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DEVELOPMENT OF KNOWLEDGE BASED THERAPY FOR CANCER IDENTIFICATION OF UNIQUE TARGETS

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ABSTRACT

Though chemotherapy has played and will continue to play key role in the treatment of cancer, it surfers from lack of selectivity. This stems from the fact that the concept of cancer chemotherapy was evolved in an era when knowledge of cancer was rudimentary. Now with the advances based as cellular and molecular biology of cancer our knowledge base has expanded enormously.
Keywords: chemotherapy; anticancer agents

INTRODUCTION

Therefore novel therapeutic agents directed against selective targets specific to different cancers are being developed. These agents when developed expected to treat cancer with minimum toxicity to normal proliferating cells. Such molecular designing will give the anticancer drugs of future which will be directed against a unique target in the cancer cell and bringing about their demise while having no action against normal cellular proliferation. In view of this we have screened several new carbazole and carboline derivatives synthesized by new our synthetic strategies. A number of systems are used to screen anticancer drugs which are mostly selected as the basis of their antiproliferative activity. Two commonly used systems are:

(1) transplantable tumor models and
(2) Cell lines established from a variety of human tumors.

Two transplantable tumors L 1210 & P 388, both derived from muose lyimphocytic leukemia, have 100% growth fraction and the tumor implanted animal dies when the tumor burden reaches 109 cells. It is possible to predict the time of death of the animal implanted with known number of L 1210 cells. P 388 behaves similarly. A drug with growth inhibitory activity would retard the growth of the tumor and prolong the life span of the animal. Drugs that prolong life by more than 20% are taken for the next phase of the study which generally involves similar testing as a number of other transplantable tumors.

Animal models are costly and somewhat unmanageable when a large number of drugs are to be screened. National cancer institute (NCI). Bethesda, USA has therefore developed a new in vitro system in which anti-proliferative activity is tested in cell lines growth in culture. A panel of sane 60 cell lines, derived from a variety of human cells (Breast, Lung, CNS, Colas, Kidney, Ovary, Melanomae etc) are used. The panel also contains cell lines exhibiting multi drug resistance.

EXPERIMENTAL

Materials and Reagents:

1. Cell line: P 388 Lenkemia (mouse lymphocytic Lenkemia) This cell line has been shown to be sensitive and superior to other leakemic lines.

2. B D F_2 mice (either sex). Body weight : 18- 21gm for male 17-20 gm for female.
3. Drugs to be tested are solubilized in D.W or in a suitable vehicle like DM 50 (10% V / V). Ethanol, Acetone.

Method:

1. Inject i.p 1 x 10^6 cells 10.2 ml P B S / mouse under aseptic conditions on D.O.
2. Randomize the tumor implanted animals. Note weight of the animals.
3. Determine the tonic dose as described below and use drug only at sub toxic level.
4. 24 hr. later (d1) inject i.p. The drug at different doses. Maximum Volume permitted is 0.1ml/10g body weight controls receive vehicle alone. (Dose determination of the drug is described at the end of this protocol).
5. Continue treatment with drug daily up to nine days. (1-9 schedule).
6. Weight animal an D5.
7. Record death, both in castrol & drug treated sets daily.
8. Calculate anti-cancer activity by increase in survival time of drug treated animals as compared to untreated controls using the formula.
9. Tumour inhibitory activity is measured using the following equation.

Mean Survival time (M.S.T) is calculated as

$$T/C\ \% = \frac{M\,S\,T\ of\ Treated\ animals\ (T)}{M\,S\,T\ of\ Control\ animals} \quad x\ 100$$

In case of same tumours (Scarcama 180) reduction in the size of the tumour (Tumour weight) is used to determine tumour inhibitory activity.

$$T\ I\ A\% = \frac{Average\ tumour\ weight\ of\ Treated\ x\ 100\ (T)}{Average\ \ tumour\ weight\ of\ \ control\ (C)}$$

PROTOCOL FOR TUMOUR – GROWTH INHIBITORY STUDY

Tumour Model	**Host strain**	**Inocculmn**	**Route (Drug administration)**	**Treatment schedule**	**Para meter**	**Activity T/C %**
P 388 Leukemia	BDF1	10^6 cells (i.p)	i.p	1-9	MST	125
L 1210 Leukemia	BDF1	10^6 cells (i.p)	i.p	1-9	MST	125
B 16 Melonoma	BDF1	10^6 cells (i.p)	i.p	1-9	MST	125
B 16 Melonoma	BDF1	4 x 10^6 cel Cells (S.C)	i.p	1-9	MST	140
Lewis Lung Carcinama	BDF1	2 x10^6 cells (i.m)	i.p	1-9	MST	140
Sarcama 180	SWISS	4x 10^6 cells (S.C)	i.p	1-9	Tumour Weight	42

TOXICITY CRITERIA

To evaluate toxicity of the test materials (or) tumour bearing animals of same strain and species are choosen. Conduct the test of described below.

1. Take normal BDF_1 animals and notes their body weights.
2. Give initially 400 mg/kg of the drug in D W or in a suitable vehicle in a single dose i.p.
3. Record body weights on D1 and D5.

4. Note survival daily.
5. The dose of the given considered toxic of

i).Grater then 34% of total animals treated die by d5.

ii) Loss of weight in treated animal as compared to controls is equal to or grater than 4g by d5

Dose determination is based on the above toxicity tests and is represented below

% deaths	Drug dose to be used
0 – 34 by d 5	400 mg/kg Body weight (No. change)
35-15 by d 5	200 mg/kg Body weight (0.50)
76 – 100 by d 55	100 mg / kg Body weight (0.25)
100 by d 1	80 mg/kg Body weight (0.20)

IN VITRO TESTING BY MTT ASSAY

A panel of cell lines has been recommended by NCI for use. In drug screening. Cells are incubated with the test drug and the degree of growth suppression is measured using a colorimetric method that correlates well with the cell number. MTT is commonly used as the subtract for this assay. It gets converted into coloured forazan which is quantitated colorimetrically. Alternatively, $3_{H\,T\,d\,R}$ can be used but the method is combursome. Procedure describes below is for any one cell line. Using microtiter plants drug action can be tested simultaneously in several cell lines.

Materials and Reagents:

1.Cells From any selected cell line(for example K 562) from NCI panel used for drug screening.
2.Microliter Plates.
3.Multi channel pipettes.
4.D M E M (or) R P M I + 10% F C S.
5.3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide(MTT,Sigma) 5 mg/ml.
6.D M S.
7. Trypsin (sigma)
8.Test-drug.

Method:

1. Detach all adherent cells from the culture flasks by adding 2-3 ml 0.25% trypsin / trypsin- EDTA.
2. Inactivate trypsin by addition of 10ml of fresh medium containing 5% FBS.
3. Separate cells into single suspension & count.
4. Determine viability by trypan blue dye exclusion. At least 90-95% cells should be viable.
5. For suspension culture, collect the cells from culture flask .Count and check viability.
6. After counting of cells, adjust the cell suspension to appropriate dilution.
7. Plate cells in 100ml/well of microliter plate at desired densities. (40000-50000/well). In castrol cells, and 100 ml medium with out cells.
8. Incubate the plates at 37℃ in a CO_2 incubator for 24hr. to allow stabilization prior to addition of drugs.
9. All test drugs are initially solubilized in DW or DMSO. Appropriate dilutions are prepared just before use in complete medium. New compounds are tested at 4 dilutions of 10,20,40 and 80mg/ml.
10. All drugs in 10ml vol./well and keep the plates at 37'C in CO_2 incubator for 48hr.
11. Add 10ml of MTT (5mg/ml) solution to each well and incubate further for 4hr.
12. Remove plates. Centrifuged and remove supernatant. Add 100ml DMSO in all cells. Wait for 15min-record OD at 540nm on Eliso plate reader. The colour development correlates well with cell number.
13. Growth inhibition is expressed as-

$$T/C\% = \frac{\text{Mean OD of individual test group} \times 100}{\text{Mean OD of control groups}}$$

N0te:

1. Using the same principle the entire panel of about 60 cells lines can be tested simultaneously
2. The test drugs do not show uniform effect on every cell line. In same cell lines may not show growth inhibition.
3. An active compound is selected for further work on the basis of its overall effect. Its effect is individual cell lines are then compared with those known compounds from the data base.
4. The final decision as whether a compound should go to the next phase of testing is taken on the basis of several parameters for which the investigator is advised to refer NCI protocol.

REFERENCES

1. H. Mitsuya, R.Yarchoan and S. Broder, *Science*, **24**9, 1533 (1990).
2. G. Saxty, S.J. Woodhead, V. Berdini, T.G. Davies, M.L. Verdonk, P.G. Wyatt, R.G. Boyle, D. Barford, R. Downham, M.D. Garrett and R.A. Carr, *Scienc*e, **28**7, 1969 (2000).
3. J.B. Gibbs, *Drug Discovery Today*, **9**, 207 (2004).
4. A.D. Westwell, *Nature Medicine*, **14**, 407 (2008).
5. A.A. Sayed, A.S. Craig, J. Thomas, J. Inglese, C.P. Austin and D.L. Williams, *J.Med.Che*m., **46**, 5579 (2003).
6. J. Kurreck, *Expert Opinion on Biological therapy*, 4, 427 (2004).
7. J.B. Gibbs, *Science*, **287**, 1969 (2000).
8. D.W. Nicholson and N.A. Thornberry, *Science*, **299**, 214 (2003).
9. T. Lawrence, T. Hageman and F. Balk, *Science*, **317**, 51 (2007).

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Scientific views end in awe and mystery, lost at the edge in uncertainty, but they appear to be so deep and so impressive that the theory that it is all arranged as a stage for God to watch man's struggle for good and evil seems inadequate.

-Richard P. Feynman