THE TRIALS IN THE CYTOLOGICAL DIAGNOSIS OF GASTRIC CANCER

PART II USE OF TETRACYCLINE FLUORESCENCE TECHNIQUE IN EXFOLIATIVE CYTOLOGY

by

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INTRODUCTION

The fact that tetracycline fluorescence may be detected in various tumors and bones following administration of tetracycline was reported by RALL, MILCH and McLEAY, and has been confirmed by many examiners. Present study was undertaken to investigate if the tetracycline fluorescence technique may aid the cytodiagnosis of gastric cancer and promise increased insight into cytochemical changes leading to malignancy.

CLINICAL MATERIALS AND METHODS

Four patients with gastric carcinoma and one patient of carcinosis from gastric carcinoma were subjected to this study.

A total doses of 6 000 mg of Cosa-tetracyn (tetracycline with glucosamine) was administered perorally 11 - 12 hours prior to the examination of gastric exfoliative cytology or the collection of ascites or blood. Two thousand mg of Cosa-tetracyn daily was given to each patient in divided doses at 6 hour interval and was continued over 3 days. Gastrectomy was performed at 34 hours after the last administration of the drug.

Surgical materials and smears from gastric cytological specimens, ascites and capillary blood obtained from these patients were subjected to the fluoroscopic examination. Surgical materials were collected immediately after gastrectomy, and examined gross under a 200 watt-ultraviolet light (3100~5400 A), and then tissue sections were cut in

* Cosa-tetracyn, supplied by Pfizer Taito Co., Ltd., Tokyo, Japan.

Fig. 1: Normal squamous epithelial cell in the cytologic specimen showing the brilliant whitish yellow nucleus and faint yellow cytoplasm. × 400.

Fig. 2: Nuclei of cancer cells and inflammatory cells in the cytologic specimen showing the brilliant gold-yellow tetracycline fluorescence. × 400.

Fig. 3: Cancer cells in ascites from the patient with carcinomatous peritonitis showing the gold-yellow tetracycline fluorescence of various grades. × 80.

Fig. 4: The section of surgical material showing the brilliant white-bluish auto-fluorescence in the serosa membrane and connective tissue. × 80.

Fig. 5: The section of surgical material showing the brilliant white-bluish auto-fluorescence in the connective tissue and the vessel wall. × 80.
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a cryostat in about 15μ thick, mounted on glass slides in glycerin and covered with
glass cover slips.

These smears and tissue sections were examined in dark field with a Carl Zeiss
microscope equipped with a photographic attachment. The ultraviolet source was an
Osram HBO 200 ultra high-pressure mercury lamp and the 3100 to 5400Å lines were
obtained with a piece of KG-1 filter and two of BG-12. Magnification of microscope
was 8×10 or 40×10. Photographs were taken with Fuji SSS film using 35 mm
Olympus PM-6 and an OG-5 filter attached to the microscope. Exposure time varied
from 10 seconds to 10 minutes.

RESULTS

Most of the cancer cell obtained in gastric exfoliative cytology and ascites yielded a
tetracycline fluorescence. These cells showed brilliant gold yellow fluorescence in nuclei
with intensely yellow granules and faint yellow fluorescence in cytoplasm. Normal
squamous epithelial cells from the oroesophageal tract showed the brilliant whitish yellow
nuclei and the faint yellow cytoplasm. Leucocytes in blood and inflammatory cells in
gastric cytological specimens showed brilliant yellow fluorescence in the nucleus and faint
blue in the cytoplasm.

As to surgical specimens, macroscopically, normal mucosa surface presented diffuse
yellow fluorescence, and tumor surface and metastased lymphonodes showed brilliant gold-
yellow fluorescence.

And microscopically, strikingly brilliant white-bluish auto-fluorescence was apparent
in serosa membrane, connective tissue and in the vessel wall. Normal cells of the gastric
mucosa showed orange-yellow fluorescence, the most brilliant in body chief cells. Nuclei
of carcinoma cells, inflammatory cells and fibroblasts presented a brilliant orange-yellow
fluorescence. Very faint yellow fluorescence was noted in the cytoplasm of these cells.

Tetracycline fluorescence diminished rapidly during the observation under exposure
of ultraviolet light. Therefore, the observation and photographing should be made as
quickly as possible, otherwise correct findings would hardly be obtainable.

DISCUSSION

The attempts have been made by FRIEDMAN5, MELLORS6,7 and BERTALANFFY8 to
utilize the various fluorescent dyes to the identification of cancer cells in the fixed
specimen in exfoliative cytology. Recently many investigators (CRAMER9, STICH10 and
RASSMUSSEN-TAXDAL11) recognized the affinity of the various fluorescent dyes to the
tumor tissue of the human being, and the mechanism of selective uptake of these drugs
has been discussed. However, on account of several drawbacks, the fluorescence techniques

Fig. 6 : The section of surgical material showing the orange-yellow tetracycline fluorescence in normal cells
of the gastric gland and the most brilliant fluorescence in body chief cells. × 80.

Fig. 7 : The section of surgical material showing the brilliant white-bluish auto-fluorescence in the
connective tissue and the brilliant gold-yellow tetracycline fluorescence in inflammatory cells
scattered in the muscle layer. × 80.

Fig. 8 and 9 : The section of surgical material showing the brilliant orange-yellow tetracycline fluorescence
in cancer cells, inflammatory cells and fibroblasts in submucosal layer. × 80 and × 400.
with these drugs have not yet contributed to the diagnosis of cancer.

Tetracycline has the benefit of prominent concentration in malignant tumor tissue without giving toxicity to living body after administration of this drug. Therefore its application to exfoliative cytology should be highly expected.

Appearance and persistence of fluorescent material in tumor tissue after tetracycline administration were demonstrated macroscopically by RALL et al. Author also observed the same phenomenon in gastric cancer specimens. Moreover, microfluorometric study of the gastric cytological specimens and the resected stomach after administration of this drug disclosed tetracycline fluorescence in malignant cells.

The precise mechanism whereby localization of induced fluorescence occurs remains unelucidated. MILCH et al. explained this mechanism with formation of a complex with new bone matrix and calcium by tetracycline. McLEAY suggested that cancer tissue had the ability to bind tetracycline, possibly through an enzyme factor in metabolism; glutamate has been suspected. HELANDER & BÖTTGER found terramycin concentrated in the reticuloendothelial system and in the liver and kidney. In the present study, author observed prominent localization and persistence of tetracycline in cancer cells, inflammatory cells, fibroblasts and normal cells of the gastric mucosa, particularly in body chief cells, but not in areas adjacent to the blood vessel, suggesting that the localization of fluorescence in these cells is closely related to prosperous metabolism and not to blood supply.

For the screening test in the exfoliative cytology, this tetracycline fluorescence technique has drawbacks such as the necessity of long interval administration of the drug before the examination, and of speedy observation on account of rapid diminution of the fluorescence in the cytologic specimens. Furthermore, it is difficult to identify the malignant cells, because the identification of the cell is to be made only through the intensity and dimension of the fluorescence, and because the fine nuclear structure is hard to obtain by this procedure. Therefore, it is not adequate to use this technique as the routine screening test of exfoliative cytology.

If a sufficient saturation of the drug in tumor cells could be obtained in a short period, and disparity between malignant cells and the other cells in their fluorescences could be increased, this technique would obtain the position of excellent screening test in exfoliative cytology.

SUMMARY

The investigation of tetracycline fluorescence was made with the gastric cytological specimens, ascites and surgical materials obtained from the patient with gastric carcinoma or carcinosis of gastric carcinoma following the administration of Cosa-tetracyn.

Malignant cells in these specimens showed very intense tetracycline fluorescence, but normal cells of the gastric mucosa, particularly body chief cells, inflammatory cells and fibroblasts also showed fairly intense fluorescence. Moreover, the fine cellular structure is not presented by this method, so that it is difficult to distinguish malignant cells from the other cells.

This fact and complicated procedure of this examination may not permit the
application of tetracycline fluorescence technique as the routine screening test in exfoliative cytology.

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References


和文抄録

胃癌細胞診の診断適中率向上を目的とする二、三の試み

第二編 胃癌細胞診へのテトラサイクリン蛻光法の応用

京都大学医学部外科学教室第二講座 (指導：吉崎安誠教授)

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4例の胃癌及び1例の胃癌による腫性腹膜炎の患者に、コサ・テトラサイクリン（グルコサミン添加テトラサイクリン）を経口的に合計6,000mgを3日間に亘って授与し、一定時間後、これらの患者から採取した胃細胞診試料、腹水及び手術材料のテトラサイクリン蛻光について、蛻光顕微鏡学的観察を行なった。これらの試料中の癌細胞は極めて強いテトラサイクリン蛻光を発することを認めた。併し、正常粘膜細胞特に主細胞、炎症性細胞並びに線維芽細胞もまた可成り強い蛻光を示した。さらに、この蛻光を以てしては、細胞、特に核の微細構造の得られなかったので、癌細胞を判別するのが困難であった。