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EFFECT OF ASCORBIC ACID ON DNA SYNTHESIS, INTRACELLULAR ACCUMULATION OF ADM AND ADM RESISTANCE OF TUMOR CELL LINES

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Objective: To determine the effect of ascorbic acid (AA) on DNA synthesis, intracellular accumulation of ADM and ADM resistance of tumor cell lines. Methods: K562, K562/ADM and KB cell lines were used to study the effect of ascorbic acid on DNA synthesis, intracellular accumulation of ADM and ADM resistance by fluid scintillometry, MTT method, spectrofluorophotometry and immunocytochemistry. Results: Results showed that AA was capable of inhibiting DNA synthesis of K562 and K562/ADM in a dose-dependence fashion, but not KB cell line, and significantly reducing ADM sensitivity in K562 and KB cell lines, as well as potentiating obviously ADM resistance in K562/ADM cell line. Conclusion: These effects of AA may be closely correlated with significant elevation of intracellular accumulation of ADM in KB cell line, and significant reduction of that in K562 and K562/ADM cell lines but possibly not correlated with the expression of P-glycoprotein.

Key words: Ascorbic acid, DNA synthesis, Drug resistance, Tumor cell lines, Experimental therapy

Ascorbic acid (AA), namely vitamin C, a kind of supplementary medicine, is used most frequently in clinic, mainly for overcoming AA shortage or inadequacy from a variety of causes. Interestingly, AA has been recently discovered to inhibit growth of tumor cells,[1-4] potentiate chemosensitivity or reverse chemoresistance of tumor cells.[5-7] Little has been reported about effect of AA on drug-resistance of tumor cells at home, even very few abroad. We reported here the effect of AA on growth or drug-resistance of tumor cells and its mechanism.

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MATERIALS AND METHODS

Major Medicines and Agents

AA, adriamycin HCl (ADM), ^{3}H-TdR, and immunocytochemical agents, such as monoclonal antibodies JSB-1 against P-glycoprotein, 353-10 against GSTPi etc., were purchased from Yanchen Pharmaceutical Manufactory in Jiangsu Province, Haimen Pharmaceutical Manufactory in Zhejiang Province, China Institute of Atomic Energy and Fuzhou Maxim Biotech Inc. respectively.

Cell Lines and Its Culture

K562 cell line was obtained from Shanghai Institute for Cell Biology and KB cell line from China Academy of Military Medical Sciences. K562/ADM cell subline resistant to ADM, derived from K562 cell line, was previously established by us.[8] These cell lines grew in medium RPMI 1640, supplemented with 15% fetal calf serum, penicillin (100U. ml^{-1}) and streptomycin (100μg. ml^{-1}). 3.8μg. ml^{-1} ADM (resistant dosage) was present in medium for K562/ADM cell line. All cell lines were incubated at 37℃ in an atmosphere of 5% CO_2 .

^{3}H-TdR Incorporation and Fluid Scintillometry for DNA Synthesis Assay

Three kinds of cell lines in exponential growth cycle were collected and inoculated into 96 well-dish, incubated for 2 h. after exposure to AA and for 3 h after exposure to 4μci. ml^{-1} ^{3}H-TdR. The resulted cells were collected on filter strips following wash with distilled water, fixation with 5% trichloracetic acid and decoloration with 95% ethanol. Dried filter strips were put into bottles containing scintillant solution, and radioactivity (cpm/10^4 cells) was detected in automatic scintillometer, with 4 wells for each group and average value from triplicate experiments. DNA synthesis inhibition rate was calculated as (cpm of control groups-cpm of treated ones)/cpm of control ones (%).

Spectrofluorophotometry Assay for Intracellular Accumulation of ADM

The procedure was carried out as previously described.[9] The cells in exponential growth cycle were treated with IC_{50} of AA (600μg. ml^{-1} for K562 cell line) for 24 h after culture for 24 h, following exposure to 2, 4, 8, 16μg. ml^{-1} ADM, each dose group for triplicate bottles. 1.5 h later, cells were collected, counted and regulated up to 1×10^6 for each bottle. After treated with mixed solution containing 0.3 mol. L^{-1} HCl and 50% ethanol, cells were placed overnight at -20℃. Next day, cells were contrifuged at 12000 r /min for 30 min at 4℃, supernate was used for assay of fluorescence intensity. Experiments were triplicated.

MTT Method for Assay of Cytotoxicity of ADM and S-P Immunocytochemistry for Detection of P-glycoprotein and GSTPi

They were carried out as previously described.[10-11]

RESULTS

Effect of AA on DNA Synthesis of Tumor Cell Lines

AA was observed to inhibit DNA synthesis of K562 and K562/ADM cell lines to a certain extent in a dose-dependent fashion, stronger in the former than in the latter, with insignificant inhibition to KB cell line, only 0.1% for DNA synthesis inhibition rate even in high concentration of AA (7.2 mmol. L^{-1})(Figure 1).

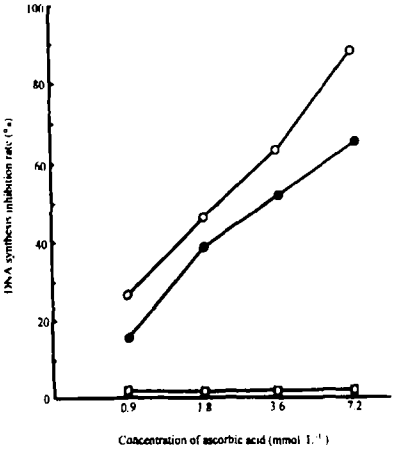

Fig. 1. Effect of ascorbic acid on DNA synthesis of tumor cells. ○-○, K562 cell line; ●-●, K562/ADM cell subline; □-□, KB cell line. Average value of triplicate experiments is represented for each point in Figure.

Effect of AA on ADM Sensitivity of Tumor Cell Lines

AA potentiated ADM sensitivity of KB cell line, reduced ADM sensitivity of K562 cell line significantly (P<0.0025) and elevated greatly ADM-resistance of K562/ADM cell line (P<0.005), and IC^{50} of AA (2.5mmol. L^{-1}), reduced that in K562/ADM (P<0.005) (Table 1).

Table 1. Effect of IC_{50} of ascorbic acid (AA) on ADM sensitivity to tumor cells[a]

Cell line	IC_{50}(nmol. l^{-1} of ADM ($\chi \pm s$)[b]		SR[c]	P value
	AA(−)	AA(+)		
K562	120.67± 1.24	216.67± 1.24	0.56	<0.05
K562/ ADM	13032.36±2.12	19165.24± 265.35	0.68	<0.005
KB	4.4± 0.04	1.30± 0.07	3.38	<0.0025

(a) MTT method for cytotoxicity

(b) Average of triplicate experiments

(c) SR(sensitization ratios)= $\frac{IC_{50} \text{ in abscence of AA}}{IC_{50} \text{ in presence of AA}}$

Effect of AA on Intracellular Accumulation of ADM in tumor cell lines

High concentration of AA (3.4 mmol. L^{-1}) elevated markedly intracellular accumulation of ADM in KB cell line ($P<0.005$), and IC_{50} of AA (3.4mmol. L^{-1}) showed a limited elevation of ADM intracellular accumulation of K562 cell line ($P>0.05$) without further elevation in presence of more than 8μg. ml^{-1} (Figure 2).

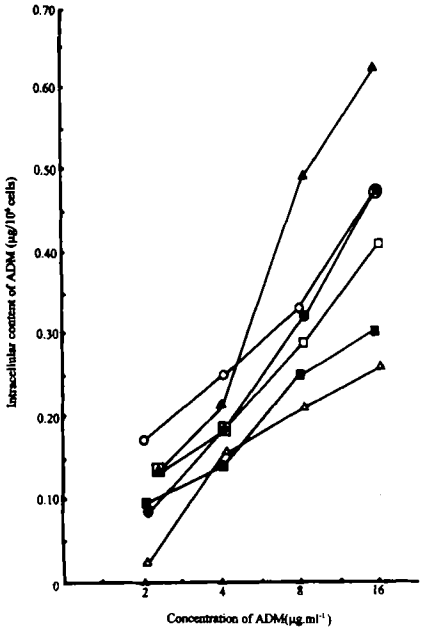

Fig. 2. Effect of ascorbic acid (AA) on intracellular accumulation of tumor cells. ○-○, K562 cell line; □-□, K/ADM cell line, △-△, KB cell line; ●-●, K562+ AA(3.4mmol. L^{-1}); ■-■, K562/ADM+AA (2.5mmol. L^{-1}); ▲-▲, KB+ AA (.mmol. L^{-1}). Average value of triplicate experiments for each point is represented in Figure 2. IC^{50} of AA are 3.4mmol. L^{-1} for K562 cell line and 2.5mmol. L^{-1} for K562/ADM cell subline respectively.

Expressions of P-glycoprotein and GSTPi

Expression of P-glycoprotein was observed (85% for positive rate) in K562/ADM cells but not in K562 cells and KB cells. Expression of GSTPi was present only in KB cells (80% for positive rate). P-glycoprotein and GSTPi were located on membrane or in cytoplasm of tumor cells (Figure 3, 4).

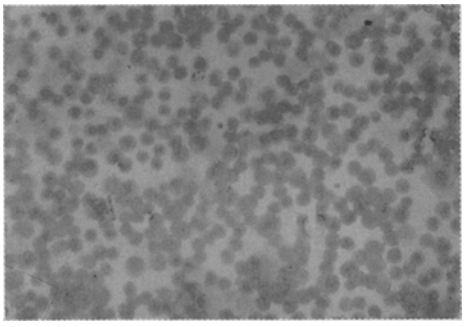

Fig. 3. -glycoproteins are located in cytoplasm of K562/ADM cell subline. S-P ICC× 200

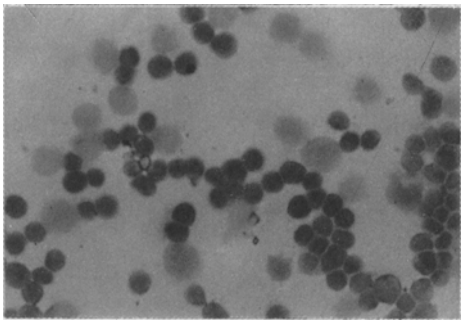

Fig. 4. GSTPi are located in cytoplasm of KB cell line. S-P ICC× 400

DISCUSSION

Our findings showed that high concentration of AA was capable of inhibiting greatly DNA synthesis of K562 or K562/ADM cell lines, but not that of KB cell line, suggesting that this effect may be associated with types of cell line. AA has been reported to induce apoptosis, such as 6-amino-6-deoxyascorbic acid, by which human tumor cells were induced to produce chromatin condensation and DNA fragments special for apoptosis, and were inhibited in growth.[12] It has not been known at present whether this induction plays a role in DNA synthesis inhibition by AA from our investigation, until further study in future.

1μmol. L^{-1} (non-cytotoxicity) or 100μmol L^{-1} (low cytotoxicity) AA potentiated the cytotoxicity by ADM, DDP and TX (Paclitaxel) from report by Kurbacher, et al.[5] Song, et al. observed the elevation of cytotoxicity and reversal of across drug resistance to colchicine by 25 μg. ml^{-1} AA (non-cytotoxicity) in PC-9/VCR cell line.[6] Some differences existed between these findings and ours. High concentration

of AA (3.4mmol. L^{-1}) assumed significant potentiation of cytotoxicity of ADM in KB cell line, and ADM resistance in K562/ADM cell line ($P<0.005$) and reduction of chemosensitivity in K562 cell line.

Effect of AA on chemosensitivity or chemoresistance may be associated with its dose, tumor cell type as well as reduction of intracellular accumulation of ADM by AA, but not expression of P-glycoprotein, since reduction of intracellular accumulation of ADM was also observed in K562 cell line lacking P-glycoprotein expression.

Our finding that elevation of ADM sensitivity in KB cell line by AA may be related to marked reduction of ADM accumulation inside tumor cell, is consistant with Chiang, et al.,[7] from whose findings AA was capable of elevating intracellular accumulation of VCR and partly reversing VCR resistance in PC-9/VCR cell line. KB cell line was found to express GSTPi, but further study is needed to understand the relationship of potentiation of ADM sensitivity by AA with GSTPi expression.

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