Development of a new contrast endoscopic method
With Techno Color blue P

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Key Words: Techno Color blue P, contrast endoscopy, dye-enhanced endoscopy, magnification endoscopy, pit pattern

ABSTRACT

Natural gardenia pigment has been known from old times as a dye and colorant and is presently used widely in that area as a food colorant and its safety has already been established. We have comparatively investigated the application of Techno Color blue P, which is the natural gardenia pigment, against normal pigments in contrast endoscopy. Techno Color blue P coagulated thereby decreasing its solubility as the pH of the solvent became acidic.

For the upper gastrointestinal tract, 10% and the lower gastrointestinal tract 20% of the Techno Color blue P solution is appropriate.

In upper gastrointestinal endoscopy, Techno Color blue P coagulates in the acid secreting areas, and is therefore unsuitable as a contrast method. However, in the pyloric gland, atrophied areas of the gastric mucosa, and duodenal villi, it was superior to Indigocarmine as a contrast method. In colonoscopy, intestinal fluids is alkaline and so Techno Color blue P showed a stable dyeing property with clearer contrasting compared to Indigocarmine. Contrast endoscopy with Techno Color blue P, is useful as a contrast method for accentuated surface morphology of the gastric mucosa, duodenal mucosa, and colonic mucosa.

Introduction

Dye-enhanced endoscopy is a method whereby various types of dyes are dispersed or sprayed onto the gastrointestinal mucosa or gastrointestinal surfaces. The minute irregularities, changes in color tone, and function of the mucosa or organ are observed endoscopically using the properties of dyes. Presently, it is becoming an indispensable method for clear and detailed diagnosis of the pathophysiology of gastrointestinal diseases.

Dye-enhanced endoscopy is classified into the contrast type, dye-enhanced type, dye-reaction type, and fluorescent endoscopy. Of these, the contrast type with Indigocarmine (IC) (Daiichi Pharmaceuticals, Tokyo) is a test that can be performed comparatively easily, and then it is
generally popular. Recently, the development and popularization of endoscopic submucosal dissection (ESD)\(^1\)-\(^2\) and less invasive surgery such as laparoscopic surgery\(^3\) in treatment of malignant gastrointestinal tumors, is gradually increasing the attention paid to the importance of diagnosing the existence and range of minute lesions. In addition, endoscopy by specialty optics such as magnified observation\(^4\)-\(^6\) and narrow band imaging (NBI)\(^7\)-\(^8\) are being performed. However, they are not common. Of these, the importance of diagnosis by the contrast method\(^9\) is again being emphasized.

We have investigated the application of Techno Color blue P (TB) (Mitsubishi Kagaku Foods Corp., Tokyo), a natural gardenia pigment\(^10\)-\(^11\) that has already been approved as a natural food additive, and there is ample information on its safety in contrast endoscopy. The aim was to further improve lesion diagnostic performance compared to the contrast method using IC.

**Materials and methods**

**I. Fundamental investigation**

TB is a pre-dried powderized substance that was obtained by reacting a mixture of iridoid glycoside that is in the liquid extracted from the rubiaceous gardenia fruit and protein decomposed residue, with \(\beta\)-glucosidase and then adding dextran, a food raw material. In addition, it is hydrosoluble and is a food additive, and that makes a substance with superior protein dying properties. To 10 mL each of distilled water prepared to a pH of 1, 2, 3, 5, 7, and 9 was added TB 0.5 g, and this was used as the 5% solution to investigate the solubility.

**II. Clinical investigation**

Dyes used

<table>
<thead>
<tr>
<th>Upper gastrointestinal tract (stomach, duodenum)</th>
<th>Lower gastrointestinal tract (colon, rectum)</th>
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<tbody>
<tr>
<td>0.1 % IC solution</td>
<td>0.1 % IC solution</td>
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<tr>
<td>10 % TB solution (pH 7)</td>
<td>20 % TB solution (pH 7)</td>
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1. **Upper gastrointestinal endoscopy**

In upper gastrointestinal endoscopy, after performing a pre-treatment with the mucous solubilizer, pronase, the gastric contents were thoroughly aspirated. Next, a conventional observation was performed using a videoendoscope and 0.1% IC solution and 10% TB solution were dispersed under direct vision using a dispersion tube. Dye-enhanced endoscopy was then performed. In addition, images obtained by magnified endoscopy and specialty endoscopy by NBI were comparatively investigated when appropriate.

In this study, gastric ulcer was classified by endoscopically classification of Sakita-Miya\(^11\), and gastric tumor by Japanese Classification of Gastric Carcinoma\(^13\).

2. **Colonoscopy**

Intestinal tract lavage fluid (polyethylene glycol solution) was administered based on the pre-treatment in the ordinary way of colonoscopy. Conventional observation was then performed using a videoendoscope. Next, 0.1% IC solution and 10% TB solution were dispersed under direct vision using a dispersion tube. Dye-enhanced endoscopy was then performed. In addition, like the upper gastrointestinal tract, a comparison was made with images obtained by specialty endoscopy.

In this study, colorectal tumor was classified by Japanese classification of Colorectal Carcinoma\(^14\), and by Kudo-Tsuruta classification in point of colonic pit pattern\(^15\).

3. **Stereo microscopic image of gastric resection specimen**

Fresh specimens of gastric tumor that were resected by ESD were observed by stereo microscopy.\(^16\)-\(^17\) 0.1% IC solution and 10% TB solution were dispersed and the structures of the glandular ducts in the tumor area were comparatively investigated.

All of these patients gave informed consent prior to participation in this study.
Results

I. Fundamental investigation
(solubility of TB)

TB clearly coagulated at pH 1 and 2 and dissolved at pH 3, 5, 7, and 9. In addition, the mean size of the coagulated particles in the 5%TB solution at pH 1 was 87.6 µm and 5.6 µm at pH 3. There was no significant difference between the values at pH 3, 5, 7 and 9 (Figure 1,2,3).

With regard to the application of TB in contrast endoscopy, TB coagulates at lower pH, and may also be diluted by the gastrointestinal contents. Therefore, 10% of TB solution were used for upper gastrointestinal endoscopy and 20% of TB solution for colonoscopy.

Fig.1 Solubility of Techno Color blue P at various pH values
TB clearly coagulated at pH 1 and 2 and dissolved at pH 3, 5, 7, and 9.

Fig.2 Observation of Techno Color blue P solution at a pH 1 (x 500)
TB coagulated at pH 1 and the mean size of the coagulated particles in the 5%TB solution was 87.6 µm.
II. Clinical investigations

1. Upper gastrointestinal endoscopy

Case 1 (Figure 4)

Upper gastrointestinal endoscopy showed a gastric adenoma\(^{38}\) of 10 mm in diameter in the posterior wall of the gastric antrum. In the conventional observation, it was recognized as an indistinct boundary of discoloration. The gastric foveola in the periphery of the lesion was enhanced by TB, enabling a clear imaging of the lesion boundary.

Fig.3  Sizes of the coagulated particles in the 5% Techno Color blue P solution at various pH values. The mean size of the coagulated particles at pH 1 was 87.6 µm and 5.6 µm at pH 3. There was no significant difference between the values at pH 3, 5, 7 and 9.

![Graph showing sizes of coagulated particles at various pH values](image)

- *** P < 0.005 vs control
- **** P < 0.005 vs pH 2

Fig.4  Gastric adenoma

The gastric foveola in the periphery of the lesion was enhanced by TB, enabling a clear imaging of the lesion boundary.

![Conventional observation vs Contrast with Techno Color blue P](image)
Case 2 (Figure 5)  
Upper gastrointestinal endoscopy showed a malignant lymphoma in the greater curvature of the upper part of the stomach. In the conventional observation, the enlarged folds of the greater curvature were observed. When TB was dispersed, it coagulated significantly. There was consistent coagulation in the fundic gland area and acid secreting areas.

Case 3 (Figure 6)  
Upper gastrointestinal endoscopy showed an artificial ulcer scar after ESD in the lesser curvature of the gastric angle. Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed superior dyeing property and the lesion could be more clearly observed.

Fig.5 Malignant lymphoma of the stomach  
When TB was dispersed, it coagulated significantly. There was consistent coagulation in the fundic gland area and acid secreting areas.

Fig.6 Gastric artificial ulcer scar after treatment of ESD  
Compared to the image obtained by IC dispersion, TB showed superior dyeing property and the lesion could be more clearly observed.
Case 4 (Figure 7)
Upper gastrointestinal endoscopy showed a type 0 IIa early gastric cancer\textsuperscript{20-21} of 20 mm in diameter in the lesser curvature of the gastric angle. In the conventional observation, the lesion was recognized as the discolored polypoid mucosa. In the image obtained by IC dispersion, a granular tumor was captured but in that obtained by TB dispersion, the boundary of the tumor was more clear and it became clear that there was formation of polypoid lesion due to the intertubular staining of the granular tumors.

Case 5 (Figure 8)
Upper gastrointestinal endoscopy showed a so called H2 stage gastric ulcer in the posterior wall of the antrum. Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed clear regeneration of the epithelium in the ulcer margin. Even with NBI, minute patterns of epithelial regeneration in the mucosa were clear.

Fig. 7 Early gastric cancer (type 0 IIa)
The boundary of the tumor was clear and it became clear that there was formation of polypoid lesion due to the intertubular staining of the granular tumors by TB.

Fig. 8 Gastric ulcer (H2 stage)
The image obtained by TB dispersion showed clear regeneration of the epithelium in the ulcer margin.
Case 6 (Figure 9)

Upper gastrointestinal endoscopy showed a normal mucosa of duodenum. In the image obtained by TB dispersion in the duodenum which is an alkaline area, individual regular villus formation, which was unclear in the image obtained by IC dispersion, could be clearly observed.

2. Colonoscopy

Case 7 (Figure 10)

Colonoscopy showed a type 0 IIa early rectal cancer with a diameter of 10 mm in the rectum. Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed good dyeing properties and so the pit pattern observation in the conventional observation was also superior. The lesion was suggested to be the type III-L pit pattern.

Fig.9 Duodenal mucosa
In the image obtained by TB dispersion in the duodenum which is an alkaline area, individual regular villus formation could be clearly observed.

Fig.10 Colonic cancer (type 0 IIa)
The image obtained by TB dispersion showed good dyeing properties and so the pit pattern observation in the conventional observation was also superior.
Case 8 (Figure 11)
Colonoscopy showed a type 0 IIa granular lesion in 1/3 of the periphery of the lumen of the sigmoid colon. Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed more contrast enhancement and so even the intermediate mucosa between the nodular tumors was clearly captured.

Fig.11 Granular lesion of colon (type 0 IIa)
The image obtained by TB dispersion showed more contrast enhancement and so even the intermediate mucosa between the nodular tumors was clearly captured.

Case 9 (Figure 12)
Colonoscopy showed a magnified observation of type 0 IIa rectal cancer with an Rb diameter of 20 mm in the rectum. Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed superior dyeing properties on the intertubular surface. Therefore, the contrast was better enhanced and a clear pit could be observed.

Fig.12 Magnified observation of colon cancer (type 0 IIa)
The image obtained by TB dispersion showed superior dyeing properties on the intertubular surface. Therefore, the contrast was better enhanced and a clear pit could be observed.
3. Stereo microscopic image of gastric adenoma from resected specimen (Figure 13)

IC and TC were dispersed onto fresh specimens of gastric adenoma and observed at a 200 x magnification by stereo microscopy.

Compared to the image obtained by IC dispersion, that obtained by TB dispersion showed high dyeing properties of the intertubular surface. It became clear that it had a glandular convolution pattern.

![Conventional observation](image1)

![Contrast with Techno Color blue P](image2)

Fig.13 Observation of stereo microscopic images of gastric adenoma (x 200)

TB showed high dyeing properties of the intertubular surface. It became clear that it had a glandular convolution pattern.

Discussion

If Lugol's solution is considered to be a pigment solution, then the history started when Schiller applied Lugol's solution for diagnosing cervical uterine cancer in 1933, and it has presently reached the diagnosis of superficial cancer of the esophagus. Furthermore, in 1963, Richart used Toluidine blue to diagnose cervical uterine cancer, and in 1968, Strong et al. reported diagnosing oral cancer using Toluidine blue. All these were aimed at the early diagnosis of cancers that are located close to the body surface, and were not applications of dyes in endoscopy. However, these studies had a significant impact on the development of dye-enhanced endoscopy.

The use of dyes in endoscopy was first reported by Niwa in 1965 when he reported using Toluidine blue in colonoscopy. For the upper gastrointestinal tract, in 1966, Chida and Okuda reported using Congo red to observe the gastric acid secretory areas, and in that same year, Tsuda and Aoki reported using blue dye to diagnose gastric lesions. These studies have formed the base for the further development of dye-enhanced endoscopy.

In Japan, classification of dye-enhanced endoscopy was proposed by the dye-enhanced endoscopy research committee in 1978. This classification consists of five classes: a) the contrast method, b) the dye-enhanced method, c) the pigment reaction method, d) fluorescence endoscopy, and e) other methods. Of these, the contrast method where the dye accumulation phenomenon is applied, is a simple method aimed at enhancing and observing the morphology of the folds of the gastrointestinal surface. The contrast method is very useful for capturing minute lesions and diagnosing the range of lesions with unclear margins, it is an essential method when biopsies, endoscopic mucosal resections and surgical operations, are being performed.

However, the basic dyes used in the contrast method are shades that are often comparable with the color tone of the mucosa. And, they must be colors that do not change or get absorbed by the mucosal surface. IC, which is a reagent for testing gastric function, is the most commonly used dye.

The TB used in this study was obtained when iridoid glycoside in solution extracted from the gardenia fruit was made to react with food enzymes. There are several types of gardenia.
enzyme pigments—gardenia blue, gardenia green, and gardenia red pigments. For this study, TB, which is gardenia blue pigment that is contrasts the color tone of the mucosa, was selected. The natural gardenia pigment has been used as a food additive from old times and its safety has been already established. IC has been reported to cause adverse reactions such as extreme shock when used as a reagent for testing renal function, however no such case has been reported when used in endoscopy.

In fundamental investigations on TB, it was found not to dissolve but rather coagulate in strongly acidic solvents at pH 1 or 2 in vitro. In order to apply TB clinically, we considered that the solubility of TB increases with increase in alkalinity in vitro, it readily coagulates in the upper gastrointestinal tract which is highly acidic, and it may be diluted by the gastrointestinal substances in the upper and lower gastrointestinal tracts. Therefore, we used 10 and 20% of the TB solution for the upper and lower gastrointestinal tracts, respectively. Even in actual endoscopy, it coagulated in the gastric fundal gland area where there is no atrophy. Therefore, it cannot be indicated as a contrast method for acid secreting areas. In other gastric parts that are not strongly acidic, TB did not coagulate as in the in vitro results. In the comparison with IC, it showed superior dyeing properties towards the crypt and intertubular surface, and the lesions could be easily identified.

As the duodenum is a more alkaline area than the stomach, clearer structures of the duodenal villi could be identified compared to IC. As the coloreutum is also an alkaline area, superior dyeing properties of the intertubular space that is also useful in the pit diagnosis of tumors was shown. Even in the stereo microscopic observation of isolated specimens, good dyeing property towards the intertubular surface was shown.

Although there has been marked advancement in magnified endoscopy and endoscopic equipments such as NBI, the diagnostic process is complicated and has not yet been generally established. Attention is being paid to the contrast method, which is simple to perform, and contrast with TB, which has higher lesion diagnostic performance compared to IC, which is commonly used in general at present, has been reported.

Conclusions

Dye-enhanced endoscopy with TB was superior to IC as a contrast method that enhances the surface morphology of the gastric mucosa which is non-acid secreting areas, duodenal mucosa, and colon mucosa. This method would be thoroughly applied clinically as an adjuvant procedure for accurately diagnosing tumor margins.

References


Received November 1, 2006
Accepted December 15, 2006