LacCur Stain for Detection of Mucin in Adenocarcinoma

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Abstract

Identification of cytoplasmic mucin, usually by Mayer's mucicarmine stain, is one of the criteria to diagnose adenocarcinoma. The inexpensive LacCur stain, made up of *Curcuma longa* (khamin-shan) and secreta of *Laccifer lacca* (krang) has been introduced. The aim of this study was to compare the Mayer's mucicarmine and LacCur stains in the detection of mucin material. The specimens included 17 adenocarcinomas of the stomach, 16 of the colon, 18 of the lung, 16 of the breast, and 12 of the bile duct. Squamous cell carcinoma and hepatocellular carcinoma (altogether 20 cases) were set as negative control. Like Mayer's mucicarmine, LacCur was capable of detecting of intracytoplasmic mucin in all adenocarcinomas of the stomach, colon and bile duct, and revealed mucin substance in 15/18 and 11/16 cases of specimens from the lung and breast, respectively. The negative control group showed a negative result. Although a little more time required in preparation, the LacCur stain is simple and very economical.

Key word : LacCur Stain, Mucin, Adenocarcinoma

WATTANASIRMKIT V, TANBOON J, SHUANGSHOTI S, SHUANGSHOTI S
J Med Assoc Thai 2004; 87: 87-91

For a long time, the secreta of *Laccifer lacca* insect known as "lac"[1] has been used for textile coloring, and it has also been reported as a hematoxylin substitute for histochemistry[2]. *Curcuma longa* (known in Thai as "khamin-shan") has been a well-known textile and food coloring since olden days[3],

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and research over the last 50 years has indicated that curcumin (diferuloylmethane), a polyphenol derived from *Curcuma longa* (commonly known as turmeric), can both prevent and treat cancer[4]. In addition, the dye extracted from *Curcuma longa* has also been found to produce a similar staining property to a metanyl yellow[3].

Using the two natural dyes, Sriplung et al, in 1995, described a polychrome method, which they coined a "LacCur" stain, for detection of mucin substance in paraffin sections[3]. The method is produced by sequential stepwise staining of hematoxylin, crude lac extract, and crude curcuma extract. The LacCur stain was claimed to produce colors comparable to the widely used Mayer's mucicarmine stain, and was less expensive. To further evaluate the efficacy of LacCur stain, in the present study the authors compared the LacCur stain with commercially available Mayer's mucicarmine stain, using a series of 79 adenocarcinomas of various organs.

MATERIAL AND METHOD

Adenocarcinomas of various organs were retrieved from the pathology file at the Department of Pathology, King Chulalongkorn Memorial Hospital. These included 17 adenocarcinomas of the stomach, 16 of the colon, 18 of the lung, 16 of the breast, and 12 of the bile duct. All were stained with LacCur[3] and Mayer's mucicarmine (Bio-Optica, Italy) stains to demonstrate mucin material. The LacCur staining method was slightly modified and summarized below (see appendix). Only intracytoplasmic staining was considered positive, regardless of the number of positive cells, whereas intraluminal mucin content was disregarded. Thirteen squamous cell carcinomas of the head and neck region (7 cases), skin (5 cases), and lung (1 case), and 7 hepatocellular carcinomas were served as negative controls for the LacCur stain, and normal colon as a positive control.

RESULT

The result of LacCur stain (Fig. 1) in the detection of mucin material in various adenocarcinomas was perfectly comparable with the commercial Mayer's mucicarmine stain. With both staining techniques, all 17 adenocarcinomas of the stomach, 16 of the colon, and 12 cholangiocarcinomas were stained positively for mucin. Intracytoplasmic mucin was demonstrated in 15/18 pulmonary adenocarcinomas and 11/16 ductal carcinomas of the breast. The number of positive cells, however, varied from case to case. All squamous carcinomas showed a negative result with LacCur stain. All hepatocellular carcinomas were also negative, but it should be noted that some tumors with pseudoglandular formation demonstrated luminal staining. The material cost of commercial Mayer's mucicarmine is 60 baht (1.5 US dollar) per glass slide while that of the LacCur is less than 5 baht (1.5 cents). Approximately 1 hour was needed for the mucicarmine, whereas five and a half hours were required for the LacCur staining procedure.

DISCUSSION

Even though significant progress has been made in diagnostic surgical pathology of neoplasms driven mainly by the advent of immunohistochemistry technique, which has become a routine diagnostic tool in many pathology laboratories in Thailand, the distinction of different carcinoma cell types remains problematic. Specifically, adenocarcinoma can be very difficult to differentiate from other malignant epithelial tumors, and, in many examples, the distinction relies largely on the demonstration of intracytoplasmic mucin substance in neoplastic cells.

In the current study, the authors were able to replicate the LacCur staining method previously described[3]. Furthermore, it was shown that the LacCur stain can be used as a perfect substitute for the commercially available Mayer's mucicarmine stain for detecting mucin material in adenocarinomas of various organs. Although a little more complicated in terms of staining procedure, which takes a longer period to complete, the cost of LacCur stain is much cheaper than that of the mucicarmine stain.

For practical diagnostic purposes, it should be kept in mind, however, that, in the present study, the LacCur stain was used only in cases with clear-cut histologic features of adenocarinoma. Such examples, generally, do not require the demonstration of mucin material. In other words, without the LacCur stain, all of these cases would have been diagnosed as adenocarcinoma anyhow. The LacCur stain might play a role in the diagnosis of poorly-differentiated adenocarinoma, in which tumor cells do not form distinct glandular structures, but this actual contribution remains to be elucidated. Nonetheless, the LacCur method should be very helpful in identifying minute nodal metastasis of adenocarcinoma cells, particularly the signet-ring cell type, in which tumor cells can easily be missed on routine stain. Of note, 10

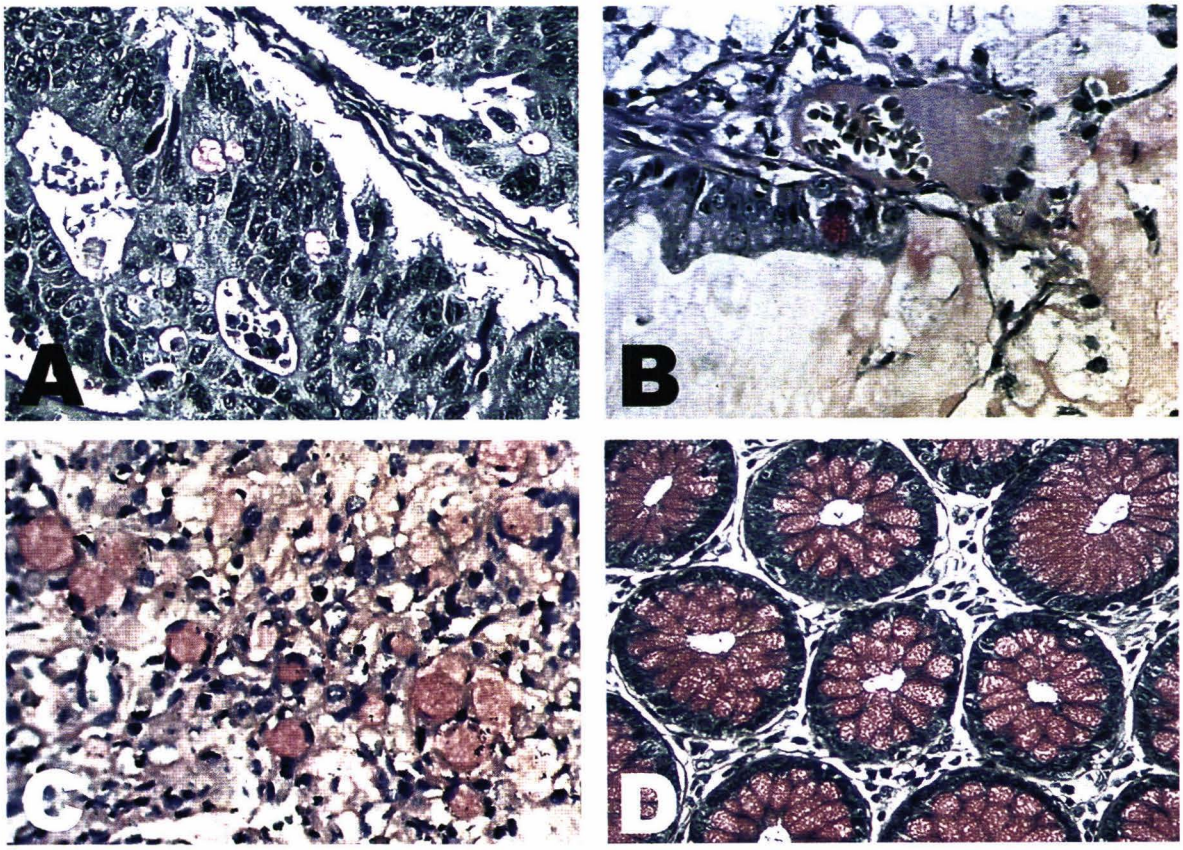

Fig. 1. **A) LacCur stain demonstrates intracytoplasmic mucin substance in colorectal adenocarcinoma, B) pulmonary adenocarcinoma, C) gastric signet-ring cell adenocarcinoma, and D) normal colonic epithelium.**

per cent of adenocarcinomas in the present series, including 5 of the breast and 3 of the lung, were negative for mucin material. Therefore, the absence of intracytoplasmic mucin does not entirely exclude the possibility of adenocarcinoma. Adjustment of the technique, for example using heat or microwave treatment, should shorten the staining time[5].

In conclusion, the LacCur stain has produced a similar result to the Mayer's mucicarmine stain in the detection of mucin substance in a series of 79 adenocarcinomas. The staining method is very simple and economical, and should be made available in pathology laboratories in Thailand.

ACKNOWLEDGEMENT

Dr. Shanop Shuangshoti was supported by The Development Grants for New Faculty Members/ Researchers, Chulalongkorn University.

(Received for publication on June 24, 2003)

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APPENDIX

LacCur stain

Solutions

Lac dye : Four grams of powdered seed lac, 0.5 gram of aluminum chloride anhydrous, and 10 ml of distilled water are mixed and heated on an electric hot plate until the lac dye dissolves, and residual shellac clumps. The solution is, then, diluted with 10 ml of 50 per cent ethanol and filtered. 3 ml of phenol is added to the filtrate to make a stock solution. The solution is kept at room temperature for at least 1 week and can be kept for months. To make a working solution, dilute 1 part of the stock solution with 4 parts of tap water.

Curcuma dye : Working solution of curcuma dye is made by mixing 10 grams of dry powdered *Curcuma longa* (khamin shan) with 100 ml of 95 per cent ethanol. Let the solution stand for 1 hour and then filter.

Procedure

1. Deparaffinize and dehydrate paraffin sections to distilled water.
2. Stain with a working solution of Weigert's hematoxylin for 7 minutes.
3. Wash well in running tap water for 10 minutes.
4. Stain with working lac dye solution for 5 hours.
5. Rinse quickly in distilled water.
6. Place in 2 per cent ferric chloride solution for 1 minute.
7. Counterstain with curcuma dye solution for 5 minutes.
8. Rinse quickly in water.
9. Dehydrate in 95 per cent ethanol, absolute ethanol, and clear in xylene.
10. Mount with permount.

Results

Mucin substance : Red.
Nuclei : Dark brown.
Other cellular and intercellular components : Brown.

สีย้อมครั่งและขมิ้น ในการตรวจหาสารเมือกในมะเร็งชนิดต่อม

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หนึ่งในเกณฑ์การวินิจฉัยมะเร็งชนิดต่อม คือการตรวจพบสารเมือกในเซลล์มะเร็งโดยการย้อมพิเศษ Mayer's mucicarmine ได้มีผู้คิดค้นวิธีการตรวจหาสารเมือกโดยใช้สีย้อม ราคาถูกที่ได้จากครั่งและขมิ้นชันและได้ตั้งชื่อสีย้อมชนิดนี้ว่าสีย้อม LacCur การศึกษานี้ได้ทำการเปรียบเทียบระหว่างการย้อม Mayer's mucicarmine และ LacCur ในการตรวจหาสารเมือกในเซลล์มะเร็งชนิดต่อม จากหลายอวัยวะ รวม 79 ตัวอย่าง ประกอบด้วย กระเพาะอาหาร 17, ลำไส้ใหญ่ 16, ท่อน้ำดี 12, ปอด 18 และเต้านม 16 ตัวอย่าง โดยใช้กลุ่มควบคุมคือมะเร็งเซลล์สความัส และมะเร็งเซลล์ตับรวม 20 ตัวอย่าง จากการศึกษาพบว่าการตรวจหาสารเมือกโดยวิธีย้อม LacCur และ Mayer's mucicarmine ได้ผลเหมือนกันทุกประการ โดยพบสารเมือกในตัวอย่างมะเร็งทุกตัวอย่าง ที่ได้จากกระเพาะอาหาร, ลำไส้ใหญ่และท่อน้ำดี พบสารเมือก 15 จาก 18 และ 11 จาก 16 ตัวอย่าง ของมะเร็งปอดและเต้านมตามลำดับ ไม่พบสารเมือกเลยในกลุ่มควบคุม แม้จะสิ้นเปลืองเวลามากกว่า แต่เมื่อเทียบกับความคุ้มทุนแล้วการย้อม LacCur น่าจะเป็นแนวทางที่ดีในการใช้ตรวจหาสารเมือก แทนการย้อม Mayer's Mucicarmine

คำสำคัญ : สีย้อมครั่งและขมิ้น, สารเมือก, มะเร็งชนิดต่อม

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