Eur J Cancer Clin Oncol, Vol. 24, No. 6, pp. 999–1003, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

Comparison of Chemosensitivity of Transplantable Non-small Cell Bronchial Tumours of Rats in Syngeneic Hosts and in Nude Rodents

H.B. KAL* and D.W. VAN BEKKUM

Radiobiological Institute TNO, 151 Lange Kleiweg, 2288 GJ Rijswijk, The Netherlands

Abstract—*Four transplantable poorly to well differentiated bronchial carcinomas, originally induced in the lungs of WAG/Rij and BN rats, were used to study their responsiveness to cytostatic drugs when growing in syngeneic hosts or in nude mice or rats. Growth delay was the endpoint determined.*

The responsiveness to drugs was specific for each tumour line and between tumours it was heterogeneous.

In general, the same tumour specific pattern of response was observed regardless whether tumours grew in syngeneic hosts or in nude mice or nude rats. These results indicate that the stroma of the host does not contribute significantly to the response of the tumour, but that the intrinsic sensitivity of the malignant cells is the prevailing factor.

INTRODUCTION

BECAUSE OF the high metastatic spread of bronchial cancers, treatment with chemotherapeutic agents presently provides the only sensible approach to the ultimate control of this disease. Although only a limited number of drugs seems to be effective, the number of combinations and sequences being investigated in clinical trials is very large. Hence, the rationale in the design of the treatment protocols needs improvement [1–3]. There is a need for more detailed information on the more specific biological properties of the various forms of cancer before a more rational approach to the improvement of treatment can be made [4]. Experimental research with animals aimed at understanding the specific responses of non-small cell bronchial cancer to radiation and chemotherapy is limited to a few observations on xenografts of human tumours in nude mice [5]. The literature contains some reports of bronchial carcinomas occurring in rats or mice following induction with radiation [6–8] or chemical carcinogens [9, 10], but these tumours have not been employed in a systematic way for studies on experimental therapy. One report of a rat lung cancer model described the growth of tumours in the lungs of rats following intrabronchial instillation of cell suspensions of a spindle cell carcinoma and a mammary adenocarcinoma, obviously rather unrealistic bronchial tumour models [11].

Accepted 22 January 1988.
*Author to whom correspondence should be addressed.

With our rat lung cancer model [12] histological and growth characteristics of tumours growing intrapulmonarily or subcutaneously can be investigated. Furthermore, the influence of the stroma of the host on the response of the tumours to treatment can be studied using syngeneic animals and nude rodents. This is of importance with regard to the question whether responses of human xenografts growing in nude rodents or in immunosuppressed animals are determined by intrinsic properties of the tumour cells or influenced by the host-derived tumour bed.

In this communication responses of tumours growing in syngeneic hosts or in nude rodents to chemotherapeutic drugs will be reported.

MATERIALS AND METHODS

Specific pathogen-free derived female WAG/Rij and BN inbred rats at the age of 3 months and weighing 130–150 g were used. For the studies with athymic rodents female C57BL/KaLwRij/nu/nu mice, 18–25 g, and female WAG/Rij/rnu rats, 135–170 g, were used. Four transplantable poorly to well differentiated bronchial carcinomas encoded L17, L37, L42 and L44 were used. The L17 and L37 carcinomas originated in the lungs of male WAG/Rij rats after implantation of iridium-192 wires in the lung and the L42 carcinoma originated in the lung of a female WAG/Rij rat after implantation of an iodine-125 seed [13]. The L44 carcinoma was discovered in the lung of a female

BN rat 18 months after local thorax irradiation with a dose of 16 Gy of 300 kV X-rays, administered at a dose rate of 0.8 Gy/min. The L33 line used in this study is a subline of the original L33 line. It was obtained from a L33 tumour regrowing after a dose of 20 Gy of 300 kV X-rays. The histological appearances and tumour volume doubling times are given in Table 1.

Tumour fragments of the carcinomas were serially transplanted in syngeneic hosts (10–74 passages) and maintained their histological and growth characteristics]14].

The volume of tumours growing subcutaneously (s.c.) in the flank was derived from measurements of three tumour dimensions *a*, *b* and *c* with vernier callipers using the formula $V = \pi abc/6$. Tumour (volume) growth delay, TGD, was used as endpoint. It is defined as the time required to reach a volume twice as large as that at the time of treatment, with subtraction of the value for the volume-doubling time, TD, of untreated tumours. For intercomparison of the responsiveness of tumours of different tumour lines with different volume doubling times, the method of specific growth delay, SGD, was used [15]. The SGD is the tumour growth delay divided by the volume doubling time of untreated control tumours, SGD = TGD/TD. We consider a tumour sensitive for a particular drug when the TGD is at least 2 TD, or SGD $\geq$ 2.

Tumours were treated with a variety of chemotherapeutic drugs. In general, the drugs were administered as a single dose either intraperitoneally, intravenously by tail vein injection or by oral gavage. The drugs, doses and routes of administration are listed in Table 2. The drug doses used in syngeneic hosts differ from those used in nude mice. The doses administered are close to the LD_{10} dose. In general, at least five tumour bearing animals per dose group were used. Individual tumour growth curves were made. From these curves the TD of individual control tumours and the TGD of each treated tumour were calculated. From these individual data mean TD or TGD values were derived.

RESULTS

Responses in syngeneic hosts

Tumours growing subcutaneously were treated with chemotherapeutic drugs (Table 2) at a volume of about 1–1.5 cm^3. Examples of typical growth curves of control tumours and of tumours sensitive to a specific drug are shown in Figs. 1 and 2, respectively. The TD derived from the growth curves of the L17 tumour (Fig. 1) is 4.7 ± 0.8 days. The TGD of the L17 tumour after TCNU treatment (Fig. 2) is 31.8 ± 9.0 days. The SGD for this treatment is therefore 31.8/4.7 = 6.8. This SGD and those of other tumour–drug combinations are given in Table 3.

The data in Table 3 clearly demonstrate that the response of this panel of tumours to drugs is quite

Table 1. Histological appearance and tumour volume doubling time of rat bronchial carcinomas growing in different hosts

Tumour line	Histology	*n*	TD ± S.E. (*d*) syngeneic	*n*	TD± S.E. (*d*) nude mice
L17	Well differentiated squam. c.c.	22	4.5 ± 1.4	13	5.2 ± 1.7
L37	Poorly to moderately differentiated squam. c.c.	14	5.9 ± 1.5	10	8.8 ± 3.7
L42	Well differentiated squam. c.c.	15	5.8 ± 1.6	18	9.3 ± 4.2
L44	Poorly differentiated adenoc.	17	2.9 ± 0.8	10	5.0 ± 1.4

Table 2. Drug doses and route of administration

Drug	Dose(mg/kg) Rats	Nude mice	Route of administration
Mitomycin C	1.5	1	i.v.
Cisplatin	6	8	i.v.
Adriamycin	7.5	6	i.v.
Methotrexate	3 × 10, every 3.5h	10	i.p.
TCNU*	20	20	p.o.
CCNU	30	75	p.o.
Ifosfamide	200	400	i.v.

*1-(2-Chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea is a new water soluble nitrosourea, kindly provided by Leo Laboratories, Helsingborg, Sweden.

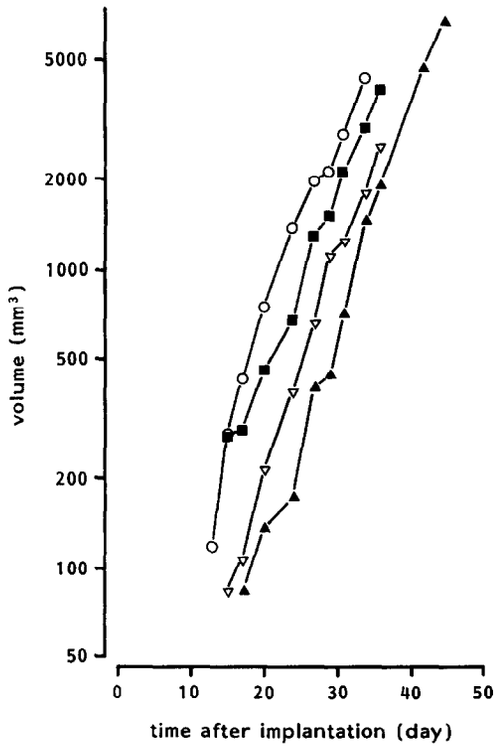

Fig. 1. Individual growth curves of untreated L17 tumours growing subcutaneously in syngeneic hosts.

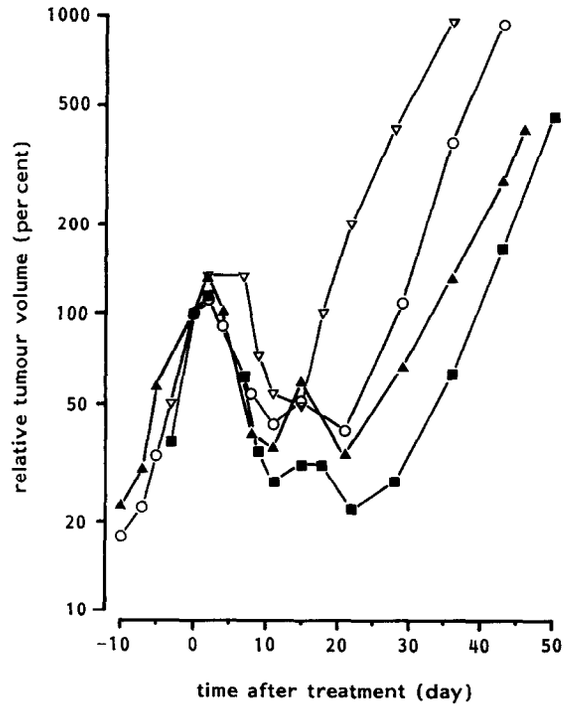

Fig. 2. Individual growth curves of L17 tumours growing subcutaneously in syngeneic hosts after the treatment with TCNU (20 mg/kg).

heterogeneous. For example, the L17 line is sensitive to methotrexate and adriamycin; the L37 line is not sensitive to any of the three drugs listed; the L42 line is responsive to five of the seven drugs tested and the L44 line only to one of three drugs.

Responses in nude rodents

The same tests were performed on tumours growing in nude mice to investigate whether the host stroma influences tumour response. The volume doubling times of tumours growing in the nude mice are, in general, larger than those of tumours growing in syngeneic hosts (Table 1). The results of the experiments on drug sensitivity are shown in Table 3. Tumour growth in nude mice after treatment with TCNU and ifosfamide could be monitored only up to about 1 month after treatment. Mice were then lost due to deteriorated health condition with tumours still in regression. The SGD values in Table 3 indicated with the '>' sign are therefore minimum values. In general, one can conclude from the data presented in Table 3 that a tumour responsive in syngeneic hosts is also responsive as xenograft in nude mice.

As shown in Table 3 the L17 tumour line is very sensitive to adriamycin, while the L44 tumour line is not. As a matter of interest, a comparison was made between the response to this drug of the L44, a tumour originating in the BN strain and the L17 tumour that originated in a WAG/Rij rat, while both were grown in opposite flanks of the same WAG/Rij/rnu rat. A dose of adriamycin (6 mg/kg, i.v.) was given and growth of the tumours was determined as a function of time after treatment. The growth curves are shown in Fig. 3. They indicate that the L17 tumour is still very responsive, the L44 tumour is not. From these data, it is not likely that the host environment did influence significantly the response of the tumours.

DISCUSSION

The responses of the rat bronchial carcinomas used in this study to treatments with various chemotherapeutic drugs were as heterogeneous as their human counterparts, varying from no response at all to prolonged growth delays and even cures. Marsoni *et al.* [16] reported that in clinical studies on patients with non-small cell carcinomas among 33 evaluable drugs, the drugs ifosfamide, adriamycin and cisplatin were active with an overall response rate (partial and complete responders) of 11–18%, respectively. The drugs CCNU and TCNU which were not included in this study of Marsoni [16] were shown in our study also to be active in at least one of the four lines tested.

The endpoint for chemoresponsiveness in our study was an SGD ≥ 2, rather than a partial or complete response as employed in patient studies. If we use complete response, i.e. a tumour volume reduction of at least 50% as the endpoint, then 11 of the 14 combinations with an SGD ≥ 2 could be considered responsive. Hence, the clinical endpoint

*Table 3. Chemosensitivity of rat lung tumours growing subcutaneously in the flank of syngeneic rats or in nude mice**

Drug	SGD(TGD/TD)† Tumour line							
	L17		L37		L42		L44	
	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse
Mitomycin C	0.1	0.5	0.2	0.2	6.3	0.2	0.4	0.5
CisPt	0.3	0.1	0.6	0.2	5.9	4.9	11	6.1
Adriamycin	>12	8.2	0.4	0.5	1.1	0.4	0	0
Methotrexate	3.7	1.0			0.9	0.6		
TCNU					5c‡	3.8		
CCNU					>12	4.3		
Ifosfamide					5c	4.2		

*Responsive combinations (SGD≥2) are underlined.
†Specific growth delay (tumour growth delay/tumour doubling time).
‡5c: cure of five animals.

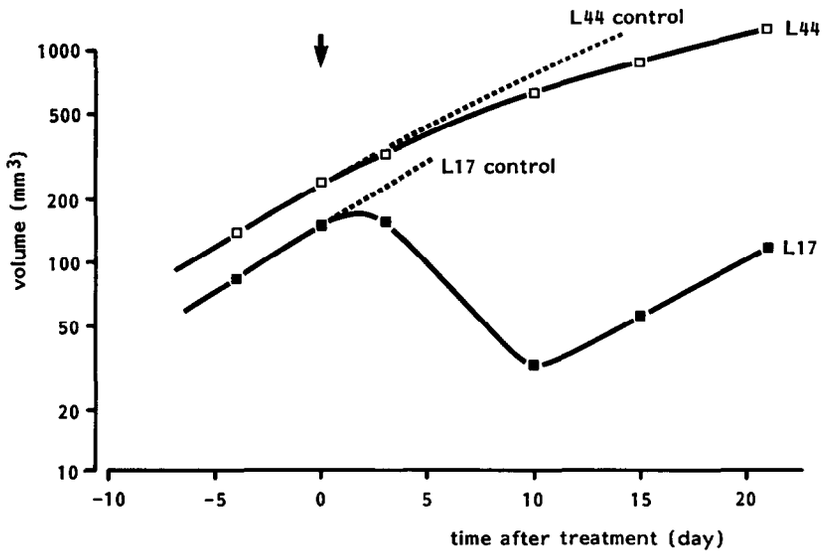

Fig. 3. Growth curves of L17 and L44 implants growing subcutaneously in the same WAG/Rij/rnu rat after treatment with a dose of adriamycin (6 mg/kg).

complete response and the SGD used in this study can be regarded as corresponding endpoints.

The data described here indicate that a single experimental lung carcinoma line cannot be used as a model for human cell carcinoma, but rather that a panel of tumours with identical histological appearances is required.

Implants in nudes had longer TDs than those growing in syngeneic rats. This is parallelled by observations of others [17] that xenografts grew slowly and irregularly in the first passage.

The chemosensitivity of tumours growing in nude mice is in general comparable to that of the same tumours growing in syngeneic rats. Fourteen of the 16 combinations tested showed a similar sensitivity. There are only two exceptions, mitomycin C in L42 and methotrexate in L17 tumours. The growth pattern of L42 tumours after mitomycin C treatment was uncommon as compared to those after other treatments. Although a long growth delay was observed in syngeneic hosts, a volume reduction after treatment was hardly observed. In fact, three of the five tumours had no volume reduction at all, one had a volume reduction of about 10% and the last one a reduction of about 40%. It may be that L42 is slightly antigenic as could be concluded from the results of an experiment in which spontaneous regression in non-treated animals (n= 55) was observed in about 30% of tumours. Regression even occurred when tumours had reached volumes of 1–2 cm^3. The decreased response of L42 tumours in nude mice to adriamycin may also be caused by the lower drug dose employed. The differences in responses to methotrexate of L17 tumours may also be caused by differences in drug dose.

In summary, the responses of four different experimental bronchial carcinomas which had originated in the lungs of rats to a variety of chemo-

therapeutic drugs were heterogeneous. A similar tumour-specific pattern of response was observed when tumours were grown as xenotransplants in nude mice. These results indicate that the responsiveness to drugs is determined by the intrinsic properties of the tumour cells and not by host influences. This observation strongly supports the notion that results of experiments performed with human xenografts in nude rodents or immunosuppressed animals are predictive for responses of tumours in human patients. It should be noted, however, that in the experiments described here, the subcutaneous location in the flank was employed, which is obviously not the natural location of these tumours in the patient.

Acknowledgements—The authors wish to thank Mrs. B.v.d. Vecht-de Jong for expert technical assistance and Mrs. H. Jense for secretarial assistance. This work was supported by a grant from the Netherlands Cancer Foundation.

REFERENCES

1. Bleehen NM. Management of inoperable squamous cell, adeno- and large cell carcinoma. In: Hansen HH, Rorth M, eds. *Lung Cancer* 1980. *II. World Conference Copenhagen*. Amsterdam, Excerpta Medica, 1980, 93–112.
2. Niell HB, Griffin JP, West WH, Neely CL. Combination chemotherapy with mitomycin C, methotrexate, cisplatin, and vinblastine in the treatment of non-small cell lung cancer. *Cancer* 1984, **54**, 1260–1263.
3. Ruckdeschel JC, Day R, Weissman CH, Stolbach LL, Creech RH. Chemotherapy for metastatic non-small cell bronchogenic carcinoma: cyclophosphamide, doxorubicin, etoposide versus mitomycin and vinblastin (EST 2575, Generation IV). *Cancer Treat Rep* 1984, **68**, 1325–1329.
4. Gazdar AF, Carney DN, Minna JD. The biology of lung cancer. In: *Lung Cancer* 1982, *General Lectures and Special Topics*. III World Conference, Tokyo 1982, 14–30.
5. Shorthouse AJ, Peckham MH, Smyth JF, Steel GG.The therapeutic response of bronchial carcinoma xenografts: a direct patient–xenograft comparison. *Br J Cancer*, Suppl IV, 1980, 142–145.
6. Deutsch-Wenzel RP, Brune H, Grimmer G *et al.* Experimental studies in rat lungs on the carcinogenicity and dose response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. *J Natl Cancer Inst* 1983, **71**, 539–544.
7. Yarita T, Nettesheim P, Williams ML. Tumor induction in the trachea of hamsters with *N*-nitroso-*N*-methylurea. *Cancer Res* 1978, **38**, 1667–1676.
8. Topping DC, Nettesheim P. Promotion-like enhancement of tracheal carcinogenesis in rats of 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res* 1980, **40**, 4352–4355.
9. Anderson EC, Holland LM, Prine JR *et al.* Lung tumorigenesis in the Syrian hamster from particulate sources of ^{147}Pm beta radiation. *Radiat Res* 1979, **79**, 349–367.
10. Ullrich RL, Jernigan MC, Adams LM. Induction of lung tumors in RFM mice after localized exposures to X-ray or neutrons. *Radiat Res* 1979, **80**, 464–473.
11. Byhardt RW, Almagro UA, Fish BL, Moulder JE. Development of a rat lung cancer model. *Int J Radiat Oncol Biol Phys* 1984, **10**, 2125–2130.
12. Kal HB, van Berkel AH, Zurcher C, Smink T, van Bekkum DW. A rat lung cancer model based on intrapulmonary implantation of tumour material. *Radiother Oncol* 1986, **6**, 231–238.
13. Kal HB, Zurcher C, van Bekkum DW. Induction of lung tumors by radioactive isotopes implanted in the rat lung. *J Natl Cancer Inst* 1986, **76**, 943–946.
14. Kal HB, van Berkel AH, van der Vecht-de Jong B, van Bekkum DW, Zurcher C. Development and application of a rat tumor model for human bronchial carcinoma. *Natl Cancer Inst Monogr* 1988, **6**, 111–114.
15. Kopper L, Steel GG. The therapeutic response of three human tumour lines maintained in immune-suppressed mice. *Cancer Res* 1975, **35**, 2704–2713.
16. Marsoni S, Hoth D, Simon R, Leyland-Jones B, DeRosa M, Wittes RE. Clinical drug development: an analysis of phase II trials, 1970–1985. *Cancer Treat Rep* 1987, **71**, 71–80.
17. Fu KK, Steel GG. Growth kinetics of a rat mammary tumour transplanted into immune-suppressed mice. *Cell Tissue Kinet* 1979, **12**, 493–499.