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• GASTRIC CANCER •

The study of chemiluminescence in gastric and colonic carcinoma cell lines treated by anti-tumor drugs

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Abstract

AIM: To study the influence of chemotherapy on proliferation activation of tumor cell by observing the change of chemiluminescence (CL) and cell cycle in various tumor cell lines after mitomycin C treated.

METHODS: BGC823 and LoVo cell lines were all cultured in RPMI-1640, and then were adjusted to a concentration of 1×10^5 cells/ml in fresh media and incubated for 24 h. Mitomycin C (100 ng·ml^{-1}) was added to each bottle. All indeses were examined after 24 h. No Mitomycin C was added in control group. Each group contained 8 samples. Flow cytometric analysis and luminol-dependent CL were used to investigate the effect of mitomycin C on two gastrointestinal carcinoma cell lines.

RESULTS: BGC823 and LoVo cell lines incubated with MMC for 24 h. We discovered that the emergence of peak of CL stimulated by PHA was postponed significantly (BGC823: 12.63±3.21 *vs* 4.50±1.04, LoVo: 13.25±2.96 *vs* 5.12±1.36, $P<0.01$) and the peak intension of CL was reduced significantly (BGC823: 120.25±16.61 *vs* 248.38±29.17, LoVo: 98.13±10.49 *vs* 267.50±18.56, $P<0.01$). The PI of cell lines was decreased significantly (BGC823: 51.87±4.82 *vs* 25.44±2.26, LoVo: 47.11±1.04 *vs* 24.23±0.37, $P<0.01$) and the apoptotic fractions changed by contraries (BGC823: 26.25±5.29 *vs* 9.83±2.51, LoVo: 33.50±3.68 *vs* 9.63±1.44, $P<0.01$).

CONCLUSION: CL can be used to measure activation of tumor cells. We discovered that the ground CL intensions of two cell lines were not high but increased rapidly after stimulation of PHA. The CL peak ranged from 4-5 minutes, and then decreased gradually. The results were not reported before. CL of tumor cell has close correlativity with the dynamics of cell cycle and can reflect the feature of oxidation metabolism and proliferation activation of tumor cell. So it can be used to observe the influence of chemotherapy drug on metabolism and proliferation activation of tumor cell and screen out chemotherapy drugs to which tumor cells are sensitive.

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INTRODUCTION

Chemiluminescence (CL) is a natural phenomenon of creatures[1]. As other cells, tumor cells also have the ability of CL. So the vigor of tumor cells could be measured by CL. At present, there were few reports about the CL of tumor cells[2]. We used gastric and colonic carcinoma cell lines to study the CL of tumor cells and to investigate the dynamics of CL and the influence of drugs on CL.

MATERIALS AND METHODS

Cell line

BGC823 is a kind of human gastric carcinoma cell line[3-5]; LoVo is a kind of human colonic carcinoma cell line[6,7]. We bought them from Shanghai Cell Biology Institute of the Chinese Academy of Sciences.

Cell culture and mitomycin C treatment

Two cell lines were all cultured in RPMI-1640 (GIBCO) containing 100 g·L^{-1} newborn calf serum (Sijiqing, Hangzhou), penicillin (100 U·ml^{-1}), and streptomycin (100 μg·ml^{-1}). Log phase cells were adjusted to a concentration of 1×10^5 cells/ml in fresh media and incubated for 24 h. Mitomycin C (100 ng·ml^{-1})[8] was added to each bottle. All indeses were examined after 24 h. No Mitomycin C was added in control group. Each group contained 8 samples.

CL analysis

Media was removed and cells were washed in phenol red free D-Hank's solution three times and adjusted to a concentration of 1×10^3 cells·ml^{-1} in the same solution. Aliquots (0.1 ml) of this cell suspension were placed into a cuvette, to which 0.7 ml of phenol red free D-Hank's solution and 0.1 ml of luminol (1 mmol·L^{-1}) were added. All cuvettes were put into sample chamber of SHG-1 bio-luminometer (Shangli measure instrument factory). CL of tumor cell was measured using T-2 procedure, configuring measure times were 30 and interval was 60 seconds. At first background of CL was measured, then 0.1 ml of phaseolus vulgaris agglutinin (PHA, 4 mg·L^{-1}) was added and shaken up rapidly. CL PHA stimulated was measured continuously for about 30 minutes. All assays were made at 37 ℃. After measurements completed, CL kinetics curve, peak value, peak time, slope function and integral were calculated using the computer. Unit of intension of CL was scintillation counting per minute[9] (Figure 1).

Flow cytometric analysis

Cells were harvested and washed in D-Hank's solution three times and adjusted to a concentration of 1×10^6 cells·ml^{-1} in the same solution. The nuclei were stained with propidium iodide at a concentration of 50 μg·ml^{-1} and then filtered through 40 μm nylon mesh before flow cytometric analysis[10]. The nuclear DNA content was analyzed with a flow cytometer (Epics XL; Coulter Co. U.S.A); 1×10^6 cells were examined in each sample. The cell cycle distribution, i.e., G_0/G_1, S, G_2/M were analyzed using a computer program (Multicycle). The mean coefficient of variation (CV) for the G_0/G_1 peak was less than 8.0 in all

cases[11]. We assessed the proliferation index (PI) to evaluate the changes of cell cycle distribution caused by treatment of MMC. $PI=\frac{S+G_2M}{S+G_2M+G_0G_1}\times 100\ \%$ We used the fraction of subdiploid peak in front of DNA histograms as apoptotic fraction[12].

Statistical analysis

Statistical analysis, i.e., one-way ANOVA, unpaired *t*-test, correlate analysis, was performed using SPSS 8.0. Data were presented as means ± standard deviation (S.D.). The level of significance was $P<0.05$.

RESULTS

CL analysis

Figure 1 and Table 1 show the time-course of CL change in control and treatment groups of BGC823. In control group a CL signal was noted almost immediately that peaked within 3-5 minutes and returned to baseline after about 10-15 min. In contrast treatment group cells responded slowly and the peak CL did not occur until after 11-18 min. The CL peak value in treatment group decreased significantly ($P<0.01$) when compared with control group. The same changes were detected in all two cell lines.

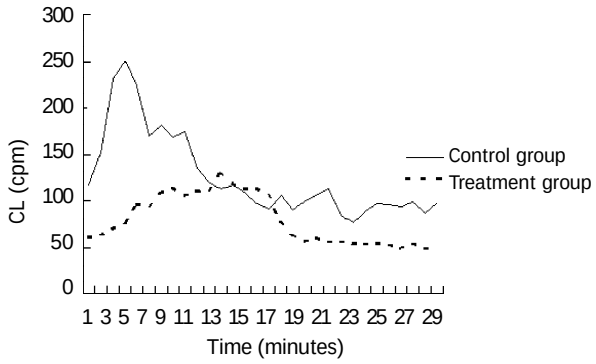

Figure 1 Time-course of CL change in control and treatment groups of BGC823.

Table 1 Effects of MMC treatment on CL of two cell lines

	BGC823 (*n*=8)		LoVo (*n*=8)	
	Control	Treatment	Control	Treatment
Peak value (cpm)	248.38±29.17	120.25±16.61[a]	267.50±18.56	98.13±10.49[a]
Peak time (minute)	4.50±1.04	12.63±3.21[a]	5.12±1.36	13.25±2.96[a]

[a]$P<0.01$, *vs* control.

Flow cytometric analysis

The PI in treatment group decreased significantly and the apoptotic fraction increased significantly when compared with control group ($P<0.01$). There was no significant difference in the change of cell cycle contribution between two cell lines (Table 2).

Table 2 Effects of MMC treatment on cell cycle distribution of two cell lines

	BGC823		LoVo	
	Control	Treatment	Control	Treatment
PI (%)	51.87±4.82	25.44±2.26[a]	47.11±1.04	24.23±0.37[a]
Apoptosis (%)	9.83±2.51	26.25±5.29	9.63±1.44	33.50±3.68

[a]$P<0.01$, *vs* control.

Correlations between CL and cell cycle distribution

There was positive correlation between CL and PI (BGC823: $r=0.92$, $P<0.01$; LoVo: $r=0.90$, $P<0.01$), and there was negative correlation between CL and apoptotic fraction (BGC823: $r=-0.91$, $P<0.01$; LoVo: $r=-0.95$, $P<0.01$) (Figures 2-5).

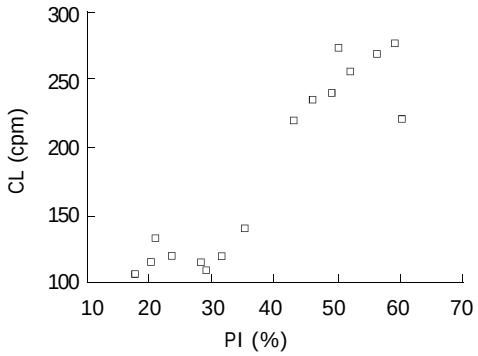

Figure 2 Correlations between CL and PI in BGC823.

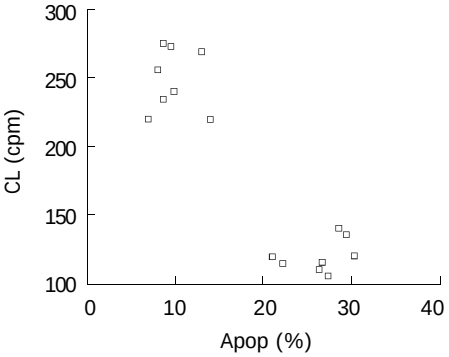

Figure 3 Correlations between CL and apoptotic fraction in BGC823.

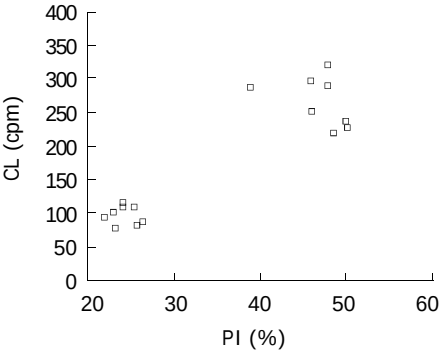

Figure 4 Correlations between CL and PI in LoVo.

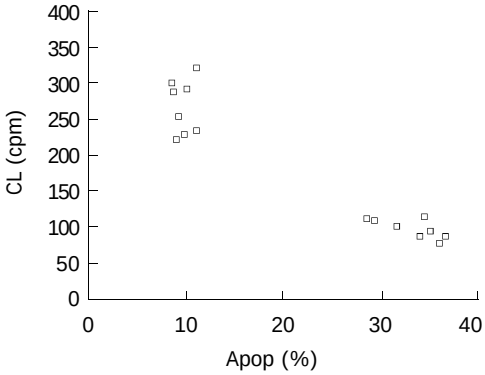

Figure 5 Correlations between CL and Apoptotic fraction in LoVo.

DISCUSSION

Oxygen radicals (OR) are intermediary products in the biochemical metabolization of cells, including $O_2^{\cdot-}$, H_2O_2, $OH\cdot$, $^1O_2$, OCl^- and lipid peroxidation oxygen radical[13]. They are produced mainly by aerobic respiration in mitochondrion and glycolysis[14,15]. OR reacts to excitable substance in cells and produces CL[16]. So we can observe activation of cells by the measurement of CL[17,18].

In the present study, luminol-dependent chemiluminescence was used as a measure of CL of human gastric carcinoma cell line BGC823 and human colonic carcinoma cell line LoVo. We discovered that the ground CL intensions of two cell lines were not high but increased rapidly after stimulation of PHA. The CL peak ranged from 4-5 minutes, then decreased gradually. The results were not reported before.

Mitomycin C is a cycle-nonspecific anti-tumor agent. The mechanism of anti-cancer effect is its alkylation which makes DNA incorporation and DNA replication inhibition and single strand rupture. MMC affects mainly G1 and early S phases. However the influence of MMC on cell cycle *in vitro* may be different in different cells[19-22]. MMC also up-regulated EpCAM and LewisY antigen expression in LoVo cell line[23]. Cell cultures were growth arrested by exposure for 5 minutes to MMC[24], and MMC retains its antiproliferative effect for at least 6 weeks[25].

The CL of BGC823 and LoVo cell lines were decreased significantly after incubated with MMC for 24 hours. We also discovered that the peak of CL was postponed significantly and the peak intension of CL was reduced significantly after the stimulation of PHA. At the same time, the proliferation index (PI) was decreased significantly and apoptotic fractions changed by contraries[26]. There was positive correlation between CL and PI, and there was negative correlation between CL and apoptotic fraction. These results revealed that the proliferation activation of tumor cell reduced, the apoptosis of cells increased and the reaction to stimulation of PHA weakened when the concentration of oxygen radicals in tumor cell decreased[27-29]. So the decrease of concentration of oxygen radicals in low range depressed the mitochysis of tumor cell[30] and prompted the apoptosis of tumor cell. These results also suggest the CL of cell can reflect change of proliferation activation of tumor cell treated by chemotherapy drugs.

In conclusion, the CL of tumor cell can reflect the feature of oxidation metabolism and proliferation activation of tumor cell, can be used to observe the influence of chemotherapy drug on metabolism and proliferation activation of tumor cell and screen out chemotherapy drugs to which tumor cells are sensitive. Compared with other methods cell chemiluminescence is more sensitive, more accurate, more rapid, less pollution and need fewer samples.

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