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• BRIEF REPORT •

Experimental study on antitumor effect of arsenic trioxide in combination with cisplatin or doxorubicin on hepatocellular carcinoma

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INTRODUCTION

The main component of a traditional Chinese drug "Pishuang", arsenic trioxide (As_2O_3), has obviously selective anti-tumor effect on human hepatocellular carcinoma (HCC) in both *in vitro* and *in vivo* studies[1-5]. Due to limited effectiveness when any anti-carcinogen is used alone and obviously increased toxicity when the dose is raised, there is no exception for As_2O_3. Furthermore, combined chemotherapy contributes to improve therapeutic effectiveness, disperse toxicity and surmount drug-resistance, in which the combination of traditional Chinese and modern medicine has more advantages and characteristics. As a result, we made an experimental study on anti-tumor effect of As_2O_3 in combination with cisplantin (PDD) or doxorubicin (ADM) on HCC, to investigate the possibility of As_2O_3 in combination with PDD or ADM and nature of interaction between them, and to provide experimental basis for clinical application.

MATERIALS AND METHODS

Materials

Drugs and reagents As_2O_3 for injection (5 mg per am poul, Lot No. 998068, provided by Professor Ma Jun of Harbin Hamaeotoloy and Oncolony Institute), PDD for injection (20 mg per vial, Lot No. 990618, Shandong Qilu Pharmaceutical Factory) or ADM hydrochloride for injection (10 mg per vial, Lot 990406, Shanxi Mlarmaceutical Co. LTD).

Cell lines Human hepatoma Bel-7402 cells were obtained from the Shanghai Cell Bank of Chinese Academy of Sciences and maintained in our laboratory. Bel-7402 cells were routinely cultured in RPMI1640 medium (Gibco) containing 100 mL·L^{-1} fetal calf (FCS) serum at 37℃ in humidified incubator with 50 mL·L^{-1} CO_2/95 mL·L^{-1} air.

Animals Mice with hepatoma HepA were obtained from the Shanghai Institute of Materia Medica of Chinese Academy of Sciences and Kunming mice (famale and male weighing, 18 g-22 g) from the Experimental Animal Center of Southerneast University Medical College.

methods

Measurement of anticancer activity *in vitro* The exponent growing Bel-7402 cells in culture flasks were harvested by 2.5 g· L^{-1} EDTA, suspended in RPMI1640 medium with 100 mL·L^{-1} FCS, adjusted to the concentration of 3×10^4 cells·L^{-1}, plated into 40-well plates (100 μL cells·well-1) and incubated at 37℃ in 50 mL·L^{-1} CO_2/95 mL·L^{-1} air until the cells were stuck with the plates. The cells were then exposed to 100 μL of various concentrations of a drug alone or combination for 48 h, and the controls to 100 μL of RPMI1640 medium with no FCS. After that, the absorption was detected by adding 20 μL tetrazolium (MTT) to each well, incubating for 4 h, sucking out the media, adding 150 μL dimethylsul foxide (DMSO) to dissolve the violet-crystal and measuring at 570 nm. Double wells were used for each drug concentration. Experiments were triplicated. The inhibitory rate was calculated as follows:

IR(%)=(1-mean absorption in experiments/mean absorption in controls)×100%

Measurement of anticancer activity *in vivo* The mice with hepatocarcinoma HepA were killed and their ascites Abstracted, adjusted to 2×10^7·mL^{-1} and implanted by subcutaneous injection 200 μL to each mouse. Sixty mice with implanted HepA tumor were randomly divided into control group (saline), and groups of As_2O_3 alone (2 mg·kg^{-1}·d^{1}), PDD alone (1 mg·kg^{-1}·d^{1}), As_2O_3 combin ed with PDD (As_2O_3 2 mg·kg^{-1}·d^{1} + PDD 1 mg·kg^{-1}·d^{1}), ADM alone (1 mg·kg^{-1}·d^{1}), and As_2O_3 combined with ADM (As_2O_3 2 mg·kg^{-1}·d^{1} +ADM 1 mg·kg^{-1} ·d^{-1}). Each group was injected intraveously 24 h after transplatation once a day for 7 days continuously. The mice were killed on the 8th day after the treatment and the tumors isolated and weighed. The inhibitory rate of tumor was calculated as follows:

Inhibitory rate of tumor (%)=(1-mean tumor weight in experiments/mean tumor weight in controls)×100%

Statistical method Analysis of variance of two-factor factoria l experiment was applied to evaluate anti-cancer activity *in vitro* and analysis of variance of random experiment was used to evaluate anti-cancer activity *in vivo*.

Evaluation of interaction of drug combination *In vitro* experiment: the interaction between As_2O_3 and PDD or ADM was evaluted by coefficient of drug in interaction (CDI), which was calculated as follows: CDI = AB/(A×B). AB is the absorption ratio between a drug combination group and controls and A or B is that between a drug alone and controls. When CDI value was equal to 1.0, or more than 1.0 or less than 1.0, the nature of the interaction between A and B was considered to be additive or antagonistic or synergistic[6]. *In vivo* experiment: the interaction between As_2O_3 and PDD or ADM was evaluated by Q value, which was calculated as follows: Q=E(AB)/[EA+(1-EA)×EB]. E(AB) is the inhibiting tumor rate and EA or EB is that of a drug alone. When Q value was equal to 0.85-1.15, or less than 0.85 or more than 1.15, additive or antagonistic or synergistic interaction was thought to occur[7].

RESULTS

The effect of As_2O_3 and/or PDD on HCC

The inhibition rates of As_2O_3 *in vitro*, in combination with PDD at various concentrations were more than that of As_2O_3 or PDD alone ($^aP<0.01$, F = 58.96), in which the inhibition rates increased more evidently at low concentrations (Figure 1). CDI values of As_2O_3 and PDD in combination at low concentration were less than 1.0 (Table 1).

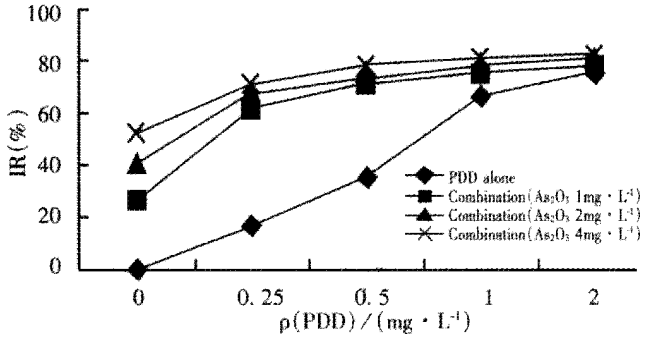

Figure 1 The effect of As_2O_3 and/or PDD on the growth of Bel-7402 cells *in vitro.*

Table 1 CDI value of As_2O_3 in combination with PDD against Bel-7402 cells

Cell line	As_2O_3 (mg·L-1)	PDD (mg·L-1)			
		0.25	0.5	1	2
Bel-7	1	0.60	0.60	1.00	1.22
	2	0.62	0.70	1.03	1.38
	4	0.60	0.65	1.13	1.50

The effect of As_2O_3 and/or ADM on HCC

In vitro the inhibition rates of As_2O_3 in combination with ADM in various concentrations were more than those of As_2O_3 or ADM alone ($^aP<0.01$, F = 64.77), in which the inhibition rates increased more evidently in low concentrations (Figure 2). CDI values of As_2O_3 and ADM in combination in low concentrations were almost equal to 1.0 (Table 2).

The effect of As_2O_3 and/or PDD on HepA implanted tumor

The inhibiting tumor rate of As_2O_3 in combination with PDD was more than that of As_2O_3 or PDD alone and *Q* value was more than 1.15 (Table 3).

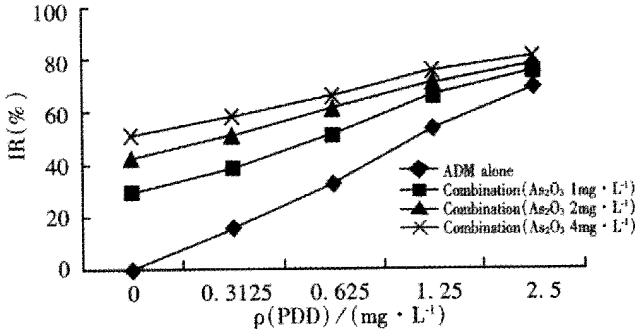

Figure 2 The effect of As_2O_3 and/or ADM on the growth of Bel-7402 cells *in vitro.*

Table 2 CDI value of As_2O_3 in combination with ADM against Bel-7402 cells

Cell line	As_2O_3(mg·L-1)	ADM (mg·L-1)			
		0.3125	0.625	1.25	2.5
Bel-7	1	1.03	1.02	1.05	1.12
	2	1.03	0.98	1.06	1.31
	4	1.00	1.05	1.08	1.28

Table 3 The effect of As_2O_3 and/or PDD on HepA implanted tumor in mice (n = 10)

Groups	Dose ($mg·kg^{-1}·d^{-1}$)	Tumor mass ($\bar{x}$±s,g)	Inhibition (%)	Q value
Control	NS	1.53±0.35		
As_2O_3	2	1.07±0.21	30.1	
PDD	1	0.82±0.11	46.2	
As_2O_3+PDD	2+1	0.40±0.05	73.9[a]	1.18

[a]$P<0.01$, F = 54.05, *vs* As_2O_3 or PDD alone.

The effect of As_2O_3 and/or ADM on HepA implanted tumor

The inhibiting tumor rate of As_2O_3 in combination with ADM was higher than that of As_2O_3 or ADM alone and *Q* value was less than 1.15 but more than 0.85 (Table 4).

Table 4 The effect of As_2O_3 and/or ADM on HepA implanted tumor in mice (n = 10)

Groups	Dose ($mg·kg^{-1}·d^{-1}$)	Tumor mass ($\bar{x}$±s,g)	Inhibition (%)	Q value
Control	NS	1.53±0.35		
As_2O_3	2	1.07±0.21		
ADM	1	0.91±0.12	40.5	
As_2O_3+ADM	2+1	0.61±0.11	60.1[a]	1.03

[a]$P<0.05$, F = 24.40, *vs* As_2O_3 or ADM alone.

DISCUSSION

As_2O_3, the main component of traditional Chinese drug "Pishuang", has been applied to treat acute promyelocytic leukemia and yielded notable results. Complete remission rate and long-term survival rate are high and the relapse rate is low in APL patients treated with As_2O_3[8-10]. The main mechanism of As_2O_3 is to induce apoptosis of leukemia cells, which is different from all-trans retinoic acid (ATRA)[11-23]. Based on the achievements, the experimental studies on anti-tumor effect of As_2O_3 in such hematopathy as malignant lymphoma[24] and myeloma[25,26] and solide tumors such as cancers of lung[27],

esophagus[28], stomach[29-32], colone[33-35] pancreas[36], mamma[37], cervix[38] and neuroblastoma[39] are in the ascendant.

The morbidity and mortality of hepatocarcinoma is high in China, which is the first cause of death among all kinds of cancers in Jiangshu Province. Due to the hidden onset, low rates of early diagnosis and rapid progression, most patients with hepatocarcinoma cannot be operated on and have to depend on chemotherapy, but the therapeutic effect of the present agents is unsatisfactory. So it is urgent and necessary to go on seeking new drugs and the improving therapeutic METHODS. Our group has taken the lead in conducting the study of As_2O_3 against liver cancer and found that As_2O_3 had obviously selective anti-tumor effect on hepatocarcinoma both *in vitro* and *in vivo*[1-5]: *in vitro* As_2O_3 inhibited the proliferation of several hepatocarcinoma cell lines but not normal human liver cells and *in vivo* inhibited implanted hepatocarcinoma in mice and prolonged the survival phase of mice with hepatocarcinoma but produced no obvious toxicity. The main mechanism is to induce apoptosis of hepatocarcinoma cells, which also has been proved by other reports[40-46].

To further investigate the best therapeutic way of As_2O_3 and raise the effect on hepatocarcinoma, we studied As_2O_3 and PDD or ADM in combination. The experiments *in vitro* showed that As_2O_3 in combination with PDD or ADM can increase the effect on HCC Bel-7402 and the increase extent varies at different concentrations, which was greater at lower concentrations. The possibl e reason is that the anti-tumor activity of an individual drug is saturated at high concentrations and difficult to increase after combination or there was antagonistic action to some extent between two drugs in combination and counteracted part of anti-tumor activity of a drug. CDI values showed that *in vitro* the nature of interaction is markedly synergistic between As_2O_3 and PDD and addictive between As_2O_3 and ADM in low concentrations. On the basis of the experiments *in vitro*, low-dose PDD or ADM combined with As_2O_3 was applied to treat HepA tumor implanted in mice, and inhibitory rate of tumor evidently increased as compared with that of a drug alone. Q value showed that *in vivo* synergistic interaction between As_2O_3 and PDD and addictive between As_2O_3 and ADM were thought to occur, which agreed with the results *in vitro*. These results suggested that low-dose PDD or ADM and As_2O_3 in combination could increase evidently anti-hepatocarcinoma effect. PDD and ADM are the main anti-hepatocarcinoma agents, but their toxicities in kidney, liver or heart restrict their clinical application, as a result patients cannot tolerate the high-dose agents whereas low dose is difficult to achieve satisfactory results. Considering selectively inhibitory effect of As_2O_3 on HCC *in vitro* and unobvious toxicity *in vivo*, the effect may be improved evidently without increased toxicities or keep satisfactory in poorly-tolerated patients with low dose of PDD or ADM when As_2O_3 and PDD or ADM in combination are applied to treat hepatocarcinoma.

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