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Similarity of Drug Sensitivity Test Results on Human Pulmonary Adenocarcinoma Xenografts Transplanted Under the Subrenal Capsules between Normal Immunocompetent and Immunodeficient Athymic Mice

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ABSTRACT

A major obstacle to control cancer growth and metastasis in patients is the widespread inappropriate use of anticancer drugs. We have known that cancers are different etiological diseases with a same pathologic characteristics of unlimited growthes. With this type of heterogenous characters, it means responses to same anticancer drugs can be various from patient to patient even though they all develop from same organs of human, or even represent with histologically same phenotype. Therefore individualized cancer chemotherapy (tailored-chemotherapy) has now begun to be recommended. As we have suggested previously two ways might be the major bases of individualized cancer chemotherapy—drug sensitivity tests and pathological or biomolecular profile information *In vivo* drug sensitivity tests are mainly from human tumor species transplanted under the subrenal capsule of mice. One of the major problems of human tumor species transplanted under the subrenal capsule of mice is whether the normal immunocompetent mice or immunodeficient athymic (nude) mice are used similarily in the tests. The immunodeficient mice (nude mice) are precious animal difficult to breed and maintain, but they have low immuno-surveillence system to reject human tumor species transplanted into nude mice. In normal circumstances, only nude or SCID mice will be used as hosts to transplant and grow human tumors. But since nude mice are difficult to breed and maintain, it seems a financial burden for a patient to use them for drug sensitivity tests. So a 4-day or 6-day subrenal capsule assay procedures (SRCP) using normal immunocompetent mice were suggested as a superseded method to do this task. In this work, we compared the drug sensitivity test result using both normal and nude mice. Our work showed that there is same drug response rates and statistically positive drugs between normal and nude mice by a week SRCP.

Keywords

Drug sensitivity tests· Individualized cancer chemotherapy· Nude mice· Bisdioxopiperazine compounds· Cancer chemotherapy· Laboratory animals

INTRODUCTION

A major obstacle to control cancer growth and metastasis in patients is the widespread inappropriate use of anticancer drugs. We have known that cancers are different etiological diseases with same pathologic characteristics of unlimited growths. With this type of heterogeneous characters, it means responses to same anti-cancer drugs can be various from patient to patient even though they all develop from same organs of human, or even represent with histologically same phenotype. Therefore individualized cancer chemotherapy (tailored-chemotherapy) has now begun to be recommended (Lu DY *et al*., 2006; Ulukaya E, 2006). As we have suggested

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before two ways might be the major bases of individualized cancer chemotherapy—drug sensitivity tests and pathological or biomolecular profile information (Lu DY *et al*., 2006).

Drug sensitivity tests encompass *in vivo* and *in vitro* means (Lu DY *et al*., 2006; Ulukaya E, 2006; Ugurel S *et al*., 2006; Salmon SE *et al*., 1978). *In vivo* drug sensitivity tests are mainly from human tumor species transplanted under the subrenal capsule of mice (Bogden AE *et al*., 1978; Stratton JA *et al*., 1984). One of the major problems of human tumor species transplanted under the subrenal capsule of mice is whether the normal immunocompetent mice or immunodeficient athymic (nude) mice are used similarly in the tests. The immunodeficient mice (nude mice) are precious animal difficult to breed and maintain, but they have low immuno-surveillence system to reject human tumor species transplanted into nude mice. In normal circumstances, only nude or SCID (severe combined immune deficient) mice will be used as hosts to transplant and grow human tumors. But since nude or ACID mice are difficult to breed and maintain, it seems a financial burden for a patient to use them for drug sensitivity tests. So a 4-day or 6-day subrenal capsule assay procedures (SRCP) using normal immunocompetent mice were suggested as a superseded method to do this task (Bogden AE *et al*., 1979; Levi FA *et al*., 1984; Aamdal S *et al*., 1985; Cunningham D *et al*., 1986). But there are many unanswered questions about this substitution and further work are needed to perfect them. In the previous work, some comparisons have been made to answer whether this substitution are acceptable. It was shown that xenografts of primary surgical explants showed positive growth more frequently in 6 days (82%) in the immunocompetent animal than in 11 days (30%) in the immunodeficient athymic nude mouse (Bogden AE *et al*., 1979). The previous work suggested the 4-day or 6-day SRCP in normal mice might be more well suited for drug sensitivity tests. This work, we compared the drug sensitivity test result using both normal and nude mice.

MATERIALS AND METHODS

Drug and reagents: Cyclophosphamide (CTX), 5-fluororacil (5-Fu), methotrexate (MTX), vincristine (VCR), cisplatin (DDP) are medical used agents purchased from commercial route. Bisdioxopiperazine compounds (Lu DY *et al*., 2010) ICRF-154, ICRF-159 (Raz) and probimane (Pro) were prepared by Prof Yun-Feng Ren, Shanghai Institute of Materia Medica, Chinese Academy of Science. Stereomicroscope was a product of Shanghai Keyi Instument Factory (C-XTL-1).

Animals and tumor models: Immunocompetent mice (ICR strain mice) were purchased from Shanghai Center of Laboratory Breeding, Chinese Academy of Sciences. Immunodeficient nude mice (Swiss-n/n nude mice) were originally obtained from Sloan-Kettering Cancer Institue, USA and later bred in Shanghai Institute of Materia Medica, Chinese Academy of Sciences at SPF conditions. The experimentations were conducted in compliance with the Guidelines for the Care and Use of Research Animals, NIH, established by Washington University's Animal Studies Committee.

Human pulmonary adenocarcinoma xenograft (*LAX-83*) (Zhang SY *et al*., 1982)were human tumor xenografted first transplanted into nude mice by SRCP from a 73 year-old male cancer patient and later serially transplanted *sc* within the nude mice.

Experiment procedure: Normal ICR and nude mice were inoculated with *LAX-83* under the renal capsule according to the procedure of Bodgen *et al* (Bogden AE *et al*., 1979). Mice were injected intraperitoneally with drugs daily during next five days after inoculation of *LAX-83*. Then mice were sacrificed, and their kidneys were taken out for measurement of tumor sizes using a stereomicroscope a week after transplantation. Tumor mean diameter was calculated as $1/2(a+b)$ and tumor volume was calculated as $1/2(ab^2)$ where a and b are their major and minor axes of the lump. Statistical tests were treated to determine whether drugs are positive or negative against tumor growth in mice.

RESULTS

Tumor growth curve and characteristics

We have make tumor growth characteristic study and shown human pulmonary adenocarcinoma xenograft (LAX-83) begin to growth from day 4-8. However, the obvious immune rejection was observed on day 8 group in normal immunocompetent mice. Tumor xenograft began to stop growth around day 7-8. Therefore, we select drug sensitivity tests on a week period.

Comparisons of positive drug rate against *LAX-83* transplanted under subrenal capsule between normal and immnodeficient mice

The experiment results are tabulated in Table 1-4. Our work showed that there is a same drug response rate and statistically positive drugs between normal and nude mice by a week SRCP are the same.

Table 1. Antitumor activities of 5 antineoplastic drugs on LAX-83 growth transplanted into normal immunocompetent mice

Drugs	Dosage mg/Kg/d	Mean body weight Initial/end (g)	No mice Initial/end	Mean tumor diameter±SD mm	Tumor volume mg	Inhibition %
Control	--	19.8 / 21.2	6 / 6	4.0 ± 0.7	32.0	--
CTX	30	19.2 / 19.3	6 / 6	1.9 ± 0.5***	3.4	89
Control	--	20.0 / 19.7	6 / 6	3.2 ± 0.3	16.4	--
MTX	1.5	20.3 / 22.0	6 / 6	2.6 ± 0.9	8.8	46
5-Fu	37.5	19.5 / 15.6	6 / 5	1.1 ± 0.5***	0.67	96
VCR	0.3	20.3 / 21.2	6 / 6	1.9 ± 0.8**	3.4	79
Control	--	19.3 /19.3	6 / 6	4.2 ± 1.2	37.0	--
DDP	1.5	19.2 / 16.8	6 / 5	3.4 ± 0.4	19.6	47

** P<0.01 *** P < 0.001 Route: ip×5; Tumor volume=1/2(width^2×length)

Table 2. Antitumor activities of 5 antineoplastic drugs on LAX-83 growth transplanted into nude mice

Drugs	Dosage mg/Kg/d	Mean body weight Initial/end (g)	No mice Initial/end	Mean tumor diameter±SD mm	Tumor volume mg	Inhibition %
Control	--	20.9 / 22.5	6 / 6	3.9 ± 0.4	29.7	--
CTX	30	21.0 / 20.9	6 / 6	1.8 ± 0.5***	2.9	90
DDP	1.5	22.8 / 21.7	6 / 5	3.1 ± 0.8	14.9	50
MTX	1.5	21.2 / 21.9	6 / 6	3.8 ± 0.2	27.4	8
5-Fu	37.5	21.7 / 21.4	6 / 6	2.3 ± 0.4***	6.1	79
VCR	0.3	20.8 / 20.8	6 / 6	1.9 ± 0.1***	3.4	89

*** P < 0.001 Route: ip×5; Tumor volume=1/2(width^2×length)

Table 3. Antitumor activities of Bisdioxopiperazine compounds on LAX-83 growth transplanted into normal immunocompetent mice

Drugs	Dosage mg/Kg/d	Mean body weight Initial/end (g)	No mice Initial/end	Mean tumor diameter±SD mm	Tumor volume mg	Inhibition %
Control	--	18.7 / 18.3	6 / 6	4.2 ± 0.5	37.0	--
ICRF-154	15	18.3 / 19.0	6 / 6	4.2 ± 1.0	37.0	--
ICRF-159	0	18.7 / 18.8	6 / 6	4.1 ± 0.6	34.5	7
Control	--	8.0 / 17.0	6 / 6	4.0 ± 0.6	32.0	--
Pro	100	18.2 / 16.5	6 / 6	2.8 ± 0.4**	11.0	56

** P<0.01 Route: ip×5; Tumor volume=1/2(width^2×length)

Table 4. Antitumor activities of Bisdioxopiperazine compounds on LAX-83 growth transplanted into nude mice

Drugs	Dosage mg/Kg/d	Mean body weight Initial/end (g)	No mice Initial/end	Mean tumor diameter±SD mm	Tumor volume mg	Inhibition %
Control	--	19.2 / 21.0	6 / 6	4.3 ± 0.3	39.8	--
Pro	80	20.0 / 19.6	6 / 6	3.3 ± 0.4***	18.0	55
ICRF-159	40	20.8 / 21.5	6 / 6	3.9 ± 0.1**	29.7	25
ICRF-154	15	18.8 / 21.0	6 / 6	4.4 ± 0.3	42.6	-7

** P<0.01 *** P < 0.001 Route: ip×5; Tumor volume=1/2(width^2×length)

DISCUSSIONS

There is a widely accepted concept to use immuno-competent mice in SRCP in drug sensitivity tests. In the previous work, the comparisons were based between the data of 4-days or 6-days SRCP in normal mice with 11-days SRCP in nude mice (Bogden AE *et al*., 1978; Stratton JA *et al*., 1984; Bogden AE *et al*., 1979; Levi FA *et al*., 1984; Aamdal S *et al*., 1985; Cunningham D *et al*., 1986). So there is no general conclusion of which is the best. However, since there is no report of a systematical comparison of positive drug rates between normal and immunodeficient mice in a same period of experiments, we are not sure whether the substitutions of normal mice with nude mice will change the fairness of drug sensitivity testings. Our work showed that there are same drug response rates and statistically positive drugs between normal and nude mice by a week SRCP are the same. Our work gives a pararoma of these situations and will provide a general bases for the future.

REFERENCES

Aamdal S, Fodstad O, Nesland JM, Pihl A. Characteristics of human tumour xenografts transplanted under the renal capsule of immunocompetent mice. *Br J Cancer*, 51(3), 1985, 347-356.

Bogden AE, Haskell PM, LePage DJ, Kelton DE, Cobb WR, Esber HJ. Growth of human tumor xenografts implanted under the renal capsule of normal immunocompetent mice. *Exp Cell Biol*, 47(4), 1979, 281-93.

Bogden AE, Kelton DE, Cobb WB, Esber HJ. A rapid screening method for testing chemotherapeutic agents against human tumor xenografts. In: Proceedings of the Symposium on the Use ofAthymic (Nude) Mice in Cancer Research. (Eds.Houchens & Ovejera), New York: Gustav Fischer, 1978, 231.

Cunningham D, Jack A, McMurdo DF, Soukop M, McArdle CS, Carter DC, Kaye SB. The 6 day subrenal capsule assay is of no value with primary surgical explants from gastric cancer. *Br J Cancer*, 54(3), 1986, 519-523.

Levi FA, Blum JP, Lemaigre G, Bourut C, Reinberg A, Mathé G. A four-day subrenal capsule assay for testing the effectiveness of anticancer drugs against human tumors. *Cancer Res*, 44(6), 1984, 2660-7.

Lu DY, Chen XL, Ding J. Individualized cancer chemotherapy integrating drug sensitivity tests, pathological profile analysis and computational coordination—an effective strategy to improve clinical treatment. *Medical Hypotheses*, 66, 2006, 45-51.

Lu DY, Lu TR. Anticancer activities and mechanisms of bisdioxopiperazine compounds probimane and MST-16. *Anti-cancer Agent Medicinal Chemistry*, 10(1), 2010, 78-91

Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *N Engl J Med*, 298, 1978, 1321-1327.

Stratton JA, Kucera PR, Micha JP, Rettenmaier MA, Braly PS, Berman ML, DiSaia PJ. The subrenal capsule tumor implant assay as a predictor of clinical response to chemotherapy: 3 years of experience. *Gynecol Oncol*, 19(3), 1984, 336-47.

Ugurel S, Schadendorf D, PfohlerC, Neuber K,Thoelke A, Ulrich J, Hauschild A, Spieth K, Kaatz M, Rittgen W, Delorme S, Tilgen W, Reinhold U. In vitro drug sensitivity predicts response and survival afterindividualized sensitivity-directed chemotherapy in metastatic melanoma: A multicenter phase II trialof the dermatologic cooperative oncology group. Clin *Cancer Res*, 12(18), 2006, 5454-5463.

Ulukaya E. Drug response assay: an increasing trend in designation of tailored-chemotherapy for more rational management of cancer patients. *Adv Mol Med*, 2, 2006, 53-58.

Zhang SY, Dai ZQ, Shu YH, Zhang JL, Lin ZQ, Wu SF, Liu YF, Xu LZ. Establishment of human lung adenocarcinoma model in nude mice and sensitivity of the transplanted tumor to antitumor drugs. *Zhongguo Yao Li Xue Bao*, 8(4), 1987, 366-369.