Chinese Journal of Cancer Research 8(4):298 — 300, 1996

A DYNAMIC STUDY OF THE CYTOTOXIC EFFECTS OF HYPERTHERMIA COMBINED WITH CIS-DIAMINE DICHLOROLPLATINUM (DDP) ON HUMAN GASTRIC CANCER CELL LINES MKN_{28} AND MKN_{45} *IN VITRO*

Chen Weixing 陈卫星 Li Youming 厉有名 Chen Liangliang* 陈良良 Ji Feng 李峰
Huang Huaide 黄怀德 Liu Xianglin 刘祥麟

Department of Gastroenterology, First Affiliated hospital, Zhejiang Medical University, Hangzhou 310003
**Department of Oncology, Chinese Traditional Medical hospital of Zhejiang*

Cytotoxic effects of hyperthermia combined with DDP on MKN28 and MKN45 cells were studied by MTT assay according to a nested design. The results showed: hyperthermia alone above 43 ℃ for 30 mins was cytotoxic; hyperthermia at temperature lower than 43 ℃ for 30 mins could increase sensitivity of cancer cells to DDP. The cytotoxic effect of simultaneous use of hyperthermia and DDP was more marked than that of sequential use of the 2 treatments. Hyperthermia combined with DDP could inhibit growth of human gastric adenocarcinoma cells regardless of their degree of differentiation.

Key words: Gastric neoplasms, Cisplatin, Hyperthermia, Induced

Single therapy on cancer is often poorly effectual, the more effective can be the combination treatment. One of them is thermochemotherapy published recently, which can enhance the cytotoxic effect on cancer cells. Otherwise over high temperature can impair the normal tissues. In order to work out the scheme for thermochemotherapy, we study the effect of hyperthermia and DDP on the human high differentiation gastric cancer cell line MKN_{28} and the lower differentiation cell line MKN_{45} measured with MTT assay *in vitro*. The best effect of temperature, duration and sequencing, and the relationship between differentiation and effect are reported.

Accepted September 2, 1996

MATERIALS AND METHODS

Cell Lines

MKN_{28} and MKN_{45} are respectively the human high and low differentiation gastric cancer cell lines,[1] which were established by Japanese and given to us be Shanghai Digestion disease Institute. The cells grew in Eagle's Minimal Essential Medium supplemented with 10% fetal calf serum. Cell cultures was kept at 37 ℃ in a incubator after seeding. All experiment were performed with logarithm growing phase cells on day 3.

Heating and DDP Exposure

Heating of cells was done in different temperature, duration and sequencing combined with DDP in different concentrations according to nested design. The cells were heated in electrothermal constant temperature water bath whose error was below ± 0.5 ℃. The cell with DDP were co-cultured for 60 mins, then washed with Hank's fluid two times and with medium one times.

MTT Assay

After heated and exposed to DDP, 100 μl cell suspension (1×10^5 cells) of each was added to 96 well culture plates. The medium without cells was acted as blanked control. The culture plates were kept in incubator for 24 hrs. 50 μl from supernatant per well was absorbed and given up. After that, 50 μl of MTT was added to each well to continually culture for 4 hrs. later on the supernatant was thrown and 100μl of DMSO was added to each well which was vibrated for 5 mins. Finally absorptivity of light in each well was measured with ELISA Scanner at wavelength 490 nm and Cytotoxicity Index (CI) was calculated.[2]

Statistics Treatment

First all data were tested with homoscedasticity. Next analysis of variance. Last Q method.

RESULTS

The Cytotoxic Effects of Hyperthermia Alone

The cytotoxic effects of hypertherma below 43 ℃ for 30 mins was weak. that above 43 ℃ for 30 mins was strong.

The Cytotoxic Effects of Hyperthermia Combined with DDP

As the temperature rose or duration of heating prolonged, the curves gone up and CI increased. The difference among groups was significantly ($P<0.01$). The cytotoxicity of DDP at thick concentration (100 μg/ml) was strong, however that at thin concentration ($P<10$ μg/ml) was weak, and when DDP was diluted in the ratio one to ten CI changed a little at 37 ℃. The slopes of the CIS curves increased as heating, but the slopes tended to even again on high levels when heated above 43 ℃. The slopes of the CIS curves increased as heating, but the slopes tended to even again on high levels when heated above 43 ℃ for 30 mins. Dose Enhancement Ratios (DER) of hyperthermia on DDP elevated as temperature rose, which was the strongest at 41.5 ℃ for 30 mins, otherwise, decreased above 43 ℃ for 30 mins.

The Effect of Sequencing

When sequencing of hyperthermia combined with DDP changed, so did the CI (Figure). The difference among groups of DDP (1 μg/ml and 10 μg/ml) combined with hyperthermia was significantly ($P<0.05$). The effect of group HD (heating and DDP exposing simultaneously) was greater than each of group H→D (heating first, then DDP exposing after 60 mins) or group D→H (DDP exposing first, then heating after 60 mins). The difference was significantly ($P<0.05$). The difference among groups was no significantly ($P>0.05$), when concentration of DDP was 100 μg/ml and 0.1mg/ml.

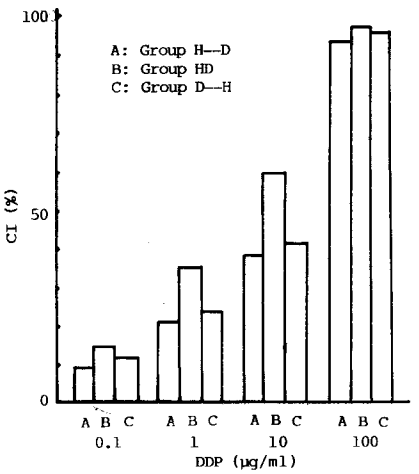

Fig. The effect of sequencing on hyperthermia combined with DDP on human gastric cancer cells.

DISCUSSION

The cytotoxicity of hyperthermia blow 43 ℃ for 30 mins alone was weak, otherwise, that above 43 ℃ for 30 mins was strong, which showed in the

experiment. 43 ℃ for 30 mins on hyperthermia is probably a critical point whose mechanism mainly is hot denaturation of protein and DNA.[3] Thus hyperthermia *in vivo* probably damage the normal tissue. Having inspected the cytotoxicity of hyperthermia combined with DDP, it was found that the concentrtion of DDP to obtaine same CI decreased in logarithm as rising of temperature or prolonging of heating and that hyperthermia could steepen the even curves. All above showed that hyperthermia could increase the sensibility of cancer cell on DDP.

Speaking of Dose Enhancement Ratios, as hyperthermia enhanced DER increased, which showed that hyperthermia could increase sensitivity of cytotoxicity of DDP. On the contrary, DER decrease as temperature rose above 43 ℃ for 30 mins. the reason was that the hyperthermia above 43 ℃ for 30 mins has strong hot denaturation and high CI, with the result that DER of DDP showed less instead. We found, similarly, DER of hyperthermia was the most obviously of all, when concentration of DDP was medium.

The best effect of consequencing was different, since drugs of different mechanism combined with hyperthermia.[4] For instance, it is the best that hyperthermia combined with Methotrexatum (MTX) in 24 hrs after heating. It is the best that the local anaesthetic Bupivacain exposed before hyperthermia. The cytotoxic effect of simultaneous use of hyperthemia and DDP was the most marked of all, which showed in our experiment. Thus it could be seen that effect of consequencing depends on the mechanism of drug.

Hyperthermia combined with DDP could inhibit growth of human gastric adenocarcinoma cells regardless of their degree of differentiation, which showed efficacy of thermochemotherapy did not depend on cells degree of differentiation.

The mechanism on synergy of hyperthermia combined DDP probably is: 1) heating enhances permeability of cell membrane on DDP; 2) heating accelerates twisting DDP and DNA; 3) heating inhibits activity of DNA repair enzyma.[5,6]

To sum up, hyperthermia alone above 43 ℃ for 30 mins has strong efficacy. Hyperthermia at medium degree combined with DDP in medium concentration can achieve the best efficacy of all and avoid possible side-effect of high temperature and high concentrating drug. The cytotoxic effect of simultaneous use of hyperthermia and DDP was more marked than each of consequential use of the 2 treatments. Undoubtedly, the study here provide some of experiment grounds about hyperthermia dynamic for clinically appliance hyperthermia combined with DDP for cancer.

REFERENCES

1. 徐光炜主编，胃癌。北京：人民卫生出版社，1987: 235.
2. Jiao H, shen W, Ohe Y, et al. A new 3- (4,5-dimethylthiazol-- 2-- yl) --2. 5--diphenyltetrazolium bromide (MTT) assay for testing macrophage cytotoxicity to L1210 and its drug resistant cell lines *in vitro*. Cancer Immunol Immunother 1992; 35: 412.
3. 黄汉贤，苏燎原，孙国器. 高温联合 ^{60}CO-γ 射线对 L5178y 细胞 DNA 断裂与修复的影响. 癌症 1989; 8: 5.
4. Kobayashi K, Fujimoto S, takahashi M, et al. The experimental and clinical study of hyperthermia with thermosensitizer for gastric cancer patients with peritoneal seeding. Can To Kagaku Ryoho 1992; 19: 1651.
5. 水野左敏。温热化学疗法の生学基础。医学のあゆみ 1989; 148: 3.
6. Kent EW, Michael WD, Gloria CL. Hyperthermic potentatiation of cis-diaminedichloroplatnum (II) cytotoxicity in Chinese hamster over cells resistant to the drug. Cancer Res 1986; 46: 6242.