6. Gamse R. Antiemetic action of 5-HT_3 receptor antagonists: review of preclinical and clinical results with ICS 205-930. *Cancer Treat Rev* 1990, **17**, 301–305.
7. Seinen H, Zonnenberg BA, Tjia P, Neijt JP. The effect of three dose levels of ICS 205-930 (a selective 5-HT_3 antagonist) on cisplatin-induced nausea and vomiting. *Eur J Cancer Clin Oncol* 1989, **25**, 1333–1335.
8. Calvert AH, Harland SJ, Newell DR, *et al.* Early clinical trials with *cis*-diamine-1,1-cyclobutane dicarboxylate platinum II. *Cancer Chemother Pharmacol* 1982, **9**, 140–147.
9. Fetting JH, Grochow LB, Folstein MF, *et al.* The course of nausea and vomiting after high-dose cyclophosphamide. *Cancer Treat Rep* 1982, **66**, 1487–1493.
10. Martin M, Diaz Rubio E, Sanchez A, Almenarez J, Lopez Vega JM. The natural course of emesis after carboplatin treatment. *Acta Oncol* 1990, **29**, 593–595.
11. Gralla R. Approaches to management of nausea and vomiting in the clinical setting. *Clinician* 1988, **6**, 26–38.
12. Gralla RJ, Itri LM, Pisko SE, *et al.* Antiemetic efficacy of high-dose metoclopramide: randomized trials with placebo and prochlorperazine in patients with chemotherapy-induced nausea and vomiting. *New Engl J Med* 1981, **305**, 905–909.
13. Marty M, Pouillart P, Scholl S, *et al.* Comparison of the 5-hydroxytryptamine$_3$ (serotonin) antagonist ondansetron (GR 38032F) with high-dose metoclopramide in the control of cisplatin-induced emesis. *New Engl J Med* 1990, **322**, 816–821.
14. Strum SB, McDermed JE, Opfell RW, Reich LP. Intravenous metoclopramide. An effective antiemetic in cancer chemotherapy. *JAMA* 1982, **247**, 2683–2686.
15. Jones AL, Hill AS, Soukop M, *et al.* Comparison of dexamethasone and ondansetron in the prophylaxis of emesis induced by moderately emetogenic chemotherapy. *Lancet* 1991, **228**, 483–487.

Acknowledgement—This study was carried out with the support of Sandoz Pharma Ltd, U.K.

Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 615–620, 1994
Elsevier Science Ltd
Printed in Great Britain

0959-8049(93)E0061-8

Intrinsic Radiosensitivity of Adult and Cord Blood Lymphocytes as Determined by the Micronucleus Assay

D.N. Floyd and A.M. Cassoni

Predictive radiosensitivity testing necessitates rapid and reliable assays of radiosensitivity. We assessed the lymphocyte micronucleus assay as such an assay. We performed repeated experiments on lymphocytes from 10 healthy donors. Levels of radiation-induced micronuclei were measured following exposures of up to 4 Gy X-rays. When measuring the slope of the dose–response, we have found more variation between individuals than between repeated experiments on the same individual (F value 12.31, $P < 0.001$). There is also greater interindividual variation in the data following a single dose of X-rays of 2 Gy (F value 3.54, $P < 0.01$) and of 4 Gy (F value 7.55, $P < 0.005$). We performed the micronucleus assay on five different samples of cord blood lymphocytes (CBLs). Their radiosensitivities were compared with the mean radiosensitivity of the lymphocytes from the normal group of donors. Comparing the level of micronuclei induced by 2 Gy, only CBL1 ($P < 0.01$) and CBL2 ($P < 0.02$) were more radiosensitive than the mean of the adult lymphocytes. At 4 Gy, CBL1 ($P < 0.001$), CBL2 ($P < 0.05$), CBL3 ($P < 0.01$) and CBL5 ($P < 0.01$) were more radiosensitive than the mean radiosensitivity of the adult lymphocytes. This was also shown when the slope of the dose–response curves were measured. We conclude that the lymphocyte micronucleus assay shows more variability when applied to lymphocytes from different individuals than when repeatedly applied to lymphocytes from the same individual, a requirement for the determination of individual radiosensitivity.

Key words:radiosensitivity, micronucleus, normal tissue tolerance
Eur J Cancer, Vol. 30A, No. 5, pp. 615–620, 1994

INTRODUCTION

IN PATIENTS undergoing radiotherapy, a range of severity of radiation-induced normal tissue damage is seen, from mild to life threatening. Turesson [1] estimates that only 20% of this variability can be accounted for by the dose received and the fractionation schedule. There appears to be a large component of patient responses due to genetic factors [1–3]. Schedules of radiotherapy are selected to achieve an acceptable incidence of normal tissue complications [4]. As there is a sigmoid dose response for tumour control by radiotherapy, and doses generally used are in the steepest part of that curve, it is possible that by altering the total dose by just a small amount, tumour control may be improved with minimal injury to normal tissues. Individualised modifications have been reported as successful for the treatment of some patients with radiosensitive, cancer prone genetic syndrome ataxia telangectasia (AT) [5]. This would be

widely applicable if those patients with low normal tissue tolerance to radiation could be identified and excluded from dose escalation. For this, rapid and reliable *in vitro* assays of intrinsic radiosensitivity are required.

A correlation has been suggested between individual normal tissue radiosensitivity *in vivo* and the *in vitro* radiosensitivity of fibroblasts [6–8], lymphoblastoid cells [9] and lymphocytes [10] as measured by clonogenic assays. The long delay in obtaining results make the clonogenic assay unsuitable for routine predictive use. DNA damage assay are more suitable since they require only short proliferation periods, or none at all [11, 12]. Persistent DNA and chromatin damage in cells following irradiation has been shown to correlate with cellular radiosensitivity *in vitro* [13–15].

The micronucleus assay is widely used to measure the toxicity of physical [16, 17] and chemical agents [18]. Micronuclei are portions of nuclear material that are excluded from the daughter nucleus at mitosis, and they consist of chromosomal fragments or whole chromosomes [19]. The evidence for a correlation between the presence of a micronucleus in a cell and the loss of proliferative capacity of that cell is currently equivocal [20–24].

Using human lymphocytes, micronucleus formation can be measured following irradiation *in vitro* with X-ray doses up to 4 Gy, and has previously been suggested as a method of predicting *in vitro* radiosensitivity [25].

In this study, we investigated the suitability of the cytokinesis-block micronucleus assay to be incorporated into a predictive protocol to determine normal tissue radiosensitivity from the *in vitro* radiosensitivity of freshly isolated human lymphocytes. We measured the reproducibility of the assay by performing repeated experiments on each of a group of 10 healthy individuals, and compared the intra-individual variation with the variation of radiosensitivity between the individuals.

Cord blood lymphocytes (CBL) have been shown to have a wide range of radiosensitivities as determined by their clonogenic capacity [10, 26], similar to that of lymphocytes from donors who are heterozygous for AT [10]. Many CBLs are more radiosensitive than normal lymphocytes [26]. We examined five samples of CBLs to determine the ability of the micronucleus assay to detect this range of radiosensitivities from a single assay on a single sample.

MATERIALS AND METHODS

Lymphocyte preparation

Heparinised whole blood (5–10 ml) was obtained from donors or placentas and diluted with an equal volume of phosphate buffered saline (PBS). Lymphocytes were obtained by layering the diluted blood on to an equal volume of Histopaque-1077 (Sigma) at room temperature in a 50 ml conical centrifuge tube, and centrifuging at 1000 *g* for 20 min at room temperature. The lymphocyte-rich layer was removed, washed twice in PBS and suspended at a concentration of 10^6 cells/ml in warm (37°C) RPMI medium (Gibco) supplemented with 10% fetal calf serum (FCS).

Irradiation

Cell suspensions (1.0 ml in 12 ml plastic culture tubes) were irradiated at room temperature with X-rays (212.5 kV, 12.5 mA). The radiation dose rate was 2.35 Gy/min.

Correspondence to D.N. Floyd.
The authors are at the Department of Oncology, University College Medical School, 91 Riding House Street, London W1P 8BT, U.K.
Revised 2 Dec. 1993; accepted 21 Dec. 1993.

The micronucleus assay

After irradiation, lymphocytes in RPMI were transferred to a 24-well plate (1 ml cell suspension per well). Phytohaemagglutinin (PHA-P) (Sigma) at 0.5 mg/ml in PBS was added to each well, to give a final concentration of 5 μg/ml. Lymphocytes were grown at 37°C in a humidified incubator, in 95% O_2, 5% CO_2. After 24 h 3 μl cytachalasin-B (Sigma) at 1.0 mg/ml in dimethyl sulphoxide (DMSO) was added [17]. Adding cytachalsin 48 h after stimulation [27] gave a smaller percentage of binucleated lymphocytes. After a total of 72 h in culture [17], the clumps of lymphocytes were disaggregated by vigorous pipetting and duplicate microscope-slide preparations were made using the Shandon™ cytospin. The slides were dried in air, fixed in methanol and then stained with May-Grünwald and Giemsa solutions. Lymphocytes were viewed at 400 × magnification. Binucleated (BN) and micronucleated (MN) cells were scored as follows: BN lymphocytes were identified as cells with an intact cytoplasm containing two circular nuclei of approximately equal size, including those with nuclear bridges, and with slightly overlapping or touching nuclei. Micronuclei, in BN cells only, were identified as being non-refractile and staining with Giemsa at an intensity equal to or less than that of the two nuclei. MN that were overlapping or touching a nucleus were counted if the nucleus was otherwise round and had a smooth edge.

Between 150 and 500 binucleated cells were counted per slide, and the radiation-induced damage was quantified as the percentage of binucleated cells that contained one or more micronuclei, i.e.

% BN with MN = [(BN with one or more micronuclei) ÷ (total BN)] × 100.

Data analyses

Dose–response curves were constructed for X-ray doses up to 4 Gy. For each set of data from each experiment a straight line was fitted by linear regression using all the available data points. An analysis of variance (ANOVA) was used to determine the variance of the magnitude of the slope of the dose response, and also the response at 2 Gy and at 4 Gy, between individuals and between different experiments on the same individual.

The 'group mean' of the slope of the dose response, and of the % (BN with MN) at 2 Gy and 4 Gy were calculated from the data from each experiment on each of the 10 donors. The group mean was calculated to allow comparison of the CBL samples with a standard measure of radiosensitivity. The radiosensitivities of each of the five CBL samples were compared with the group mean by the method of linear contrast [28].

RESULTS

Individual radiosensitivity in vitro*: interindividual and intraindividual variation*

The group of normal donors consisted of 9 females and 1 male. The age range was 23–70 years at the start of the study. Included in the group were one exsmoker (donor 6), one occasional smoker (donor 2) and two asthmatics (donors 3 and 9).

Figure 1 shows representative dose–response curves for 4 donors obtained from repeated experiments on each donor over a 15-month period. Data have been corrected for micronuclei in unirradiated control cells. The dose response between 1 and 4 Gy is approximately linear. There is little variation in measured individual radiosensitivity over time. The accuracy of a single assay cannot be improved by either setting up separate cell cultures or counting thousands of BN cells. In our preliminary

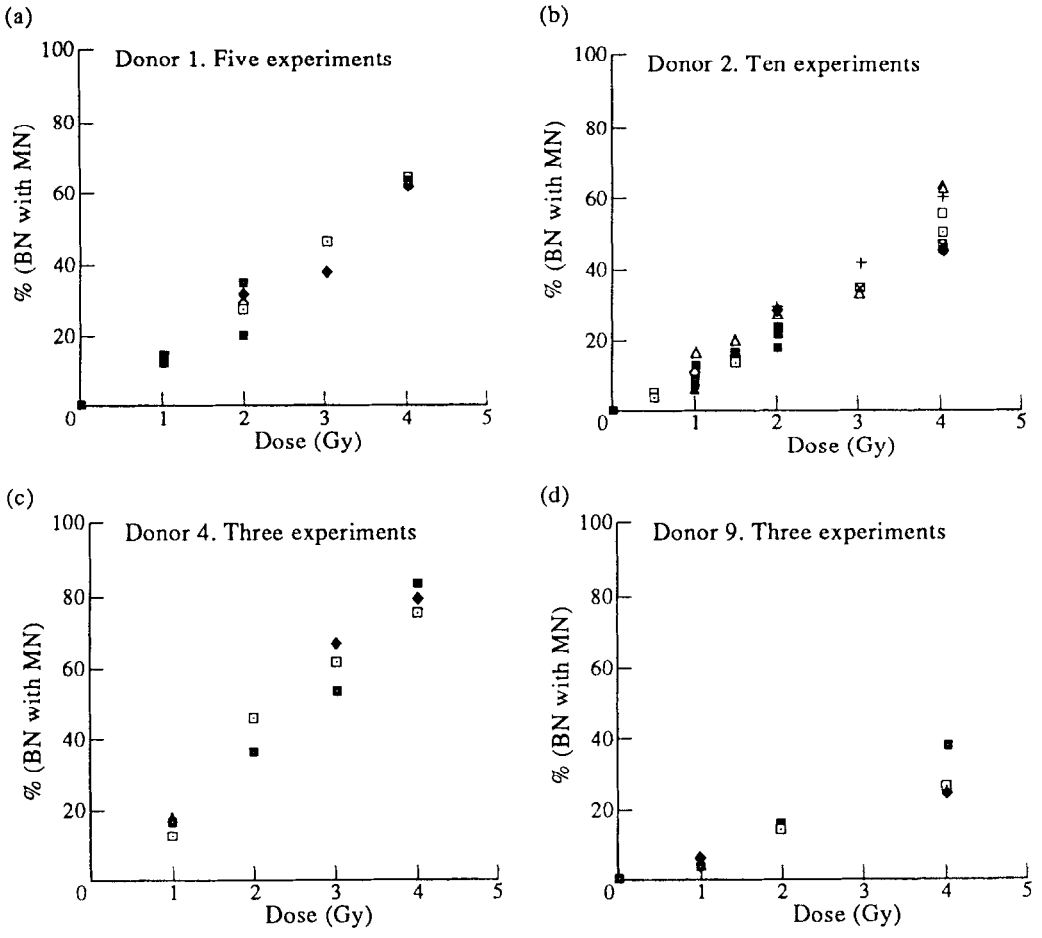

Figure 1. Dose–response data for radiation-induced micronuclei in lymphocytes from the blood of healthy adult donors following *in vitro* irradiation. Data from repeated experiments spanning a 15-month period are shown for 4 donors representative of the group of 10 adult donors. (a) Smallest data spread from repeated experiments; (b) largest data spread from repeated experiments; (c) steepest dose response; (d) shallowest dose response.

work we found that replicate cultures established from the same lymphocyte sample gave almost identical dose–response curves [different points at the same dose varied by between 0.2 and 2.0% (BN with MN)]. Counting errors were kept to below 2.0% (BN with MN) when more than 200 cells were counted.

Figure 2 shows the interindividual differences in the percentage of micronucleated BN cells, corrected for spontaneous micronuclei, following 2 and 4 Gy. For each individual a difference can be seen between the percentage of micronucleated BN cells following 2 and 4 Gy. Comparing the responses of the 10 individuals, the percentage of micronucleated BN cells for donor 9 is seen to be below the range of other donors, whilst that from donor 4 is above the others.

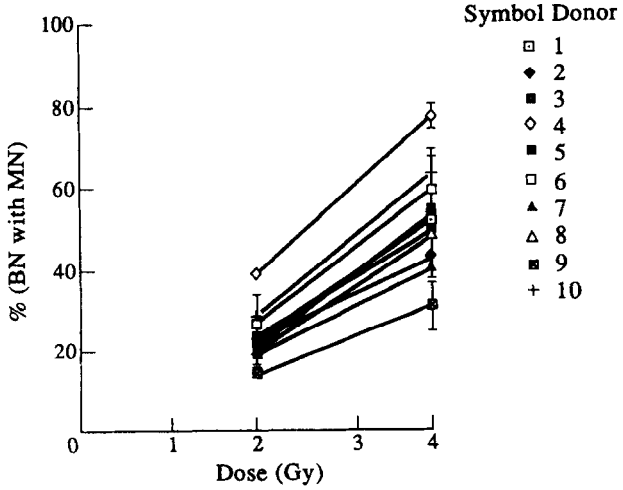

Figure 2. Micronucleus induction following X-ray doses of 2 and 4 Gy. The mean % (BN with MN) is shown for each donor. Error bars represent the standard deviation of the data for each individual.

The results of the analysis of variance (ANOVA) performed on the data obtained using adult lymphocytes are summarised in Table 1. The ANOVA data imply that a single observation at 2 or 4 Gy could be used to determine the radiosensitivity of an individual in a future experiment. There is a greater variation between individuals' lymphocytes in their response to irradiation

Table 1. ANOVA data comparing the discriminatory capacity of a single dose of radiation (2 and 4 Gy) and the magnitude of the slope of the dose–response curve

	F value	Degrees of freedom	*P*
% (BN with MN) at 2 Gy	3.54	9, 27	<0.01
% (BN with MN) at 4 Gy	7.55	9, 27	<0.005
Slope of dose response [% (BN with MN)]	12.31	9, 27	<0.001

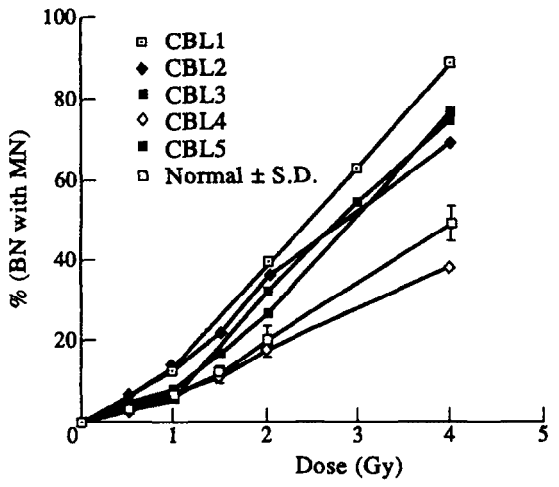

Figure 3. Micronucleus induction in cord blood lymphocytes. The dose–responses of five separate CBL samples are compared with the mean dose response from repeated experiments on lymphocytes from a single adult donor.

with 4 Gy (F value 7.55, $P<0.005$) than at 2 Gy (F value 3.54, $P<0.01$).

Using linear regression, we fitted a straight line to each dose–response curve for each experiment for each individual. The correlation coefficients varied from 0.891 to 1.000. A linear-quadratic curve could also be fitted to the data (correlation coefficients—0.97 to 1.00). We favoured a straight line for two reasons. The first was that a linear fit emphasised the high-dose data, where the resolution appeared better. Secondly, the errors associated with the parameters describing linear and exponential parts of a linear-quadratic fit would make comparisons of individual dose–response curves difficult. The differences between individuals appear greater when the slope of the dose response is measured (F value 12.31, $P<0.001$) than when the response at a single dose is measured.

In vitro *radiosensitivity of cord blood lymphocytes*

CBLs have been shown to have a wide range of radiosensitivities *in vitro* [26]. We performed the micronucleus assay on five CBL samples to assess the ability of the assay to detect this wide range of radiosensitivities. The dose–response curves for five samples of CBLs are shown in Figure 3. As with normal lymphocytes, we used linear regression of the data to fit a straight line to the dose–response data for each CBL sample. Table 2 gives the percentage of micronucleated BN cells at 2 Gy and at 4 Gy and the slope of the dose response for each sample, together with the value for the group mean.

The level of radiation-induced micronuclei at 2 Gy show CBL1 ($P<0.01$) and CBL2 ($P<0.02$) to be more radiosensitive than the group mean for adult lymphocytes. There is no significant difference for the other samples ($P>0.05$). The resolution is improved by measuring the level of micronucleation following 4 Gy ($P<0.05$), and further by measuring the slope of the dose–response curve—all samples were significantly different ($P<0.02$). Of the five samples, one is less sensitive that the group mean ($P<0.05$ at 4 Gy). The others were more radiosensitive, especially CBL1 ($P<0.001$ at 4 Gy). No assumption about the radiosensitivity of cord blood lymphocytes as a group can be made since not enough samples have been tested.

DISCUSSION

The lymphocyte micronucleus assay has been suggested as a potential method of measuring individual radiosensitivity [25]. An important aspect of any predictive test is the effect of the reproducibility on the resolution; that is the difference between interassay variability when the assay is performed on cells from 1 individual, and observed variation when the assay is performed on more than 1 individual. We performed repeated experiments on lymphocytes from 10 healthy individuals in order to compare the extent of assay variability and resolution.

We chose to measure the percentage of micronucleated cells, not the more commonly used micronucleus frequency. There are possible errors associated with both methods of counting. Grote and colleagues [21] showed that a single micronucleus in the cytoplasm of a living diploid cell correlated with a loss of the proliferative capacity of that cell. The micronucleus frequency therefore may overestimate the level of radiation-induced cell death, while counting the number of micronucleated BN cells may underestimate cell death due to the inclusion of micronuclei in one of the macronuclei. Practically, it is less time consuming to count the number of bionucleated cells containing micronuclei than the micronucleus frequency, and there is a degree of subjectivity in delineating each micronucleus in a BN cell containing multiple micronuclei.

Many CBLs have a radiosensitivity greater than that of normal lymphocytes, although the ranges overlap (mean inactivation dose ranges 1.24–2.12 Gy compared with 1.60–2.50 Gy for normal adult lymphocytes [26]). It is noteworthy that CBLs have a similar range of radiosensitivities to lymphocytes from AT heterozygotes (mean inactivation dose 0.70–2.20 [10]). We have shown that four of five cord blood lymphocyte samples can be identified as more radiosensitive ($P<0.05$) than the mean radiosensitivity of normal lymphocytes using the micronucleus assay (Figure 3, Table 2). This difference could be shown by the level of damage measured following irradiation with 4 Gy, or

Table 2. Micronucleus data obtained following the irradiation of five different samples of cord blood lymphocytes (CBL1–5). The 'group mean' values, calculated from the data obtained using adult lymphocytes, are given for comparison. The level of significance of the difference between each CBL sample and the group mean (P value) is given in brackets

	% (BN with MN) at 2 Gy	% (BN with MN) at 4 Gy	Slope of dose–response [% (BN with MN) per Gy]
CBL1	39.00 ($P<0.01$)	89.00 ($P<0.001$)	22.80 ($P<0.001$)
CBL2	37.00 ($P<0.02$)	70.00 ($P<0.05$)	18.00 ($P<0.01$)
CBL3	28.00 ($P>0.05$)	77.00 ($P<0.01$)	19.70 ($P<0.001$)
CBL4	18.00 ($P>0.05$)	39.00 ($P<0.05$)	9.95 ($P<0.02$)
CBL5	30.00 ($P>0.05$)	75.00 ($P<0.01$)	19.95 ($P<0.001$)
Group mean	23.28	52.81	13.68

with greater resolution by comparing the magnitudes of the slopes of the dose–response curves.

Huber and colleagues [25] also suggested that the lymphocyte micronucleus assay could be used to determine individual radiosensitivity, whilst Nakamura and colleagues [29], using a lymphocyte colony assay, concluded that the individual variation of intrinsic radiosensitivity is too small to be detected. This may be due to the variability of the assay. However, no large-scale study has yet been performed, either to measure the *in vitro* radiosensitivity of individuals, or to correlate any observed elevated radiosensitivity with normal tissue tolerance to ionising radiation. Such a study is necessary to confirm or deny that individual radiosensitivity can be predicted by a single measurement of the *in vitro* response of lymphocytes to radiation. If the micronucleus assay is to be used in such a study, two major problems must be addressed. These are (a) the choice of a defined 'standard' radiosensitivity and (b) overcoming the intrinsic assay variability to improve the discriminatory power of the assay.

The problem of chosing a source of control cells is important in defining the standard value of *in vitro* radiosensitivity to which unknown test samples would be compared. Although we have found cryopreserved lymphocytes to be as dependable as fresh lymphocytes from the same donor, it is impractical to use lymphocytes from donors where the *in vitro* radiosensitivity is accurately known, since large volumes of blood would be required. A diploid cell line could be used providing that the percentage of micronucleated BN cells at 72 h was large enough to allow comparison with the test lymphocytes. This would in part rely upon the cell kinetics. A cell line would offer the possibility of being less variable than lymphocytes in their behaviour in culture. A third possibility is using the mean response of the tested population. We have employed this method here. For our work, the 'group mean' values of slope and percentage of micronucleated BN cells at 2 and at 4 Gy took into account assay variability and interindividual differences in radiosensitivity. Such a control has the advantage of being recalculated as more data are acquired. This would in turn more accurately reflect the true population mean, thus possibly allowing improved discriminatory power.

The other problem when considering predictive radiosensitivity testing is the intrinsic variability of the assay. In the case of the micronucleus assay, this variability is quite small. Whilst it is possible that some of the temporal variation seen in individual radiosensitivity may be due to altering proportions of immunologically distinct subgroups of T-lymphocytes, there is currently no evidence that these subgroups have different radiosensitivities ([30, 31] Floyd and Michie, unpublished data). Possibly, the use of more than one assay would be most effective.

We are now using the micronucleus assay to investigate the relationship between *in vitro* radiosensitivity and radiation-induced normal tissue damage in patients who have received radiotherapy.

We have shown that statistically significant differences ($P<0.05$) between individual *in vitro* radiosensitivities are detectable and reproducible. We have also shown that the assay can detect a wide range of radiosensitivity of cord blood lymphocytes. We conclude that the lymphocyte micronucleus assay has the potential to be used as a predictive assay of individual radiosensitivity.

1. Terry NHA, Brock WA, Hendry JH. Future directions for predictive assays. *Int J Radiat Biol* 1993, **64**, 35–338.
2. Arlett CF. Human cellular radiosensitivity—the search for the diagnostic holy grail or a poisoned chalice. *Adv Radiat Biol* 1992, **16**, 273–292.
3. Deschavanne PJ, Debieu D, Fertil B, Malaise EP. Re-evaluation of *in vitro* radiosensitivity of human fibroblasts of different genetic origins. *Int J Radiat Biol* 1986, **50**, 279–293.
4. Horwich A. The future of radiotherapy. *Radiother Oncol* 1990, **19**, 353–356.
5. Hart RN, Kimler BF, Evans RG, Park CH. Radiotherapeutic management of medulloblastoma in a pediatric patient with ataxia telangectasia. *Int J Radiat Oncol Biol Phys* 1987, **13**, 1237–1240.
6. Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR, Peacock JH. Prediction of normal-tissue tolerance to radiotherapy from *in vitro* cellular radiosensitivity. *Lancet* 1992, **339**, 1570–1571.
7. Loeffler JS, Harris JR, Dahlberg WK, Little JB. *In vitro* radiosensitivity of human diploid fibroblasts derived from women with unusually sensitive clinical response to definitive radiation therapy for breast cancer. *Radiat Res* 1992, **121**, 277–231.
8. Plowman PN, Bridges BA, Arlett CF, Hinney A, Kingston JE. An instance of clinical radiation morbidity and cellular radiosensitivity, not associated with ataxia-telangectasia. *Br J Radiobiol* 1990, **63**, 624–628.
9. Chen PC, Lavin MF, Kidson C, Moss D. Identification of ataxia-telangectasia heterozygotes, a cancer prone population. *Nature* 1978, **274**, 484–486.
10. Cole J, Arlett CF, Green MHL, *et al.* Comparative human cellular radiosensitivity: II. The survival following gamma-irradiation of unstimulated (G_0) T-lymphocytes, T-lymphocyte lines, lymphoblastoid cell lines and fibroblasts from normal donors, from ataxia-telangectasia patients and from ataxia-telangectasia heterozygotes. *Int J Radiat Biol* 1988, **54**, 929–943.
11. Arrand JE, Michael BD. Workshop report: recent advances in the study of ionising radiation damage and repair. *Int J Radiat Biol* 1992, **61**, 717–720.
12. Whitaker SJ, Powell SP, McMillan TJ. Molecular assays of radiation-induced DNA damage. *Eur J Cancer* 1991, **27**, 922–928.
13. Lynch TH, Anderson P. Wallace DMA, Kondratowicz GM, Beaney RP, Vaughan ATM. A correlation between nuclear supercoiling and the response of patients with bladder cancer to radiotherapy. *Br J Cancer* 1991, **64**, 867–871.
14. Powell SN, Whitaker SJ, Edwards SM, McMillan . A DNA repair defect in a radiation-sensitive clone of a human bladder carcinoma cell line. *Br J Cancer* 1992, **65**, 789–802.
15. Taylor YC, Duncan PG, Zhang X, Wright WD. Differences in the supercoiling response of irradiated cell lines from ataxia-telangectasia versus unaffected individuals. *Int J Radiat Biol* 1991, **59**, 359–371.
16. Fenech M. The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat Res* 1993, **285**, 35–44.
17. Mitchell JC, Norman A. The induction of micronuclei in human lymphocytes by low doses of radiation. *Int J Radiat Biol* 1987, **52**, 527–535.
18. Yang W-L, Klopman G, Rosenkranz HS. Structural basis of the induction of micronuclei. *Mutat Res* 1992, **272**, 111–124.
19. Miller BM, Werner T, Weier H-U, Nüsse M. Analysis of radiation-induced micronuclei by fluorescence *in situ* hybridisation (FISH) simultaneously using telomeric and centromeric probes. *Radiat Res* 1992, **131**, 177–185.
20. Geard CR, Chen CY. Micronuclei and clonogenicity following low- and high-dose rate γ irradiation of normal human fibroblasts. *Radiat Res* 1990, **124**, S56–S61.
21. Grote SJ, Joshi GP, Revell SH, Shaw CA. Observations of radiation-induced chromosome fragment loss in live mammalian cells in culture, and its effect on colony forming ability. *Int J Radiat Biol* 1981, **39**, 385–408.
22. Peacock JH. The micronucleus assay as a predictor of intrinsic radiosensitivity (abstract). *Br J Radiol* 1993, **66**, 960.
23. Scott D, Bryant PE, Second LH. Gray workshop report. *Int J Radiat Biol* 1992, **61**, 293–297.
24. Wandle EO, Ono K, Kain R, Herbsthofer T, Hienert G, Höbarth K. Linear correlation between surviving fraction and the micronucleus frequency. *Int J Radiat Biol* 1989, **56**, 771–776.
25. Huber R, Braselman H, Bauchinger M. Intra- and inter-individual variation of background and radiation-induced micronucleus frequencies in human lymphocytes. *Int J Radiat Biol* 1992, **61**, 655–661.

26. Waugh APW, Beare DM, Arlett CF, Green MHL, Cole J. Comparative human cellular radiosensitivity: IV. The increased sensitivity of human neonatal cord blood lymphocytes to gamma-irradiation compared with lymphocytes from children and adults. *Int J Radiat Biol* 1991, 59, 767–776.
27. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. *Mutat Res* 1985, 147, 33–38.
28. Armitage P, Berry G. *Statistical Methods in Medical Research*, 2nd edition. Blackwell, Oxford, 1987.
29. Nakamura N, Sposto R, Kushiro J-I, Akiyama M. Is interindividual variation of cellular radiosensitivity real or artifactual? *Radiat Res* 1991, 125, 326–330.
30. Ban S, Cologne JB. X-ray induction of micronuclei in human lymphocyte subpopulations differentiated by immunoperoxidase staining. *Radiat Res* 1992, 131, 60–65.
31. Nakamura N, Kusunoki Y, Akiyama M. Radiosensitivity of CD4 and CD8 positive T-lymphocytes by an *in vitro* colony formation assay. *Radiat Res* 1990, 123, 224–227.

Acknowledgements—We wish to thank Drs Neil Gillies and John Peacock, and Prof. Robert Souhami for extensive discussions during the preparation of the manuscript. Dr Paul Nicholson performed the statistical analyses and helped us with their interpretation. Finally, we thank Dr Colin Mitchie for supplying the CBLs. Work is supported by the North East Thames Regional Health Authority.

Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 620–626, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(94)E0039-7

Primary Chemotherapy and Delayed Surgery for Non-metastatic Telangiectatic Osteosarcoma of the Extremities. Results in 28 Patients

G. Bacci, P. Picci, S. Ferrari, L. Sangiorgi, A. Zanone and A. Brach del Prever

28 patients with telangiectatic osteogenic sarcoma of the extremities were treated between March 1983 and March 1990 with neoadjuvant chemotherapy according to two different protocols activated successively. With the first protocol, patients preoperatively received high dose methotrexate (HDMTX)–cisplatinum (CDP) and postoperatively, depending on the histological response, either HDMTX–CDP-doxorubicin (ADM) or ADM–"BCD". With the second protocol patients were preoperatively treated with HDMTX–CDP–ADM and postoperatively either with the same drugs or with the same drugs plus ifosfamide and VP-16. Preoperatively, CDP was delivered intraarterially. A good histological response (tumour necrosis >90%) was observed in 25 patients (89%) and at a mean follow-up of 5 years (2–9 years) 23 patients (82%) remained continuously disease-free and 5 developed lung metastases. These results are better than those obtained in 272 contemporary cases of conventional osteosarcoma of the extremities treated with the same protocols (62% good histological responses and 61% continuously disease-free survival).

Key words: chemotherapy, telangiectatic osteogenic sarcoma
Eur J Cancer, Vol. 30A, No. 5, pp. 620–626, 1994

INTRODUCTION

TELANGIECTATIC OSTEOSARCOMAS represent a rare subtype of high grade osteosarcoma with distinctive radiological gross and microscopic features, and certain prognostic implications [1, 2]. According to several authors [1, 3, 4], the prognosis of telangiectatic osteosarcoma, when treated with surgery alone, is worse than that of conventional high grade osteosarcoma. In a series from a major centre where large numbers of patients with bone tumours were seen, only 1 of the 25 patients treated with surgery alone survived for more than 5 years [4].

In the last few years, many papers have demonstrated that adjuvant and neoadjuvant chemotherapy dramatically improve prognosis of conventional osteosarcoma [6–10]. There are only two conflicting reports in the literature concerning the efficacy of adjuvant and neoadjuvant chemotherapy in telangiectatic osteosarcoma. In fact, while Rosen and colleagues [11], in a series of 16 patients treated according to two different protocols of neoadjuvant chemotherapy, found that this rare variant of

Correspondence to P. Picci.
G. Bacci and S. Ferrari are at the Department of Chemotherapy; P. Picci and L. Sangiorgi are at the Bone Tumor Center, Istituto Ortopedico Rizzoli, Via Pupilli 1, 1-40136 Bologna; A. Zanone is at the Department of Anestesiology; and A. Brach del Prever is at the Pediatric Clinic, University of Turin, Italy.
Revised 21 Jan. 1994; accepted 24 Jan. 1994.