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FIRST STEP VIEW OF THE EFFICACY OF ANTI-NEOPLASTIC AGENTS

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A human K562 leukaemia cell line and acute adult leukaemia patient samples have been used to test the efficacy of antineoplastic agents with MTT assay. All 18 drugs were invoved. According to the purpose of experiment these drugs were applied at different opportunities or combinations. The drug efficacy has been observed and summarized as four different conditions: 1. The change of the time (Δ T) closely related with drug effacacy, during the duration the change of drug concentration (Δ C) at certain extent has almost no influence; 2. The Δ C closely related with the efficacy, the Δ T has no influence; 3. The Δ C and Δ T effect the results together; and 4. The Δ C and Δ T effect not the result. And then draw a conclution that the process of drug effacacy has a multiple function with flat district.

Key words: Antineoplastic agents, Pharmacodynamics, Acute leukaemia, Drug effective test.

The concentration of drug accumulation in tumor cells is a very important fact which is tightly closed together with the efficacy of antineoplastic agents.[1-3] But investigation of cellular accumulation and pharmacodynamics of anticancer drugs is still at an early stage of development and facing many technological challenge. Before we can overcome the barriers there is possible to search the process of how the drug killing tumor cells, which could impress us with the knowledge of the efficacy of antineoplastic agents *in vitro*, then increasing the judgement to the real effective drugs. To establish correlations between research data and clinical response leukaemia cells were conducted in this experiment, which were relatively accessible.

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MATERIALS AND METHODS

Leukaemia Cells and Patients

The K562 human leukaemia cell line was provided by Department of Immunology, Kiel University, Germany, and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin and streptomycin, at 37℃, 5% CO_2 and 100% humidity. When K562 cells were routinely used in MTT assay, the culture medium contented only 5% fetal calf serum and a density of 1×10^5 cells per hole in 96 well microtitre plate was reached.[4]

Fifty-four acute adult leukaemia patients were accepted in research.[5] Patient leukaemia cells were seeded in 96 well plate at 2×10^5 per hole.

Antineoplastic Agents and the Concentrations

All 18 drugs were involved. They were Homoharringtonine(Hom), Daunorubicin(DNR), Cytosine arabinoside(Ara-C), Vincristine(VCR), Vindesine (VDS), Etoposide (VP-16), Mitoxantrone(Mitox), Amsacrine(AMSA), L-asparaginase(ASP), Doxorubicin(Dox), Epirubicn (Epi), 5-Fluorouracil (5-Fu), Fluoro deoxyuridine (Fudr), Aclacinomycin (Acla), Mitomycin C (MMC), Cyciocytidine (CC), Methotrexate(MTX), Dexamethasone(DXM). All drugs were dissoved in Hank's or appropriate solutions and stored at -20℃ at 10 times the desired final concentration. According to the purpose of experiment these drugs were applied at different opportunities.

To evaluate the optimal conditions 10 drugs were used, and the drug concentrations included a 4-fold difference in magnitude. The details were published at previous report.[4]

To test the drug concentrations, which were slowly changed, 15 drugs were used, and the theoretical maximal drug concentration (C_{max})[4,5] as well as a series of 10 doses with reduced 0.1 C_{max} each were included (Table 1). Two days drug exposure and 4 h. MTT incubation were selected in this investigation.

Table 1. Drug and their C_{max} ($\mu g \cdot ml^{-1}$)

drug	Hom	DNR	Ara-c	VCR	VDS	Vp-16	CC	Mitox	AMSA
C_{max}	1.6	16	60	0.8	1.6	40	16	6.0	40
drug	Acla	Asp	Dox	5-Fu	Fudr	MTX	Epi	MMC	DXM
C_{max}	8.0	2.0	64	400	200	200	16	3.2	4

To approach the relationship between the test data and the clinical response 13 drugs and their protocols were examined and the drug were tested at 0.1 C_{max} and 0.2 C_{max}. About 2 d drug exposure and 8 h MTT incubation were selected as reported before.[5]

MTT Assay

The methods as same as the previous work.[4,5] Briefly, leukaemia cells were seeded at 96 well plates in 135 μl per hole, 15 μl of drug stock solution was added. The plates were incubated and reincubated with MTT. When the plate reached the endpoint of the experiment, it was then inverted into a pad of blotting paper to remove the medium. The formazan crystal was dissolved in 100 μl acid-isopropanol and them the OD values were read, the tumor inhibitive rates were calcurated.

RESULTS

The onset of investigations was to extablish the does-response and time-response cures. The main results was published previously.[4] The initial data were reported here (Table 2). According to the standard error, the disparity of tumor inhibitive rate (IR), which was beneath the 5%, was arbitrarily defined eguiralance with each other. In order to observe easily, a under line was marked under the group of equal efficacy of drug series. When the concentraction of Hom, Ara-c, MTX changed 50- or 100-folds, there was no different about IR. Also, a double vertical line was marked at the right of data, where the IR was no significant changes as the drug expouse time increase from 2 d to 5 d. So there was only in a certain extent the linear relationship was existent.

To establish whether there was linear

relationship, when the drug concentrations were changed slowly range from 1.0 C_{max} to 0.1 C_{max}, the further experimental data were shown in Table 3.

The same observational method was used. A underline was marked under the group of equal efficacy of drug series. A vertical line was marked, where the IR was changed larger than 5% between the two drug points, which expressed a certain extent of slope was existent.

To Hom and Ara - C in full series IR were high and almost stable. To DNR, VP-16, Mitox and AMSA IR were high and at the lower end have some decrease. To VCR, VDS, CC, ACla, 5-Fu, Fudr and MTX, IRs were low and have stable districts in some regions. To Dox, at the middle IR was stable and at the both high and low ends the IR has some changes.

The relationship between the MTT assay and the clinical response was well as expected.[5] On the basis of observation and understanding, the ideal condition was selected, i. e. about 2 d drug exposure and 8 h MTT incubation at 0.1 C_{max} and 0.2 C_{max}. The true - positive rate, true - negative rate, predictive accuracy, sensitivity, specificity were 71.4%, 50%, 66.7%, 83.3% and 33.3% respectively.

DISCUSSION

For many years, how shall we select correct drug concentrations and drug exposure time studing *in vitro* drug sensitivity of patient samples, is a difficult problem. Previous technics, like human tumor stem cell clonogenic assays, the differential staining cytoxicity (DiSC) assay, nucleic acid precursor incorporation, their advanteges and disadvanteges were evaluated throughly;[6-9] however, we have still lack the knowledge about how the processes and the patterns of antineoplastic agents killing the tumor cells. Since MTT assay has involved into the test of chemosensitivity of cell lines and clinical leukaemia samples, the different designs such as the dose - response curves or drug cut - off points were prefered according to the users;[5] therefore, a standard MTT assay, which would be presentable, shoule be deliberated. We proposed our design before and here review and summarize the data detailedly so that it would be enrich experience of the tumor inhibitive processes, and then have a full look.

In the seventies a concentration - time pro - ducts (C × T) was considered an important pharmacodynamic parameter, just like the area under the dose response curve (AUC), and a hypothesis was proposed, which think that C × T is a special effective parameter of drug exposure, when the chemosensitivity was compared to a poorly differentiated carcinoma of human pancreas (H × 32) mice model *in vivo* and the tumor clonigenic assay *in vitro*.[10,11] In spite of the limitation because of speial tumor cell treatment, the clinical trials were performed in some myeloma and ovarian carcinoma patents ten years ago.[11,12]

However, as the time go on, the concentration or accumulation of drugs in tumor cells was noticed,[1-3] and the accumulation can be deduced as the basis of the cytotoxicity. Since the intrinsic tumor cell specificity, such as indentical histo-pathologic type, the cycle of cellular metabolism and the cellular damage and repair; also since many unclear mechanisms, such as the network and its buffer effect, it would be disposed to think that drugs have superior limit and inferior limit, between them they having same effects. Review of the past data, on the dose- or time-curvers some flat districts were existed not unusual.[13-15] Commbining our data they documented four different conditions: 1. The change of the time (Δ T) closely releted with drug efficacy; during the duration the change of drug concentration (Δ C) at certain extent has almost no influence. 2. The Δ C closely related with the efficacy; the Δ T has no influence. 3. The Δ C and Δ T effect the results together. 4. The Δ C and Δ T effect not the result.

In this report, to show the tumor inhibitive grade imaginatively, underline and vertical line were used in Table 2 and Table 3. Thus, the flat as well as the districts with different sloples was stressed. Therefore, it conducted a new view: The process of

Table 2. Inhibitive rate of 10 drugs on K562 cells after 1, 2, 5 d drug exposure

(drug concentration in a five or six series, each has the difference of fivefold to tenfold)

Drug	C	R	d*	Series					
	C			50	10	1	0.1	0.01	0.001
Hom	R	1	d	74	74	72	66	28	9
		2	d	98	98	98	84	36	16
		5	d	93	94	89	64	43	8
	C			10	5	1	0.1	0.01	0.001
DNR	R	1	d	71	51	56	19	15	12
		2	d	100	95	87	38	27	25
		5	d	96	96	86	38	22	11
	C			500	100	50	5	0.5	0.05
Ara - C	R	1	d	25	27	23	23	22	9
		2	d	53	57	61	49	43	
		5	d	90	91	93	80	52	26
	C			10	1	0.1	0.01	0.001	
VCR	R	1	d	38	36	33	13	5	
		2	d	55	53	37	6	6	
		5	d	95	91	63	18	1	
	C			10	5	1	0.1	0.01	0.001
Dox	R	1	d	55	52	29	23	18	11
		2	d	68	64	45	24	19	21
		5	d	96	95	79	31	12	
	C			50	10	2	0.2	0.02	0.002
Epi	R	1	d		35	31	8	0	0
		2	d	62	62	63	17	14	2
		5	d		98	97	49	8	0
	C			500	100	50	5	0.5	
5 - Fu	R	1	d	39	30	26	15	5	
		2	d	59	40	30	19	9	
		5	d	97	90	79	28	9	
	C			5000	500	100	50	5	0.5
Fudr	R	1	d	53	48	41	39	27	23
		2	d	74	74	73	68	14	
		5	d	99	74	72	67	16	12
	C			50	10	2	0.2	0.02	0.002
MMC	R	1	d	68	45	26	24	23	23
		2	d	92	85	55	29	15	8
		5	d	100	100	77	39	9	
	C			100	50	5	0.5	0.05	0.005
MTX	R	1	d	47	47	45	46	41	20
		2	d	58	57	57	57	51	13
		5	d	60	60	59	62	59	12

C: drug concentration $\mu g \cdot ml^{-1}$; R: inhibitive rate %; d: drug exposure day

efficacy of antineoplastic agents has a multiple function with flat district. The new view, richer than C × T hypothesis, is that it presents a wide range of tumor cell actual conditions and concretizes the dependence of the drug efficacy upon time and concentration.

Table 3. Inhibitive rate of 15 drugs on K562 cells after 2 d drug exposure (drug range from 1.0 C_{max} to 0.1 C_{max})

Drug	Inhibitive rate									
	C_{max} 1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Hom	73.2	72.8	68.1	71.1	68.1	71.1	69.5	70.6	66.4	69.0
DNR	100	99.8	99.3	93.5	90.1	83.3	79.1	74.7	74.4	73.1
Ara-c	62.7	61.0	61.1	60.8	58.2	58.6	56.5	57.2	55.8	55.2
VCR	42.7	38.8	35.0	33.2	32.6	32.3	29.5	25.6	26.4	25.3
VDS	44.0	40.7	37.8	36.3	34.5	34.3	30.0	29.6	28.9	29.4
VP-16	99.9	98.9	95.1	91.4	88.3	82.0	76.0	69.4	48.9	40.8
CC	53.3	54.7	53.3	51.1	48.9	46.4	43.6	40.9	38.0	26.5
Mitox	78.9	77.3	71.7	67.2	62.4	57.7	39.2	31.1	28.3	18.5
AMSA	96.4	95.5	99.5	97.3	93.4	94.7	95.1	91.7	84.3	43.9
Acla	55.9	51.3	50.8	48.1	47.2	46.4	41.8	44.8	40.0	31.8
Asp*	13.6	13.2	14.7	11.3	12.6	12.4	13.8	14.8	8.4	6.2
Dox	76.0	64.0	58.4	58.1	61.2	61.2	60.2	56.0	49.5	44.9
5-Fu	42.5	35.3	31.8	30.9	33.7	35.1	32.9	35.4	30.8	28.7
Fudr	44.1	32.9	33.9	30.9	33.7	35.1	32.9	35.4	30.8	28.7
MTX	42.1	36.4	31.3	29.7	32.1	30.2	30.5	31.4	33.1	32.3

* ASP should be activited *in vivo*, so it has low effect *in vitro*.

A rational principle of detecting effective drug's method should generally make most clinical effective drugs showing higher tumor inhibitive ability than uneffectives. According to K562 cell line experiance as well as guiding by the new view, the 0.1 C_{max} and 0.2 C_{max} 2 d exposure and 8 h MTT incubation were adopted with clinical leukaemia samples and got promising results.

Considered as a whole, it could be concluded that drugs go into effect with those which have to do with the tumor cell cycles and damage - repair mechenisms, the balance between the injury and anti-injury of the tumor cells, which couldn't be counted geometrically, so that the process of drug efficacy has a multiple function with flat district.

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