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INDEPENDENT AND SYNERGIC INHIBITION OF VERAPAMIL AND ELECTRIC BEAM RADIATION ON CLONOGENIC GROWTH IN K562 AND K562/ADM CELL LINES *IN VITRO*

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It was first reported here that verapamil (VP) and electric beam radiation (EBR) were capable of inhibiting, independently or synergically, clonogenic growth in two kinds of K562 cell lines, adriamycin (ADM)-sensitive and ADM-resistant (K562/S and K562/ADM). Results showed that clonogenic rate (CGR) decreased by 3%–99.9% in the presence of dependent dose-ADM (3.8 μg/ml) in K562/ADM cell lines, while treated with 0.5μM–6μM of VP. VP was capable of potentiating radiosensitivity in K562/S and K562/ADM cell lines, whether before or after exposure of them to electric beam radiation, and significantly reduced CGR in these kinds of cell lines ($P<0.01$).

Key words: Verapamil, Radiation, K562 cell line, K562/ADM cell line.

As a calcium channel blocker, verapamil (VP) has been recently reported to not only inhibit proliferation of human brain tumor cells[1] but also potentiate chemosensitivity of tumor cells to a lot of anticancer-drugs.[2-4] Little has been known as yet about whether VP is capable of potentiating radiosensitivity of tumor cells. Adriamycin resistant K562 cell line has been recently established in our laboratory[5] which showed cross-resistance to Cisplasin, Vincristin, 5-Fluorouraci and Nitrocaphamn. And then, we studied the effect of VP and electric beam radiation (EBR) on clonogenic growth of ADM-sensitive K562 cell lines (K562/S) and K562/ADM cell line by double layer agarose-culture technique.

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MATERIALS AND METHODS

Cell Lines

K562/S cell line, derived from human myelogenous leukemia, was obtained from Shanghai Institute for Cell Biology. K562/ADM cell line selected by serial passages in the presence of increasing concentration of ADM after two years of drug exposure of K562/S cell line, ADM resistant degree of which was 114.7-fold higher than that of K562 cell line. These two kinds of cell lines were incubated at 37°C in an atmosphere of 5% CO_2.

Monolayer Cell Culture and Agent Exposure

K562/S- and K562/ADM cell lines grew in RPIM medium 1640, supplemented with 15% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). VP was obtained from Shanghai 10th Pharma-ceutical Factory. K562/S and K562/ADM cell lines were exposed to radiation. After various 50% clono-genic inhibition concentration (ID_{50}) was obtained, K562/S and K562/ADM cell lines were exposed to VP at 14 μM and 3 μM respectively in the groups of expo-sure to radiation.

Double Layer Agarous Culture and Agent Exposure

As previously described but with some modifi-cations,[6] it was carried out 1 day after exposure of suspension cells to radiation. In all 35 mmculture dishes, bottom feeder layer contained agar, McCoy5A, fetal calf serum and horse serum, and top layer, agar, K562/S cell lines or K562/ADM cell lines (500 cells/dish), RPIM medium 1640 and horse serum. 14 μM and 3 μM of VP were added to top layers containing K562/S or K562/ADM cell lines respectively. Incubation condition was the same as suspension cell as mentioned above. At the end of the 15-day culture period, colonies containing at least 50 cells were counted under a binocular dissecting microscope. Clonogenic rate (CGR) and clonogenic inhibition rate (CIR) were defined as:

$$CGR = \frac{\text{mean number of colonies per dish}}{\text{mean number of inoculated cell per dish}} \quad (\%)$$

$$CIR = \frac{\text{mean CGR of control groups - mean CGR of V-treated or EBR-exposed group}}{\text{mean CGR of control group}} \quad (\%)$$

Each mean value of CGR or CIR was obtained from repeated experiment for four times.

Radiation Exposure

Some groups of K562/S cell lines were exposed to electric beam radiation (6 MeV Clinac 1800 Linear Accelerator). After 50% clonogenic inhibition dose (ID_{50}) was obtained, the other of K562/S and K562/ ADM cell lines were exposed to 6 MeV electric beam radiation at 0.5 Gy (ID_{50}).

RESULTS

Inhibition of VP or EBR Alone on K562/S- and K562/ADM Cell Lines

As illustrated in Figure 1, CGR reduced signifi-cantly ($P<0.05$) in the K562/S and K562/ADM cell lines after exposure of the cell lines to 3 Gy or allow 3 Gy of EBR in a dose-dependent fashion, and decreased by 83.9% in the K562/ADM cell lines in the presence of 3.8 μg/ml, after exposure of the cell line to 0.5 Gy of EBR.

As illustrated in Figure 2, clonogenic growth was inhibited obviously by VP in the K562/S and K562/ ADM cell lines in a dose-dependent fashion. CGR decreased by 1.3%–99.6% in the K562/S cell lines, after treatment with 4μM–100μM of VP ($P<0.05$) and did by 1.3%–99.9% in the K562/ADM cell lines in the presence of 3.8 μg/ml of ADM, after that of 0.5μM–6μ M of VP.

Synergic Inhibition of VP and EBR on K562/S Cell Lines

CGR decreased by 86.8% and 85.7% respectively in the K562/S cell lines treated with 14 μM of VP before exposure to radiation, compared with in that

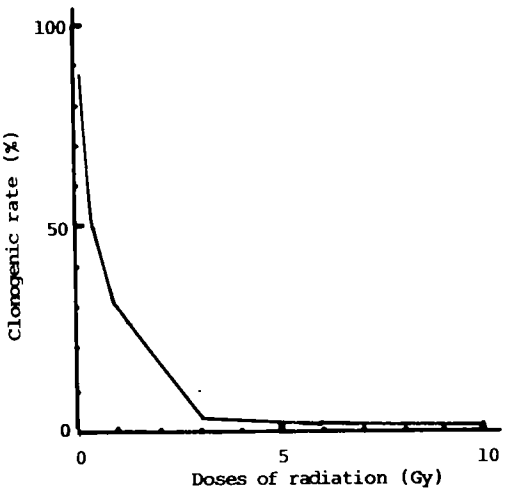

Fig. 1. Effect of EBR on clonogenic growth in K562/S cell lines

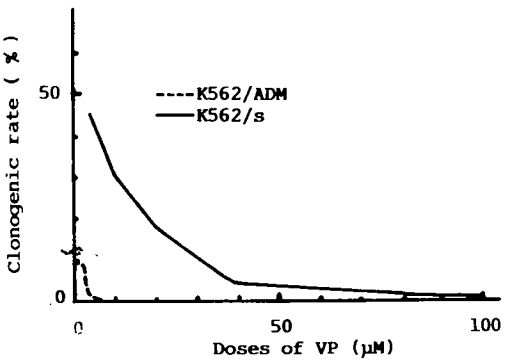

Fig. 2. Effect of VP on clonogenic growth in K562/S and K562/ADM cell lines

pre-exposed to 0.5 Gy of EBR and in VP-free that and CGR did by 73.7%, 73.9%, 71.5% and 71.9% respectively in the K562/S cell lines pretreated with 14 μM of VP before exposure to EBR or pre-exposed to 0.5 Gy of EBR before treatment with VP, compared with that only treated with 14 μM of VP and only exposed to 0.5 Gy of EBR. There was no significance for CGR between VP pretreated with and pre-exposed to EBR 562/ADM cell lines (P>0.05) (Table 1).

Synergic Inhibition of VP and EBR on K562/ADM Cell Lines

CGR decreased by 99.5%, 91%, 96.7%, 92.5%, 84.9% and 53.3% respectively in the K562/ADM cell lines pretreated with 3 μM of VP or pre-exposed to 0.5 Gy of EBR in the presence of 3.8 μg/ml of ADM, compared with VP-free and radiation-free that, and treated that with 3 μM of VP or that exposed to 0.5 Gy of EBR.

Table 1. Effects of ID_{50} of VP and o.5 Gy of EBR of CGR in K562/S- and K562/ADM cell lines

Cell lines	VP-free and EBR-free (%)	Treatment with VP (%)	Exposure to EBR (%)	Pretreatment with VP prior to EBR (%)	Pre-exposure to EBR prior toVP (%)
K562/S	45.4	22.8	23	6	6.5
K562/ADM	9.3	4.6	1.5	0.05	0.7

ID_{50} of VP was 14 μM and 3 μM respectively in K562/S and K562/ADM cell lines. 0.5 Gy was ID_{50} of EBR to K562/S cell line. 3.8 μg/ml of ADM were added to all groups of K562/ADM cell lines.

DISCUSSION

The result suggested that EBR is capable of potentiating significantly sensitivity of K562/ADM cell lines to ADM, mechanism of which is likely to be associated with P-glycoprotein acting as a molecular pump that could extrude a wide variety of drugs (including ADM).[7] The P-glycoprotein has been reported previously to express in K562/ADM cell lines but not in K562 cell lines.[8] It is possible for EBR to damage the P-glycoprotein or its expression.

As a calcium channel blocker, VP was capable of potentiating sensitivity of K562/ADM cell lines to a variety of anticancer-drugs,[2-4] which was considered to involve a mechanism that VP binded compactly P-glycoprotein with those anticancer-drugs. But, besides P-glycoprotein-mechanism, the sensitivity by VP also involves cytotoxicity of itself from our findings, since it is capable of inhibiting significantly clonogenic growth in the K562/S cell lines while used alone. In this sense, our findings shows consistence with Schmidt's that VP inhibited significantly proliferation of tumor cell from human brain while used alone with lack effect of it on calcium channel in the membrane of the tumor cells.

The result also suggests that VP is capable of potentiating radiosensitivity in the K562/S and K562/ADM cell lines, which is likely to involve a mechanism in association with cytotoxicity of VP but not P-glycoprotein, since it is possible for EBR to damage P-glycoprotein or its expression as mentioned above so that the Pglycoprotein-mechanism for VP does not work.

REFERENCES

1. Willian FS, Klaus RH, Robert SE, et al. Antiproli - ferative effect of verapamil alone on brain tumor cells *in vitro*. Cancer Res 1988;48:3617.
2. Ishida Y, Shimada Y, Shimoyama M, et al. Synergic effect of cyclosporin A and verapamil in overcoming vincristine resistance of multidrug resistant cultured human leukemia cells. Jpn J Cancer Res 1990;81:834.
3. Hu XF, de Luise M, Martin TJ, et al. Effect of cyclosporin A and verapamil on the cellular kinetics of daunorubicin. Eur J Cancer 1990;26:814.
4. Hall KS, Endresen L, Schjerven L, et al. Verapamil potentiates 4' - epidoxorubicin cytotoxicity in a rat hepatoma cell line and in a substrain resistant to 4' - epidoxorubicin. Pharmacol Toxicol 1990;67:402.
5. 沈世仁,苏颖,黄秀清,等. K562/AD 耐药细胞株的建立及其生物学特性的初步观察. 癌症 1992;11:222.
6. 鄂征. 组织培养技术. 第六版. 北京:人民卫生出版社. 1988;P157.
7. Borst P. Genetic mechanisms of drug resistance. Acta Oncology 1991; 30:87.
8. Yusa K, Tsuruo T. Reversal mechanism of multidrug resistance by verapamil direct binding of verapamil to P - glycoprotein on specific sites and transport of verapamil outward across the plasma memebrane of K562/ADM cell. Cancer Res 1989;49:5002.