWI did not enhance non-specific staining remarkably. MWI made the goblet cell and mucin positive for GSA-1 binding sites as well. Even though the detectable GSA-1 binding sites increased after MWI in carcinoma, the binding sites in adenoma and non-neoplastic mucosa were still negative. These results suggested MWI could reveal more carcinoma-associated GSA-1 binding sites in sigmo-rectal polyps in comparison with the results of the previous report\textsuperscript{3).} Hewicker-Trautwein et al. reported the efficacy of MWI on lectin histochemistry of microglial cells in paraffin-embedded sections in 1995, and reported that since the binding of lectins to glycoconjugates was based on their specific affinity to sugar residues, it might be speculated that the application of MWI in lectin histochemistry might facilitate access of the lectins to their receptors\textsuperscript{15).} Shi reported that though the mechanism concerning recovery and enhancement of antigenicity with microwave oven was not clear, it was possible that the cross-linking of protein caused by formalin fixation might be altered by MWI in a similar way to that achieved by enzymatic predigestion\textsuperscript{12).} These phenomena would play roles for the enhanced detection of GSA-1 binding sites in sigmo-rectal polyps. To confirm this improvement by MWI, more case studies would be necessary.

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