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Tumor Animal Models Used for Evaluating the Antineoplastic Activity of Platinum Coordination Complexes*

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Abstract

The number of organometallic platinum complexes synthesized and tested for antitumor activity, after the discovery of the antineoplastic properties of *cis*-dichlorodiammineplatinum(II) (cisplatin), is very high, indicating the interest of numerous scientists in this field. Many experimental models of transplantable animal tumors have been employed for estimating the antitumor potency of these derivatives. Rodent tumors growing in ascitic form, such as Ehrlich ascites carcinoma, sarcoma 180, L1210 lymphoid leukemia and P388 lymphocytic leukemias were widely employed and their use has characterized the first 10 years of research. Solid tumors were used as well. The interest was first addressed to the solid forms of s.c.-implanted Ehrlich ascites carcinoma and of sarcoma 180, and particularly to the s.c.-growing ADJPC6 Plasmocytoma on which many analogs have been tested. B16 melanoma, Lewis lung carcinoma, and specialized forms of implant of human and animal xenografts, in the nude mouse and in the subrenal capsule of the mouse respectively, have elicited the interest of scientists in recent years. In addition, several other transplantable animal tumors have been used: Yoshida sarcoma, Walker 256-carcinosarcoma, VX2 carcinoma, mouse fibrosarcomas. Leukemias such as Dunning ascitic leukemia, Pausher leukemia, MPOC 104E plasmocytoma, and myeloid and lymphatic leukemias of the rat have been used during the last 15 years, giving a clear indication of the broad spectrum of the antineoplastic activity of cisplatin. It is worth noting that, in many instances, more than one single tumor model has been used, although L1210 lymphoid leukemia seems to be the tumor line which has received the most interest, being present alone or with other tumors in almost all of the scientific works from the end of the Sixties. Detailed examination of the tumor models employed in the research for cisplatin analogs shows that, although some old tumors such as Ehrlich ascites carcinoma are still used for studying drug–cell interactions, an even greater interest is devoted by researchers towards an improvement in the selection of experimental tumors endowed with more similarities with those commonly encountered in humans, which are thus more predictive of activity in the human patient.

Introduction

To the pharmacologist involved in studies on antitumor drugs, the animal model of neoplastic disease represents the way to evaluate unproven therapies or new drugs without risk to human patients. The animal tumor model thus represents the means of recognizing new drugs of potential value in the treatment of human malignancies, either as better tolerated substitutes of already known drugs or to be introduced into clinical use as new means for treating cancers where traditional modalities of treatment fail.

Considering that the efficacy of newly synthesized drugs cannot be tested directly on humans, preclinal models are of indisputable value, although many limitations occur because of the differences between animals and humans. Despite the many questions about this problem, the need for improved preclinical models has been the object of research in the last two decades. The result is that the attributes of validity, selectivity and predictability are now even more frequently associated with the *in vivo* test system used in preclinical studies, and the risk of discovering false-positive drugs is miminized by a better predictability of the model(s) employed [1]. On the other hand, the contribution of experimental studies on laboratory animals, despite the many important behavioral characteristics shared by animal and human tumors, without doubt gave important suggestions about the 'curability' of human neoplasms.

Screening Strategy: the Choice of the Tumor Model

Although none of the *in vivo* animal tumor system tests, not even xenografts, are perfect predictors of

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TABLE I. Tumor Models[a]

Models with high predictivity of action on solid tumors
Carcinomas (Lewis lung carcinoma, RL-67, 173, 491 and AKATOL); sarcomas (myosarcoma ISM, Ridgway's osteogenic sarcoma, sarcoma 37); melanoma B16
Models with good analogy with tumors of lymphoreticular type
Lymphoid L1210 leukemia; lymphocytic P388 leukemia
Models with any direct specificity for human tumors
Adenocarcinoma 755; Walker 256 carcinosarcoma; Yoshida ascites sarcoma; Ehrlich ascites carcinoma; Plasmocytoma MOPC406 and Plasmocytoma ADJ/PC6

[a]Obtained and modified from ref. 6.

TABLE II. Evolution of National Cancer Institute Screening Program[a]

1955	Sarcoma 180, carcinoma 755 and lymphoid L1210 leukemia
1960	Lymphoid L1210 leukemia and two further models chosen in a panel of 21 tumors
1965	Lymphoid L1210 leukemia and Walker 256 carcinosarcoma
1968	Lymphoid L1210 leukemia for synthetics and lymphocytic P388 leukemia for natural products
1972	Lymphoid L1210 leukemia for synthetics, lymphocytic P388 leukemia for natural products and B16 melanoma and Lewis lung carcinoma for further special tests
1975	Lymphocytic P388 leukemia for initial screen plus a panel of 8 models, including human xenografts for further testing of compounds active on P388

[a]Obtained from ref. 2.

drug response in human disease, the results of a retrospective study on the predictability of the tumor models can be summarized as in Table I.

Experimental tumors are thus necessary to discover new drugs and represent the starting point for clinical evaluation. Their role in the development of antitumor drugs has often been submitted to criticisms because of the unproven assumption that a correlation exists between activity in experimental tumors and therapeutic effectiveness against human cancer. This made necessary the evolution of a preclinical tumor model more reliable than those presently available; an example is given by the evolution made at the screening program of the National Cancer Institute (U.S.A.) (NCI) (Table II).

The strategy used for the discovery of potential clinically effective drugs by means of transplantable tumors in mice is thus part of a continuous evolution tending to improve the 'quality' of the tumors involved [2, 3]. The main result of this is the elimination of L1210 as determinant screen for positivity: this is the result of the observation that a leukemia cannot be taken as the dependable predictive model for human malignancies where many drugs, after preclinical studies, are then introduced into the clinical treatment of solid neoplasms or of their metastases. Nevertheless, the last proposed strategy can be thought definitive, and the so-called 'two-stage strategy' actually used since 1975 is now submitted to criticism and a perfected strategy is proposed in Table III.

TABLE III. Perfected Strategy[a]

1st stage prescreen – P388
$T/C < 125\%$ (drop), $T/C \geq 125\%$ (continue)
↓
2nd stage screens – B16, L1210, MX-1
negative in all screens (drop), positive in at least one screen (continue)
↓
3rd stage screens – Co38, LL, CX-1
negative in all screens (low priority), positive in at least one screen (high priority for Phase I and II clinical trials)

[a]Abbreviations: P388 = Lymphocytic P388 leukemia; B16 = B16 melanoma; L1210 = lymphoid L1210 leukemia; MX-1 = infiltrating duct cell carcinoma (human breast xenograft in the nude mouse); Co30 = colon adenocarcinoma; LL = Lewis lung carcinoma; LX-1 = carcinoma (human lung xenograft in the nude mouse). Modified from ref. 2.

The search for antitumor platinum analogs falls into all the considerations presented above [4]. This research, since the discovery of the antitumor properties of *cis*-dichlorodiammineplatinum(II) (cisplatin), has considered more than 1500 compounds which were synthesized and tested in experimental models according to the strategy of the current time. The number of experimental neoplasms employed in this research is rather large and, besides those which constitute the panel of NCI, many other tumors in ascitic or solid form were utilized.

Sarcoma 180, Adenocarcinoma 755, Ehrlich Ascites Carcinoma

Historically, these tumors have been widely used in many laboratories in the preliminary evaluation of the antitumor activity of many substances of either synthetic or natural origin, and of cisplatin and its analogs as well. Sarcoma 180 and adenocarcinoma 755 represent two models of tumors growing in a solid form, established in mice by weekly passages of tumor fragments in the right axillary region of non-inbred (sarcoma 180) and syngenic BD2F1 recipients (adenocarcinoma 755). Ehrlich ascites carcinoma, normally transplanted i.p. in non-inbred mice in ascitic form, can also be grown as a solid tumor, although this latter form, among the experimental tumors which are the product of laboratory manipulations, can be considered the most artificial. Tumor cell sensitivity to cytotoxic action and mainly the reduction of tumor mass (growth) after treatment have been the parameters of drug evaluation employed with these tumors [5].

Lymphoid L1210 Leukemia and Related Leukemias

Lymphoid L1210 leukemia initially belonged to the same panel of tumors previously described, differing from them mainly because of its characteristics of malignant tumor with elevated similarities with acute human tumors of lymphoreticular type [6]. Furthermore (unlike sarcoma 180, adenocarcinoma 755 and Ehrlich ascites carcinoma, whose use has been discontinued), this tumor, 'born' in 1948 in spleen and lymph nodes of mice painted on the skin with 3-methylcholanthrene, is still present and used in many laboratories. An important basis for the choice of this system was its reproducibility; untreated BD2F1 or CD2F1 mice inoculated i.p. with 10^5 tumor cells regularly die between day 8–10 from tumor inoculation. Additionally, positive controls (highly effective agents) have also regularly demonstrated good reproducibility of the T/C ratio (%):

$$\frac{T}{C} = \frac{\text{mean survival time of treated animals}}{\text{mean survival time of control animals}} \times 100$$

The protocol used for this system, for screening chemical agents, specifies at least 5–6 BD2F1 or CD2F1 animals (DBA/2 for propagation) per test-dose, inoculated i.p. with 0.1 ml of diluted ascitic fluid containing 10^5 leukemic cells; a T/C value equal to 125 is the minimum required for a compound to be 'presumed active' [7]. Being the principal animal model employed for many years, L1210 leukemia was used to study in detail the kinetics associated with curability of experimental leukemias, giving important results on the: lethality of a small number of leukemic cells; dynamics of the proliferation of leukemic cells *in vivo*; percentages of various-sized leukemic cell populations killed by given dosages and schedules of therapeutic agents, estimated by either statistical or biological methods; kinetic mechanisms by which chemotherapeutically induced increase in life-span can be achieved; importance of leukemic cell population, drug level and treatment schedule to obtain 'cures'; involvement of the cerebral district.

The dynamics of L1210 cell proliferation concern a two-day lag phase after transplantation followed by a period of log phase proliferation with a generation time of approximately 0.55 days, which continues until death (total host's leukemic cell population of about one billion cells); brain involvement appears when the host's peripheral leukemic population is about 10^6 cells [8].

Besides L1210 leukemia, other tumors of 'leukemic-type' may be used, the kinetic characteristics of which being not much different from those of L1210 leukemia. Of particular interest is the lymphocytic P388 leukemia which, in the last 10 years, has substituted L1210 in the prescreen in the NCI program [2]. This tumor, also induced by skin painting with 3-methylcholanthrene, appeared in 1955 in a DBA/2 mouse. The weekly propagation and testing are made by i.p. transplantation of 0.1 ml of diluted ascitic fluid containing 10^6 tumor cells in the same animals used for L1210 leukemia [9]. Its higher sensitivity, in comparison to L1210 leukemia, reduces the risk of exclusion of potentially active agents in prescreen evaluations.

Among other ascitic tumors of 'leukemic-type' MCVD-12 leukemia (Rauscher leukemia, virus-induced) grown i.p. in BALB/c mice and the MOPC 104E myeloma implanted i.v. in the same strain of animals should be mentioned. Furthermore, rat leukemias, such as Dunning ascitic leukemia, i.p.-implanted in Fischer 344 rats (5×10^6 or more cells/animal), RBA-Le (acute myelogenous leukemia), i.v.-inoculated (10^4 cells/animal obtained from spleens of rats similarly inoculated 10–15 days before) in Sprague-Dawley rats of both sexes, or leukemia L5222 of BD IX rats implanted i.p. (10^6 to 5×10^6 cells/animal) were also used in some instances to either characterize the spectrum of activity of cisplatin or to study the antitumor potential of newer platinum analogs (Table IV).

TABLE IV. Further Tumor Lines Used *in vivo* in Pre-clinical Screens[a]

Denomination	Recipient and day of transplantation
Carcinoma, adrenal cortex	Syrian hamster, 10–12
ADJ-PC-22 plasma cell	BALB/c mice, 27
Spontaneous AKR lymphoma	AKR mice
AKR lymphoma (transplanted)	AKR mice, when spleen is 600 mg
ADJ-PC-20 plasma cell	BALB/c mice, 21
ADJ-PC-5 plasma cell	BALB/c mice, 21
ADJ-PC-6plasma cell	BALB/c mice, three weeks
VX2 carcinoma	New Zealand white rabbits
FSa fibrosarcoma	C3Hf/Kam mice, when 10–12 mm width

[a]Data modified from refs. 27 and 28.

Lewis Lung Carcinoma

It is widely accepted that, for an improvement of the cure of cancer patients with solid malignancies, a better understanding of the metastatic process and treatment is needed. The Lewis lung carcinoma model offers unique possibilities to study these aspects of tumor disease: it grows slowly after s.c. and i.m. implantation, giving rise to a solid mass which regularly and spontaneously produces metastases to the lung [10]. Since its characterization, arisen spontaneously in 1951 as carcinoma of the lung in a C57BL/6 mouse, it has been accepted in many laboratories and can be considered to be the best known model of solid tumor, relevant for drug-activity comparisons between different laboratories. Similarly to human neoplasms, it has a poor responsiveness to chemotherapeutic treatment with many agents of already established clinical use [11]. Indeed, as a metastasis model, Lewis lung carcinoma is a good tool to differentiate between specific effects of drugs on metastases as opposed to effects on primary tumor growth [10]. The tumor is *in vivo* maintained by serial passages, every two weeks, of 10^6 single viable tumor cells (cell suspension prepared by disaggregation of the primary tumor mass) i.m.-implanted in the calf of the hind leg or of tumor fragments implanted with a trocar in the axillary region of C57BL/6 mice; BD2F1 hybrids are usually employed for propagation in test groups [12]. Knowledge of the growth-kinetics of this tumor has to be attributed to Simpson-Herren *et al.* [13]:

(a) the proportion of proliferating tumor cells decreases with tumor age for both primary and spontaneously formed lung metastases;

(b) at advanced stages of tumor growth, cell-cycle time was shorter for spontaneous metastases (14–16 h) as compared with primary s.c. tumor (24 h);

(c) volume-doubling time was longer for the primary tumor (8–10 days) than for spontaneous metastases (2 days).

Lung metastases with Lewis lung carcinoma can also be obtained by i.v. implantation of tumor cells (artificial metastases). The kinetic characteristics of the resulting lung nodules are almost similar to those of nodules spontaneously formed from a primary neoplasm, except for the volume-doubling time which is similar to that of the primary lesion. The tests made to ascertain the responsiveness of this model to chemotherapeutic drugs suggest that i.v. Lewis lung appears to be a useful solid tumor model for the evaluation of potential antitumor agents [14].

Lewis lung carcinoma has served more recently to develop two further models of spontaneous liver metastases in mice: the intrasplenic implant [15] and the caecum tumor model [16]. The appearance of liver metastases after intrasplenic implantation of Lewis lung carcinoma cells appears to be spontaneous and the model provides a useful tool to study different aspects of liver metastases. Liver metastases are spontaneously generated also after implantation of Lewis lung carcinoma in the caecum of syngenic mice. The most interesting property of this model is that, after resection of the primary tumor (14 days after implantation), residual micrometastases in the liver (mainly) and transperitoneally and in the lungs provide the opportunity to test drug-sensitivity in a way closed to the human situation [14].

Melanotic melanoma B16 also represents a good substrate for the study of malignant tumor metastasis. This tumor, arisen spontaneously in 1954 on the skin at the base of the ear in a C57BL/6 mouse, received particular attention because of its similarities to human malignant melanoma. The transplant and propagation for tests are the same as for Lewis lung carcinoma [17] and produce spontaneous and, after i.v. inoculation, artificial metastases with high regularity. More recently, a model of melanoma implanted intracutaneously in DBA/2 syngenic mice has been proposed which better represents the human malignant melanoma growth. This model is a rapid and efficient system to study the dynamics of melanoma growth and the effects of treatment of either systemically or topically applied antitumor drugs on melanoma proliferation [18].

Specialized Models for Preclinical Tumor Drug-Sensitivity

The models which will be mentioned and discussed in this paragraph mainly represent the results of efforts made to develop models for predictivity of drug action on human cancers of low cost, high efficiency and rapid execution, to be easily applied in clinical situations. The concept of individualized cancer chemotherapy is attractive and allows one to select the best treatment regimen(s) with little or no toxicity for the patient. *In vivo* xenograft models offer several advantages over *in vitro* human colony-forming assays [19]:

(a) tumor cell morphology is preserved;

(b) pharmacokinetic characteristics of host metabolisms and host response to neoplastic tissue operate and toxicity for normal tissue can be evaluated;

(c) the relationship between tumor cell and stroma factors, vascularization and angiogenesis and other paraneoplastic phenomena are not excluded;

(d) human-derived tumor lines can be passaged and maintained by transplant in immunosuppressed animals.

These approaches depend upon the ease with which various human tumors will grow and maintain their identity in immunologically subnormal animals. The use of congenitally athymic nude mice, deficient in their ability to reject allograft or xenograft of tumor tissues have been successfully used to grow a large variety of human tumors although with percentages of takes lower than 100% [20]. Indeed, in the nude mouse some immunological functions are fully represented, among which natural resistance can be responsible for interactions with tumor growth, thus interfering with drug-activity testing. This consideration has prompted the search for privileged sites where natural resistance could be limited, suggesting that i.c. tumor implants in the nude mouse appear to be suitable for human tumor cell growth [21]. Satisfactory growth of human tumor xenografts can be achieved also by using immune-deprived mice [22] or mice immunosuppressed by pharmacological treatment [23].

Bodgen *et al.* have, more recently, introduced the subrenal capsule assay (SRCA) for human heterotransplantation [24]. This model is based on *in situ* measurement of small changes with time in the size of biopsy specimens from tumor transplanted under the renal capsule of immunodeficient mice; this has more recently been extended with some success to normal animals [25], with results obtained within 6–10 days. Morphological examination of tumors growing in this model indicates that tissue structure of the specimen and surface microvilli, mucin and CEA production are preserved. Indeed, detailed examination of this model, still stressing its relevance, indicates that precautions have to be observed to achieve successful results [26]. Mainly: (i) in the graft malignant tumor and necrotic tissue must be separated; (ii) work in a strictly aseptic way; (iii) avoid introduction of contaminants which can give rise to inflammatory processes and epitheliocellular granulomas; and (iv) compare microscopic evaluations with macroscopic measurements of tumor dimensions.

With these precautions, the SRCA furnishes interesting data and can be better defined as an *in vivo* organ culture with self-replenishing medium, retaining fully operating drug activation and detoxification mechanisms.

Conclusions

The use of transplantable tumors for studying the antitumor properties of chemotherapeutic agents has led to the persuasive conclusion that chemotherapy can be frequently curative against low tumor burdens. Nevertheless, evidence from clinical experience contradicts this encouraging view of experimental chemotherapists. Although murine tumors and human cancer share similar behavioral characteristics, such as metastasization, re-arrangement of tumor heterogeneity and response to a given treatment, it must be noted that experimentalists work in a well-defined and easier field as compared with the clinical event. Without entering the problem of predictivity, which is not the topic of the present paper, a brief note of caution to excess of enthusiasm should be presented remembering that the clinician is not as fortunate as the experimental chemotherapist, and in many instances adjuvant treatment fails because of large tumor burdens, the presence of occult tumors, or of undetectable micrometastases remaining after surgery or radiotherapy. The existence of murine models for testing new drugs and studying new strategies cannot guarantee complete cancer cure and cannot be considered as conclusive for cancer patients.

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