Individualized chemotherapy for colorectal cancer based on the collagen gel droplet-embedded drug sensitivity test

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Received March 20, 2012; Accepted June 26, 2012

DOI: 10.3892/ol.2012.823

Abstract. The leucovorin (FOL) and fluorouracil (5-FU) plus oxaliplatin (l-OHP; FOLFOX) or FOL and 5-FU plus irinotecan (SN-38; FOLFIRI) regimens with or without molecularly-targeted drugs are widely used as first-line chemotherapy in the treatment of advanced colorectal cancer (CRC). Whether FOLFOX or FOLFIRI is administered first is not significant, however, it is essential that full administration of the targeted dosages of all 3 drugs, 5-FU, l-OHP and SN-38, is achieved. However, this is not always possible and second-line chemotherapy must be abandoned in certain cases. Where possible, the most effective regimen should be selected as the first line of treatment. The aim of this study was to determine whether first-line chemotherapy may be individualized using the collagen gel droplet-embedded drug sensitivity test (CD-DST). Specimens of primary tumors were obtained from 43 CRC patients who had received no preoperative chemotherapy. Informed consent to measure drug sensitivity was obtained from all patients. The CD-DST allows evaluation of drug sensitivity using isolated, 3-dimensionally cultured tumor cells in a small collagen gel droplet. The CD-DST was performed and the growth inhibition rate (IR) was obtained under incubation conditions (5-FU with l-OHP at 6.0 and 3.0 μg/ml, or 5-FU with SN-38 at 6.0 and 0.2 μg/ml, respectively, for 24 h). The cumulative distributions of the growth IRs under each condition were evaluated based on the evidence that the clinical response rates to FOLFOX and FOLFIRI were almost the same. Individualization of first-line treatment was possible in all patients, with FOLFOX and FOLFIRI showing higher efficacy in 26 and 15 patients, respectively, and equal efficacy in 2 cases. This method has the potential to facilitate the establishment of individualized first-line chemotherapy for CRC and improve the prognosis in such patients.

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Key words: colon cancer, individualized chemotherapy, collagen gel droplet-embedded drug sensitivity test, leucovorin and fluorouracil plus oxaliplatin, leucovorin and fluorouracil plus irinotecan

Introduction

The leucovorin (FOL) and fluorouracil (5-FU) plus oxaliplatin (l-OHP; FOLFOX) or leucovorin and 5-FU plus irinotecan (SN-38; FOLFIRI) regimens with or without molecularly-targeted drugs are widely used as first-line chemotherapy in the treatment of advanced colorectal cancer (CRC) (1-12). Whether FOLFOX or FOLFIRI is administered first is not significant, however, it is crucial that full administration of the targeted dosages of all 3 drugs, 5-FU, l-OHP and SN-38, is achieved. However, this is not always possible and second-line chemotherapy must be abandoned in certain cases due to disease progression, adverse effects or high medical costs (13,14).

Where possible, the most effective regimen should be selected as the first line of treatment. A previous study using the collagen gel droplet embedded culture-drug sensitivity test (CD-DST) reported that FOLFIRI should be selected as the first line of chemotherapy in the treatment of poor responders to 5-FU (15).

The aim of the current study was to determine whether first-line chemotherapy may be individualized using the CD-DST.

Patients and methods

Patients. Specimens of primary tumors were obtained between March 2008 and September 2011 from 43 CRC patients who had received no preoperative chemotherapy. Informed consent for measuring drug sensitivity was obtained from all patients. The study was approved by the ethics committee at Juntendo University School of Medicine, Tokyo, Japan.

Table I. Patient characteristics.

Characteristics	n
No. of patients	43
Age (years), mean (range)	64.3 (42-78)
Gender (male/female)	24/19
Histological type	
Well-differentiated carcinoma	7
Moderately differentiated carcinoma	29
Poorly differentiated carcinoma	2
Mucinous carcinoma	5
Colon/rectum	35/8
Dukes' stage (A/B/C/D)	2/13/19/9

Methods. Tumor tissue was excised from primary surgical specimens and subjected to the CD-DST. The CD-DST allows for the evaluation of drug sensitivity using isolated, 3-dimensionally cultured tumor cells in a small collagen gel droplet and was used to evaluate the sensitivity of the tumors to 5-FU, performed as described by Kobayashi *et al* (16,17). Each specimen was washed 5 times with 50 ml saline, followed by further washing 5 times with 50 ml antibiotic fluid containing 1.0 mg/ml piperacillin and 0.5 mg/ml kanamycin. The transport bottle contained 1.0 mg/ml piperacillin, 0.5 mg/ml kanamycin and 2.5 μg/ml amphotericin B. Tissue (1 g) was treated for 2 h with a dispersion enzyme cocktail containing 1.0% collagenase. Dispersed cell suspensions were inoculated into pre-culture media on collagen-coated flasks overnight, after which viable tumor cells were recovered by 0.05% collagenase treatment. Recovered cells were embedded in 30-μl collagen gel droplets.

The embedded cells were cultivated in culture media containing 5-FU and l-OHP at 6.0 and 3.0 μg/ml, or 5-FU and SN-38 at 6.0 and 0.2 μg/ml, respectively, for 24 h. Following the removal of the anticancer agent-containing media, the cells were further cultured for 7 days in serum-free culture media to prevent the growth of fibroblasts. Viable cells were stained with neutral red solution and counted using the imaging colorimetric quantification method. The surviving cell number ratio between the drug-treated and control group, which received no drug treatment, was calculated. A growth rate <0.8 was considered indicative of successful culture.

The frequency distributions of the growth inhibition rates (IRs) under the two conditions were evaluated based on the evidence that the clinical response rates to FOLFOX and FOLFIRI were almost the same.

Statistical analysis. Histograms were analyzed with the D'Agostino-Pearson omnibus normality test using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). $P<0.05$ was considered to indicate statistically significant differences.

Results

Individualization of first-line chemotherapy is possible in patients with advanced CRC. Patient characteristics are shown in Table I. The individual growth IRs under each of the two conditions are shown in Table II. With 5-FU and l-OHP at 6.0 and 3.0 μg/ml, respectively, the median, mean, standard deviation and standard error of the mean were 63.20, 65.69,

Table II. Individual growth inhibition rates.

Patient no.	5-FU/l-OHP 6.0/3.0 μg/ml for 24 h	5-FU/SN-38 6.0/0.2 μg/ml for 24 h
1	80.1	82.9
2	71.3	79.2
3	81.2	83.4
4	60.0	68.7
5	29.9	66.5
6	69.7	89.6
7	58.7	63.2
8	73.0	85.2
9	63.2	75.9
10	77.9	85.5
11	76.3	85.6
12	53.6	62.6
13	41.9	60.7
14	81.3	80.9
15	42.3	70.2
16	84.8	86.8
17	75.9	83.9
18	59.2	76.4
19	69.9	85.5
20	57.0	49.7
21	79.2	83.0
22	86.1	89.1
23	67.3	74.4
24	81.3	85.2
25	60.4	71.9
26	93.4	98.6
27	62.6	84.9
28	58.5	54.8
29	81.2	84.0
30	66.5	73.3
31	81.3	78.1
32	59.9	74.1
33	53.3	65.0
34	49.3	48.3
35	44.7	49.3
36	68.8	72.1
37	59.7	69.4
38	50.8	59.3
39	51.6	56.5
40	57.9	70.2
41	58.5	63.7
42	62.4	72.5
43	82.9	84.3

5-FU, fluorouracil; l-OHP, oxaliplatin; SN-38, irinotecan.

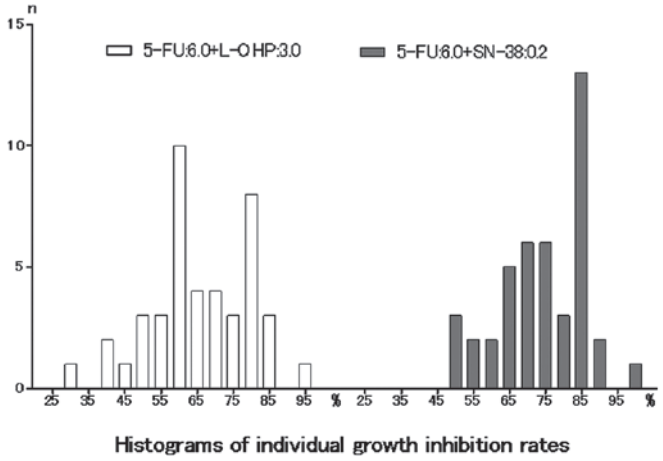

Figure 1. Histograms of individual growth inhibition rate (%) in culture media containing 5-FU and l-OHP at 6.0 and 3.0 μg/ml, respectively, for 24 h or 5-FU and SN-38 at 6.0 and 0.2 μg/ml, respectively, for 24 h. 5-FU, fluorouracil; l-OHP, oxaliplatin; SN-38, irinotecan.

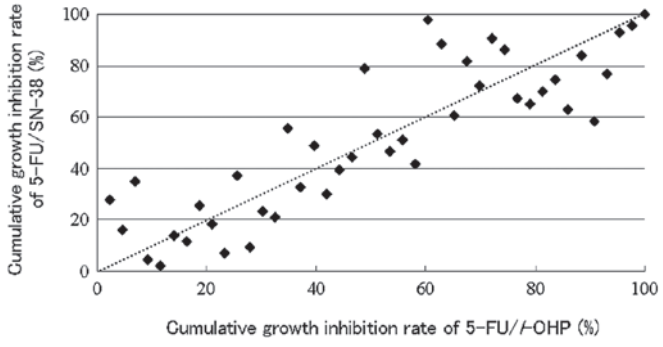

Figure 2. Correlation between cumulative distribution of individual growth inhibition rate between each condition. Dotted line indicates equivalence in efficacy. FOLFIRI was superior to FOLFOX in upper left half. FOLFOX was superior to FOLFIRI in lower right half. 5-FU, fluorouracil; l-OHP, oxaliplatin;FOL, leucovorin; FOLFOX, FOL, 5-FU and 1-OHP; SN-38, irinotecan; FOLFIRI, FOL, 5-FU and SN-38.

14.02 and 2.138, respectively. With 5-FU and SN-38 at 6.0 and 0.2 μg/ml, respectively, the median, mean, standard deviation and standard error of the mean were 74.40, 74.06, 12.12 and 1.848, respectively. Histograms of the individual growth IRs (%) under each of the two conditions are shown in Fig. 1. The histograms passed the normality test (α=0.05; 5-FU and l-OHP, P=0.7265; 5-FU and SN-38, P=0.3756).

The cumulative distribution of the individual growth IRs between the two conditions is shown in Fig. 2. There are individual differences of the efficacies between the two regimens.

Individualization of first-line chemotherapy was possible in all patients, with 5-FU plus l-OHP and 5-FU plus SN-38 showing higher efficacy in 26 and 15 patients, respectively, and equal efficacy in 2 cases (Fig. 2).

Discussion

The addition of molecularly-targeted anticancer agents enhances the effect of FOLFOX/FOLFIRI therapies. Moreover, individualized chemotherapy with molecularly-targeted anticancer agents may be implemented based on the genetic characteristics of the individual patient (6-12,18-20). Several studies have investigated individualization in 5-FU-based chemotherapy (21,22). The three enzymes that have been identified as the most significant in the metabolism of 5-FU are orotate phosphoribosyl transferase (OPRT), thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD). The most significant phosphorylation enzyme of 5-FU is OPRT, while the degradation enzyme is DPD and the main enzyme of DNA synthesis is TS. In general, high expression of TS correlates with poor efficacy of 5-FU, while low expression correlates with good efficacy. The activity of these enzymes has been reported to be extremely useful in individualization of 5-FU chemotherapy in CRC (23). Moreover, the individual 50% inhibitory area under the concentration curve of 5-FU using the CD-DST has been reported to be useful in determining individualized chemotherapy in CRC patients (24,25). Previous studies have investigated the correlation between the efficacy of irinotecan and topoisomerase-1, or that between the toxicity of irinotecan and uridine diphosphate glucuronosyltransferases 1A1 (21,22,26-29). It has been reported that there is a correlation between the efficacy of oxaliplatin and the excision cross-complementing gene (21,22,26). However, individualization in 5-FU-based chemotherapy remains to be implemented clinically.

Grothey *et al* reported that while it was not significant whether FOLFOX or FOLFIRI was administered first, it was crucial that full administration of the targeted dosages of all 3 drugs, 5-FU, oxaliplatin and irinotecan, be achieved. However, certain randomized controlled trials noted that, even with the best prognosis, full administration of all 3 drugs was not possible in approximately one-quarter of patients (13,14). It has also been reported that second-line chemotherapy could not be carried out in approximately one-third of patients (1,2,4,30,31). First-line chemotherapy is usually administered over a long period of time (5). Therefore, a more effective regimen should be selected. That is, if prognosis is to be improved, then individualization of first-line chemotherapy is indispensable.

It has been reported that the clinical response rates of FOLFOX and FOLFIRI are almost the same, at approximately 50% (5) and thus the efficacies of FOLFOX and FOLFIRI are considered to be almost equivalent (1,2,4,5,30). However, it remains to be clarified whether this holds true if considered on a case-to-case basis. In this study, when the clinical response rates of FOLFOX and FOLFIRI were 50%, responders were identified using the median based on the histograms of the individual growth IRs. The results also demonstrated that the efficacies of FOLFOX and FOLFIRI were not exactly equivalent in all the individuals in this study. Therefore, the more effective regimen for each individual was identified based on the cumulative distributions of the individual growth IRs between FOLFOX and FOLFIRI. FOLFOX was recommended as first-line chemotherapy in 26 patients, while FOLFIRI was recommended as first-line chemotherapy in 15 patients. Thus, individualization of first-line chemotherapy was possible in all patients.

The results from the present study suggest that this method has the potential to facilitate the establishment of individualized first-line chemotherapy for CRC and is likely to improve

the prognosis in such patients. This method requires further prospective randomized study.

Acknowledgements

The authors would like to thank Associate Professor Jeremy Williams, Tokyo Dental College, for his assistance with the English of the manuscript.

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