Predictive value of MTT assay as an *in vitro* chemosensitivity testing for gastric cancer: One institution's experience

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**Abstract**

**AIM:** To investigate the predictive clinical value of *in vitro* 3-(4,5-dimethylthiazol-2)-2, 5-diphenyltetrazolium bromide (MTT) assay for directing chemosensitivity in patients with gastric cancer.

**METHODS:** Results of a total of 353 consecutive patients with gastric cancer treated with MTT-directed chemotherapy or physician's empirical chemotherapy from July 1997 to April 2003 were reviewed and analyzed retrospectively.

**RESULTS:** The overall 5-year survival rate of MTT-sensitive group (MSG) and control group (CG) was 47.5% and 45.1%, respectively. The results of subgroup analysis with Cox proportional-hazards model were favorable for the MSG-sensitive group. However, no statistically significant difference in survival rate was observed between the two groups.

**CONCLUSION:** Individualized chemotherapy based on *in vitro* MTT assay is beneficial, but needs to be confirmed by further randomized controlled trials.

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**Key words:** Gastric cancer; Chemosensitivity testing; Chemotherapy; MTT assay; Survival rate

**Materials and Methods**

**Patients**

This was a retrospective study. The medical records of patients who had gastric cancer were reviewed from July 1997 to April 2003. The patients were treated with MTT-directed chemotherapy or physician's empirical chemotherapy. The overall 5-year survival rate of MTT-sensitive group (MSG) and control group (CG) was 47.5% and 45.1%, respectively. The results of subgroup analysis with Cox proportional-hazards model were favorable for the MSG-sensitive group. However, no statistically significant difference in survival rate was observed between the two groups.
patients registered for adjuvant chemotherapy from July 1997 to April 2003 were reviewed. The criteria for case inclusion were as follows: (1) a diagnosis of histologically or cytologically proven gastric cancer, (2) without prior chemotherapy or radiotherapy, (3) adequate blood counts (hemoglobin ≥ 10 g/L, WBC count of 3000/µL, and platelets of 100000/µL), normal renal function (creatinine clearance ≥ 60 mL/min), and normal liver function (serum transaminase level less than double the normal upper limit). Patients with esophageal cancer, small cell carcinoma, lymphoma, and squamous cell carcinoma were excluded from the study. A total of 353 eligible records of patients were collected and analyzed. The patients were divided into MTT-sensitive group (MSG) and control group (CG). One hundred and fifty-seven patients in the MSG-sensitive group were treated by chemotherapy containing at least one sensitive drug based on the MTT assay results, and 196 patients received physician’s empirical chemotherapy. The chemotherapeutic drugs used were cisplatin (CDDP), 5-fluorouracil (5-Fu), mitomycin (MMC), doxorubicin (DOX), paclitaxel (PAC) and docetaxel (DOC). The protocols of chemotherapy have been described elsewhere.[6,16,17).

**MTT assay**

Fresh tumor tissue obtained from the surgically resected specimens was tested within 6 h. The tumor tissue was cut into pieces (smaller than 1 mm³) and passed through No. 100 and No. 200 stainless steel meshes respectively into a complete medium containing RPMI 1640 solution, 100 µg/mL penicillin, and 100 µg/mL streptomycin, and washed twice gently with the same solution. The viable cells were assessed using trypan blue exclusion method. Cell viability was measured by MTT assay to assess the chemosensitivity of tumor cells. Cell suspension was collected into sterile 96-well flat-bottomed microtiter plates (1 × 10⁶ cells/well) with or without chemotherapeutic agents. The drug and testing drug concentrations used were 25 µg/mL cisplatin (CDDP), 100 µg/mL 5-fluorouracil (5-Fu), 10 µg/mL mitomycin (MMC), 4 µg/mL doxorubicin (DOX), 100 µg/mL paclitaxel (PAC) and 30 µg/mL docetaxel (DOC). Each drug was tested in triplicate. The plates were then incubated at 37°C in a humidified atmosphere containing 50 mL/L CO₂ for 72 h. Microtiter wells containing tumor cells but no anticancer agents were used to control cell viability, in which the total number of tumor cells was equivalent to that in the test wells, and wells containing only a complete medium were used as blank controls for nonspecific dye reduction. After incubation, MTT solution was added to each well at a final concentration of 1 mg/mL per well and the plates were incubated at 37°C for another 4 h. Then the mixture containing the medium, the drug, and the unconverted MTT was removed. DMSO was added to each well to dissolve the formazan and absorbance was read at 550 nm using a spectrophotometric microplate reader (Labsystems, Finland). The inhibition rate of tumor cells for each drug with different concentrations was calculated following the formula: inhibition rate (％) = (1 - OD_{drug exposed}/OD_{control}) × 100. The effective anticancer activity was regarded as sensitive when the tumor inhibitory rate was greater than or equal to 70%.

**Toxicity**

All patients who started treatment were considered assessable for toxicity. Toxicity was analyzed following the National Cancer Institute Common Toxicity Criteria (version 2.0).

**Statistical analysis**

All statistical analyses were done using the SAS 6.12 statistical software (SAS Institute, Cary, NC). The clinical and pathological characteristics, including gender, age, cancer stage (TNM), and histological type (differentiated versus undifferentiated type), were evaluated by Mann-Whitney’s U-test and the Kruskal-Wallis test. The overall probability was calculated using the Kaplan-Meier method for censored failure time data, and the statistical significance was analyzed by the log-rank test for comparison of survival rate between the two groups. The Cox proportional-hazards model was used to calculate the hazard ratios. P < 0.05 was considered statistically significant. All P values were two-tailed and unadjusted for potential multiple comparisons.

**RESULTS**

**Patient characteristics**

The clinical and pathological characteristics of the patients are outlined in Table 1. Between the MSG and CG arms, there was no significant difference in baseline clinical characteristics and pathological findings which were considered to be related to the prognosis of gastric cancer patients.

**Overall survival analysis**

The overall 5-year survival rate of the patients was 47.5% and 45.1% in the MSG-sensitive group and CG group, respectively, with no statistical difference (Figure 1). The
hazard ratio for deaths in the MSG-sensitive group, as compared with the CG group, was 0.92 [95% confidence interval (CI) = 0.69 to 1.23, \( P = 0.57 \)].

**Subgroup analysis**

The overall survival rate of the patients was analyzed according to sex, age, cancer stage (TNM classification), and histologic type. The hazard ratio of deaths was favorable for the MSG-sensitive group (Figure 2). There were no significant interactions between the two groups and any of the variables studied.

**Adverse events and treatment compliance**

Data on the 157 patients in the MSG-sensitive group and 196 patients in the CG group were analyzed for adverse events. The main emergent adverse toxicities (grades 3 and 4) related to treatment are listed in Table 2. The severe adverse events (defined according to NCI-CTC version 2.0), including hematologic and nonhematologic toxic effects, did not occur more frequently in the MSG-sensitive group than in the CG group.

**DISCUSSION**

Conventional chemo-/radio-therapy for gastric cancer is limited to improve the treatment outcomes and quality of survival/life of human patients\(^{[18,19]}\). Physicians’ empirical choice of chemotherapeutic regimen for gastric cancer is based on the data obtained from clinical trials\(^{[20]}\). However, even the same gastric cancer behaves so differently that the response rate of cancers to the chemotherapeutic agents varies. These variations are partly contributed to the failure of treatment of gastric cancer patients. The effectiveness of current chemotherapies for cancer is limited mainly due to its heterogeneity\(^{[21]}\). To overcome this problem, selecting a sensitive chemotherapeutic regimen in vitro for individual gastric cancer patients appears to be an attractive way. Chemosensitivity testing is an ex vivo means of determining the cytotoxic and/or cytostatic, or apoptosis-inducing effect of anticancer drugs. The most common in vitro assays include MTT and ATP-TCA assays, etc\(^{[22]}\). These assays have been successfully used in the assay-guided chemotherapy for certain cancers, including breast, ovarian, melanoma and colorectal cancers\(^{[23-25]}\). MTT assay has been most widely used in different cancers, and is sensitive, accurate, and efficient in the in vitro evaluation of anticancer or immunological agents prior to preclinical and clinical testing. Some research groups have used MTT assay to guide individual adjuvant chemotherapy for gastric cancer\(^{[10]}\), showing that the therapy based on the chemosensitivity testing can improve the clinical outcomes of cancer patients. In the present study, based on the criteria for chemosensitivity in vitro, we predicted and evaluated the efficacy of chemotherapy for 353 gastric cancer patients according to the result of MTT assay. The overall survival rate of the patients, treated with chemotherapy regimen containing at least one sensitive agent, was higher in the MSG-sensitive group than in the CG group treated with the physicians’ empirical therapy. The hazard ratio of most subgroups was favorable for the MSG-sensitive group as demonstrated in Cox proportional-hazards mode. However, no significant difference between the two groups was observed. These results indicate that MTT
Background

Since cancer patients with histologically similar tumors respond differently to standard drug treatment, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) chemosensitivity assay is performed to provide predictive information to help physicians choose sensitive chemotherapeutic agents for eliminating potentially ineffective agents used in chemotherapeutic regimens for each cancer patient.

Research frontiers

At present, several new chemosensitivity assays, such as histoculture drug response assay (HDRA), collagen gel-droplet-embedded culture drug sensitivity test (CD-DST) and fluorometric microculture cytotoxicity assay (FMCA), are used in selection of an appropriate chemotherapeutic drug, showing the predictive value of chemotherapy for cancer patients.

Innovations and breakthroughs

There is no evidence for the clinical benefits of MTT chemosensitivity assay. The present study evaluated the clinical usefulness of MTT chemosensitivity assay in gastric cancer patients, and showed no significant differences in clinical outcomes between the MTT-sensitive group (MSG) and the control group (CG), indicating that the potential value of MTT assay for patients with gastric cancer is limited.

Applications

Although some studies have shown a potential clinical benefit for patients with drug-sensitive cancer, MTT assay of chemosensitivity is not widely accepted by physicians because there is no sufficient evidence obtained in the clinical setting. The potential clinical benefits of individualized chemotherapy based on chemosensitivity assay for gastric cancer patients need to be confirmed by further randomized controlled trials in comparison with standard chemotherapy.

Terminology

MTT assay is a laboratory test and a standard colorimetric assay for measuring cellular proliferation. Yellow MTT is reduced to purple formazan in the mitochondria of living cells. A solution (usually dimethyl sulfoxide) is added to dissolve the insoluble purple formazan products into a colored solution. The absorbance of this colored solution can be quantified at a certain wavelength with a spectrophotometer.

Peer review

This is an interesting report on the predictive value of MTT assay as an in vitro chemosensitivity testing for gastric cancer patients. Individualized chemotherapy based on in vitro MTT assay has clinical benefit, but needs to be confirmed by further randomized controlled trials.

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