APOPTOTIC THRESHOLD OF ADRIAMYCIN AND CISPLATIN IN HEPATOCELLULAR CARCINOMA

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ABSTRACT

Objective: To investigate the apoptotic threshold of adriamycin (ADM) and cisplatin (CDDP) on hepatocellular carcinoma (HCC). Methods: Sensitivities of ADM and CDDP on HCC were studied by primary cell culture. Results: The apoptotic threshold of ADM and CDDP were 1.0 μg/ml and 1.5μg/ml respectively (its clinical dosage was 20 mg and 30 mg respectively). Conclusion: Understanding apoptotic threshold of anticancer drugs may reduce clinical dosages of anticancer drugs and reduce the incidence of multidrug resistance (MDR).

Key words: Apoptotic threshold, Hepatocellular carcinoma, Primary cell culture, and Multidrug resistance

Chemotherapy is one of major strategies against hepatocellular carcinoma, and failure of chemotherapy is usually caused by toxicities of anticancer drugs and multidrug resistance. Recently, it has been shown that many anticancer drugs, such as ADM, VCR, CDDP, MTX, etc. kill tumor cells by promoting apoptosis.[1] Apoptotic threshold is defined as the minimum dose of anticancer drug to cause apoptosis. This study will investigate the apoptotic threshold of anticancer drugs ADM and CDDP in hepatocellular carcinoma in primary cell culture.

MATERIALS AND METHODS

Agents and Samples

RPMI-1640 (Gibco), Hoechst33342 (Sigma), PI (Sigma), and RNase A (Sigma) were used. HCC samples from patients without prior chemotherapy were obtained from the First Affiliated Hospital of Medical College, Jinan University and the Affiliated Tumor Hospital of Zhongshan Medical University. Human hepatocellular carcinoma cell line hepG-2 was from the Pathology Department of Medical College, Jinan University.

Methods

Tumor samples were put in RPMI-1640 medium containing pencillin 100U/ml, streptomycin 100 μg/ml after resection, then stored at 4°C and processed to suspension within 4 hours. Suspensions were made from specimens by cold trypsin digestive method. The specimens were cut into pieces about 1 mm^3, cold trypsin (0.05%) added, and stored at 4 ℃ for 6–8 hours, then digestion was stopped, the suspension filtered, and cells were incubated in RPMI-1640 medium.

Drug concentration was defined as following:

Drug concentration (μg/ml)=(drug × BSA*) BW*× (100/60)

*BSA: Body surface area. BW: Body weight.

The suspension was adjusted to 10^6 cells/ml, then suspensions were divided into six groups (A,B,C,D,E,F). They were cultured in RPMI-1640 in a humidified atmosphere of 5% CO_2 at 37°C. Then 24 hours later, ADM or CDDP were added at different concentration. The final concentrations of ADM were: group A: 0 μg/ml, group B: 0.125 μg/ml, group C: 0.25 μg/ml, group D: 0.5 μg/ml, group E: 1.0 μg/ml, group F: 2.0 μg/ml; that of CDDP were: group A: 0 μg/ml, group B: 0.1875 μg/ml, group C: 0.375 μg/ml, group D: 0.75 μg/ml, group E: 1.5 μg/ml, group F: 3.0 μg/ml. Then 24 hours later, cell numbers in suspension were adjusted to 10^5 cells/ml and analyzed with fluorescence microscopy and flow cytometry.

1 ml of each suspension was stained with Hoechst 33342 solution 1 μl (Hoechst 33342 final concentration is 1 μg/ml) for 15 minutes and analyzed by DNA fluorescence under fluorescence microscopy.

The suspensions were fixed with 70% ethyl in

Accepted for publication: October 18, 1999

This work was supported by a grant from the Key Project Foundation of Guangdong province Science and Technology Committee.

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alcohol at 4°C for 24 hours, washed with PBS and centrifuged, added 5 mg/ml RNase A 50 μl, 50μg/ml PI 450 μl were added, stored at 4°C for 30 minutes, then cell cycle analysis was performed under flow cytometry. The cells with lower DNA quantities than those of G1 stages were apoptotic cells.

The above process was carried out with all samples.

The data were analyzed for statistical significance by chi-square test.

RESULTS

Under fluorescence microscopy, the normal cells showed spherical nuclei and uniform chromatin distribution, the apoptotic cells showed chromatin condensation, margination and nuclear fragmentation,[3,4] (Figure 1).

Under flow cytometry, diploid peak decreased (G1 cell) in column diagram, hypodiploid peak (sub-G1 peak) appeared in left side of G1 peak, lower front astigmatism appeared in light astigmatism diagram.[3]

Apoptotic ratio of group B and C were not significantly different from that of group A ($P>0.05$) while the apoptotic ratio of two cases in group D and ten cases in groups E, F were significantly different from that of group A ($P<0.05$).

The results showed the concentration of group E was apoptotic threshold of ADM (1.0 μg/ml) and CDDP (1.5 μg/ml) (Table 1).

Table 1. Sensitivity of 18 cases with HCC to different concentration ADM and CDDP

Group	ADM		CDDP	
	Sensitive	not sensitive	Sensitive	no sensitive
Group A	0	18 (100%)	0	18 (100%)
Group B	0	18 (100%)	0	18 (100%)
Group C	0	18 (100%)	0	18 (100%)
Group D	2 (11.1%)	16 (88.9%)	2(11.1%)	16(88.9%)
Group E*	10(55.6%)	8(44.4%)	10(55.6%)	8(44.4%)
Group F*	10(55.6%)	8(44.4%)	10(55.6%)	8(44.4%)

*: Significantlt different with other groups ($P<0.05$)

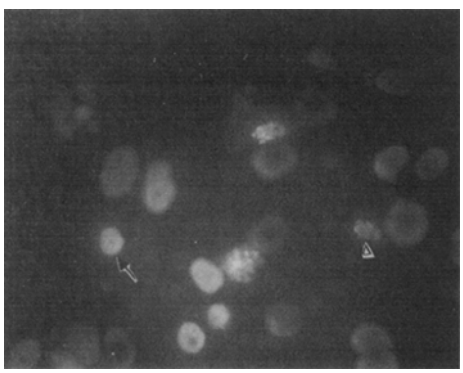

Fig. 1. DNA fluorescence under fluorescence microscopy (× 500)

→: The normal cell shows spherical nuclei and uniform chromatin distribution.

△ : The apoptotic cell shows chromatin condensation, margination and nuclear fragmentation.

DISCUSSION

Apoptosis is cell death different from necrosis. Kerr, et al. described the histomorphology and DNA changes characteristic of apoptosis in 1972.[5] Recent studies have shown many anticancer drugs, such as ADM, CDDP, MTX, VCR, etc, kill tumor cells by promoting apoptosis.[1]

Our study demonstrates both ADM and CDDP can promote HCC's apoptosis. The apoptotic ratio is different and related to different drug concentration. At certain drug concentrations, apoptotic ratio increases significantly. This drug concentration is the apoptotic threshold. Apoptotic thresholds of ADM, CDDP are 1.0 μg/ml, 1.5 μg/ml respectively in our study. According to the drug distribution estimates, their clinical dosages are 20 mg and 30 mg respectively.

Understanding apoptotic threshold of anticancer drugs is of important value for chemotherapy. Anticancer drugs used clinically have toxicity and side effects. With higher doses, more toxicity and side-effects are seen, so chemotherapy use is limited. Meanwhile, laboratory experience of inducing drug resistance in tumor cell lines has shown several points that may be of clinical relevance. In inducing resistance, if drug concentrations are rapidly increased, multidrug resistance in a short time. If drug concentrations are increased slowly, multidrug resistance more slowly, with an average time of 4–6 months.[6-8]

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