# THE USE OF INVITRO SCREENING TEST TO PREDICT CHORIOCARCINOMA CELLS RESPONSE TO CYTOTOXIC AGENTS

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## SUMMARY

**The reliability of in vitro screening test to predict choriocarcinoma cell response to chemotherapy was done in 3 cases.**

*Introduction*

The treatment of gestational trophoblastic neoplasm has changed dramatically, from primarily surgical to primarily medical. Cancer chemotherapy remains an empirical science, drugs being selected for patients on the basis of the results of past clinical trials. However, with the explosion in medical knowledge about the correlation between cell kinetics and mechanism of action of anticancer drugs, the value of chemotherapy against malignancy has become more clear. But the most challenging problem, in the field of chemotherapy is the selection of anticancer drug for an individual patient on an individual basis. Salmon *et al* (1980), Holmes (1978) and Dendy (1980) established that variation exists in sensivity between tumours and in tumours even of a similar histopathology.

The present study was undertaken to assess the reliability of *in vitro* screening test to predict choriocarcinoma cells response to chemotherapy on an individual basis.

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*Material and Methods*

Tissue samples were collected from the endometrial cavity (3) and secondary deposit in the suburethral region (1) aseptically in the tissue culture medium M199 from 3 patients of choriocarcinoma. A detailed clinico-pathological study was carried out in all these patients.

Culture medium consisted of synthetic medium M199 (GIBCO, USA), supplemented with 10% foetal bovine serum (Flow Laboratories, U.K.). Antibiotics—penicillin (100 iu/ml) streptomycin (100 ug/ml) were routinely added to the medium.

To set up cell cultures, tumour tissue was minced and treated with trypsin (0.25% in Hanks' balanced salt solution) for about 20 minutes at 37°C to dissociate tumour cells from the tissue. The dissociated tumour cells were separated by centrifugation. The supernatant trypsin was discarded and the pellet was suspended in the culture medium. Equal aliquots of cells were distributed in the 24 wells of

the disposable culture plates (Costar, U.S.A.). The cultures were incubated in the desiccator at 37°C in the humidified gas phase of 5% $CO_2$ in air.

Cultures were treated with drugs 24 hours after explantation. Suitable controls were maintained for every test. Drugs were tested individually as also in 4 different combinations of 2-5 drugs. The dose of the drug(s) also varied accordingly in the combinations. Concentration of the drugs in the culture medium was determined according to the dose of the drug given to the patient at a time. The *in-vitro* concentrations of the drugs are shown in Table I. Drugs used for choriocarcinoma (*in vitro*) were as follows:

TABLE I
*Drugs and Treir Invitro Dose*

| Drugs | Doses invitro |
|---|---|
| Methotrexate | 20 ugm/ml |
| Actinomycin-D | 0.1 ugm/ml |
| Adriamycin | 5.0 ugm/ml |
| Vincristine | 0.2 ugm/ml |
| Cyclophosphamide | 0.1 mg/ml |

1. Methotrexate
2. Actinomycin-D
3. Methotrexate + Actinomycin D
4. Methotrexate + Actinomycin = D + Vincristine + Adriamycin + Cyclophosphamide.

*Results*

*Explantability of choriocarcinoma cells*

Since the cultures were maintained for about 60 hours only, growth of the cells was not expected during this period. A short term primary culture was the requirement for the study. This ability of tumour cells to form a primary culture is termed as explantability of the tumour in this study. Tissues explantation was done on 4 occasions from 3 cases of choriocarcinoma. Explant was successful on 2 occasions (50 per cent). However, the other 2 cases did not show explantability. The reason was that, in one case choriocarcinoma cells could not be detected in the explant and in endometrial curettings on histopathology. In the second case tissue culture as well as blood culture grew *E. coli* organism and patient was having high grade fever.

*Clinical profile of the three cases*

*Patient No.* 1

A 26 year old woman, P 3 + 0 presented with amenorrhoea, lump in the lower abdomen and pain in the lower abdomen. She had no history of antecedent molar pregnancy or abortion. She was diagnosed as a case of Nephrotic Syndrome 3 years ago. The general and systemic examination revealed that she was pale, BP 180/120, pedal odema ++, Hepatomegaly of 4 cms below the costal margin and uterus was enlarged to 20 weeks.A provisional diagnosis of fibroid uterus with pregnancy was made. Patient was taken for surgery to perform hysterotomy and tubal ligation. At laparotomy a minimal amount of haemorrhagic fluid was detected in the peritoneal cavity. Uterus was soft, congested (purplish in colour) and enlarged to 20 weeks size of pregnancy. On opening the uterus there was a neorotic, purplish colour growth involving 2/3rd of the posterior wall of the uterus. The growth had involved the whole thickness of the myometrium except the serosa. Both the ovaries were normal. Intestine, omentum, liver and rest of the pelvic organs were normal. Total hysterectomy was done. X-ray chest and liver Scan done postoperatively were normal. Serum HCG was 3000 miu/ml.

Histopathology of the tumour was choriocarcinoma. *In vitro* tissue culture and drug sensitivity to various drugs was performed from the tumour specimen taken from the posterior wall of the uterus. The predictive test revealed that most effective drug (*in vitro*) was Methotrexate. Patient was given this drug selected by the predictive test and patient went into complete remission. But she developed burst abdomen on the 7th postoperative day (Methotrexate toxicity). Patient was in complete remission for 32 weeks.

*Patient No.* 2

A 35 year old P 0+5 presented with irregular vaginal bleeding following a history of five repeated molar pregnancies. Vacuum aspiration was done, but curettings were negative for choriocarcinoma cells. Serum HCG level was 2700 miu/ml. X-Ray chest and liver scan were Normal. *In vitro* culture was unsuccessful.

*Patient No.* 3

A 52 year old woman presented with high fever, irregular bleeding and foul smelling discharge per vaginum following a spontaneous abortion. She had a growth $1 \times 1\frac{1}{2}$ cm. in the suburethral region on the anterior wall of the vagina. It was purplish in colour with areas of necrosis and it bled on touch. Biopsy of the growth revealed the diagnosis of choriocarcinoma on histopathology. X-Ray chest showed canon ball type of secondaries in the lung. *In vitro* culture and blood culture grew *E. Coli* type of organism, and thus the tumour tissue could not be explanted due to *E. Coli* injection.

*Discussion*

Various culture methods are being currently used by Oncologists for *in vitro* evaluation of anticancer drugs. Hamburger *et al* (1978) developed a soft agar gel method utilizing clonogenic ability of malignant cells. However, this assay takes 2-3 weeks to assess the drug effect. But the objective of our study was to make available invitro test results as quickly as possible for the benefit of the patients. We, therefore, tried to make the test simple, quick and straight forward. In view of this, the standard tissue culture method with morphological criteria of cells survival was adopted.

The success of growing the tumour is variable. It depends upon the type of the tumour and the source of the tumour specimen. Wilson and Neal (1980) obtained 52.0 per cent success rate with solid specimens. The reported incidence of success with the invitro tissue culture varies from 50 to 70 per cent with solid tumour specimen—Table III.

To date choriocarcinoma cells have not been cultured to predict tumour response to chemotherapy. In the present study invitro culture of choriocarcinoma cells was successful on two occasions (2/4—success rate 50 per cent). The result of the present study corrabrates with that of other workers (Table III).

One of the most important application of this invitro culture, is its use in attempting to predict the chemosensivity of an individual patients tumour. Invitro drug sensivity could be performed successfully only on two occasions. Patient was treated with the drug that was found most effective in the invitro test. Patient went into complete remission and was in complete remission for 32 weeks. (Table II). Therefore, the invitro drug sensivity correlated with the clinical response (invivo). Thus the predictive accuracy of the test .was good (2/2-100 per cent). The predictive accuracy reported by vari-

TABLE II
*Review Incilence of Successful Cultures*

| Author | Year | Successful Culture | Method |
|---|---|---|---|
| Wseeler et al | 1974 | 70.0 | — |
| Berry et al | 1975 | 50.0 | Monolayer |
| Wilson et al | 1981 | 52.0 | Monolayer |
| Present study | 1982 | 50.0 | Monolayer |

ous workers varies from 64-80 per cent (Table IV).

TABLE III
*Predictive Accuracy*

| Author | Year | Predictive Accuracy Sensivity |
|---|---|---|
| Epstein et al | 1980 | 64.0 |
| Alberts et al | 1981 | 73.0 |
| Wilson et al | 1981 | 80.0 |
| Present Study | 1982 | 100.0* |

* Number is small (2 explants).

Cancer patients needing the help of chemotherapy should be treated on a scientific basis by selecting drug (s) by the invitro screening test, instead of putting the patient on "blind" drug schedule to which the patient may not respond. With the use of this test, one can select and give the most effective drug and thus improve the prognosis of the patient by avoiding the use of resistant, non effective and toxic drugs.

The *invitro* culture of human tumour represents a powerful tool for clinical and biological investigations. It helps in choosing an appropriate drug for the treatment of an individual patients tumour, to detect primary and secondary drug resistance, and select the next best drug once the tumour had become resistant to a particular drug and screen new anticancer drugs (s) that would be most effective and least toxic.

This developing discipline of medical science needs further work on a larger scale.

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