

Success Rate of Collagen Gel Droplet-embedded Culture Drug Sensitivity Test in Colorectal Cancer: Are Antibiotics a Prerequisite for Specimen Irrigation?

Takeshi Yamada¹, Hayato Kan¹, Satoshi Matsumoto¹,
Yoshikazu Kanazawa¹, Michihiro Koizumi¹, Junpei Sasaki¹,
Aya Tani¹, Kimiyoshi Yokoi^{1,2} and Eiji Uchida¹

¹Surgery for Organ Function and Biological Regulation, Graduate School of Medicine, Nippon Medical School

²Department of Surgery, Nippon Medical School Chiba Hokusoh Hospital

Abstract

The collagen gel droplet-embedded culture drug sensitivity test (CD-DST) is one of the best chemosensitivity tests owing to its high success rate. However, CD-DST is often a culture method, and contamination is a serious problem, especially in the case of colorectal cancer, which is contaminated by enteric bacteria. It has been reported that the success rate of CD-DST is 64.0% in the case of colorectal cancer. Therefore, the sampling and washing of specimens before culture are extremely important. By washing specimens carefully with normal saline containing antibiotics, we achieved a success rate of 85.3% in the case of colorectal cancer. To improve the success rate, we started specimen irrigation with a large amount of normal saline in January 2007. As a result, a success rate exceeding 90% was acquired. For the success of CD-DST for colorectal cancer, it is important to irrigate specimens many times with a large amount of normal saline.

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Key words: collagen gel droplet-embedded culture drug sensitivity test, colon cancer, chemosensitivity test

Introduction

The progress of colorectal cancer chemotherapy has been remarkable. Excellent treatment guidelines are available, and the standard treatment regimen is FOLFOX (leucovorin, fluorouracil, and oxaliplatin) or FOLFIRI (leucovorin, fluorouracil, and irinotecan) with or without molecularly targeted agents. Although response rates range from 40% to 50%^{1,2}, many patients have adverse effects or do not benefit

from chemotherapy. Consequently, determining whether an anticancer drug will be effective for individual patients would be of enormous benefit, as would personalized therapy.

Anticancer drug chemosensitivity tests support personalized therapy by predicting the effect of chemotherapy, and one of the best such tests is the collagen gel droplet-embedded culture drug sensitivity test (CD-DST)³. However, the CD-DST is a culture method, and contamination is a serious problem, especially in the case of colorectal cancer,

Correspondence to Takeshi Yamada, Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

E-mail: y-tak@nms.ac.jp

Journal Website (<http://www.nms.ac.jp/jnms/>)

which is contaminated by enteric bacteria. Therefore, the sampling and washing of specimens before culture are extremely important.

We have worked toward improving the success rate of the CD-DST in colorectal cancer. To that end, preventing contamination is most important. We believe that irrigation of specimens with a large amount of normal saline is required. The purpose of this study was to evaluate the effect of specimen irrigation with a large volume of normal saline on the success rate of the CD-DST in colorectal cancer.

Patients and Methods

Patients

Our research subjects were 87 patients with colorectal cancer who underwent surgery from January 2004 through August 2010 in our department. We divided the subjects into 2 groups: group A (36 patients) underwent surgery from January 2004 through December 2006, and group B (51 patients) underwent surgery from January 2007 through August 2010.

Specimen Preparation

At the end of surgery, we obtained specimens by stripping off the surface of a cancer tissue. We can collect the largest number of cancer cells from the surface. The specimens from group A were irrigated 5 times with 20 mL of saline containing 200 U/mL penicillin (Gibco, Grand Island, NY, USA), 200 µg/mL streptomycin (Gibco) and 50 µg/mL amphotericin B. On the other hand, specimens from group B were irrigated 10 times with 40 mL of saline without antibiotics. After irrigation, the specimens from both groups were stored in Eagle's minimal essential medium (Gibco) at 4°C until the start of the CD-DST.

CD-DST

We performed the CD-DST with the method of Kobayashi et al³. This sensitivity test was performed in the BML Laboratories (Saitama, Japan). We considered the CD-DST to be successful when the culture in a dish with an anticancer drug and that in a dish without an anticancer drug (control) were both successful.

Bacteriological Analysis

To judge the effect of specimen irrigation with a

Table 1a Patient background

	Group A (n = 38)	Group B (n = 45)
Sex (M : F)	31 : 7	23 : 22
Age (years)	67.4	67.2
Pathologic type (well-differentiated : moderately differentiated : poorly differentiated)	19 : 15 : 4	27 : 13 : 5
T classification (1 : 2 : 3 : 4)	4 : 7 : 22 : 5	2 : 4 : 36 : 3

Table 1b CD-DST success rates

Group	A	B
Total cases	38	45
Successful cases	29	36
Success rate	76.3%	80.0%
Contaminated cases	6	4
Contamination rate	15.8%	8.9%

large volume of normal saline, we counted the number of bacterial cells in the irrigation solution of specimens from 5 patients. We counted the number of bacterial cells in the first irrigation solution (sample 1), the fourth irrigation solution (sample 2), and the last irrigation solution (sample 3).

Results

There were no cases from which we could not gather a sufficient number of cells. There were 2 cases each in group A and group B which showed a poor proliferation capability of cancer cells, and these 4 cases were excluded from the study. Thus, 34 cases belonged to group A, and 49 cases belonged to group B.

There were no significant differences in clinicopathologic factors between the groups (**Table 1a**). The success rate did not differ significantly between group A (85.3%) and group B (91.8%; **Table 1b**).

In all cases, the number of bacterial cells decreased with irrigation (**Table 2**). We found 10^3 to 10^5 bacterial cells per milliliter of the first irrigation solution but only 10^3 bacterial cells or less per milliliter of the last irrigation solution.

Table 2 Bacteriological analysis

Case	Age (years)	Sex	Number of cells		
			Sample 1	Sample 2	Sample 3
1	73	F	10 ⁵	10 ⁴	10 ³
2	67	M	10 ³	0	0
3	76	M	10 ⁵	10 ⁴	10 ³
4	79	M	10 ⁵	10 ⁴	10 ³
5	66	M	10 ⁴	10 ⁴	10 ³

Discussion

In cases of lung or breast cancer, the success rate of the CD-DST is 80% or higher⁴. However, in cases of colorectal cancer the success rate of the CD-DST is 64.0%⁵, which indicates the limitation of the CD-DST in playing a key role in personalized therapy for colorectal cancer.

The most important problem of CD-DST is contamination, especially in colorectal cancer. Because bacterial contamination cannot occur in the absence of bacteria, the success rate can be increased by minimizing the number of bacterial cells before starting the CD-DST. Therefore, careful irrigation of specimens is extremely important. To our knowledge, there have been no reports of how many bacterial cells adhere to colonic specimens or how much irrigation solution is needed to prevent contamination.

We have succeeded in performing the CD-DST by washing specimens carefully with normal saline containing high concentrations of antibiotics. To improve the success rate, we started irrigating specimens with large amounts of normal saline without antibiotics in January 2007. The present study shows the importance of specimen irrigation with a large amount of normal saline, and our success rate is higher than previously reported rates⁵. By irrigating 10 times, the number of bacterial cells decreased to 1/1,000, and the irrigation solution contained 10³ or fewer bacterial cells per milliliter. Although 4 irrigations (total volume, 160 mL) decreased the number of bacterial cells to 1/10, many bacterial cells still adhered to the specimen. If the number of bacterial cells adhering to a specimen is 1,000 or less, antibiotics contained in

the culture solution will prevent contamination in almost all cases. As a result, a success rate exceeding 90% was obtained. With this high success rate, we can establish personalized therapy on the basis of the CD-DST. To establish the CD-DST as an accurate method, we must increase its success rate and review the sampling and irrigation methods. Can an increase in the amount of irrigation solution decrease the number of bacterial cells adhering to colonic specimens and increase the success rate of CD-DST? These questions must be addressed by future studies.

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