Eur J Cancer, Vol. 29A, No. 16, pp. 2239–2243, 1993.
Printed in Great Britain

0959-8049/93 $6.00 + 0.00

Compensatory Renal Response after Unilateral Partial and Whole Volume High-dose Irradiation of the Human Kidney

L. Dewit, M. Verheij, R.A. Valdés Olmos and L. Arisz

Renal function was prospectively analysed in 26 patients treated with radiotherapy for various types of malignancies. In patients with gastric non-Hodgkin's lymphoma stage I–II (gNHL, n = 5), the ^{99m}Tc-diethylene-triamine-penta-acetic acid (^{99m}Tc-DTPA) renal uptake and the relative ^{99m}Tc-dimercapto-succinyl acid (^{99m}Tc-DMSA) accumulation decreased gradually and concomitantly in the high-dose, whole-volume irradiated left kidney (40 Gy/5, 5 weeks), down to 25 ± 10% (mean ± 1 S.E.M.) and 31 ± 11%, respectively, after 6–9 years. The absolute ^{99m}Tc-DMSA uptake in the left kidney declined down to 33 ± 12% whereas in the low-dose, whole-volume irradiated right kidney (12–13 Gy/3 weeks) it increased up to 187 ± 11%. When considering renal volume changes with single photon emission computed tomography, the left kidney in the gNHL patients was reduced to 30 ± 13%, with, surprisingly, a contralateral enlargement up to only 119 ± 7% ($P < 0.05$). The overall renal function in this group of patients, as assessed by creatinine clearance and by [^{125}I]iothalamate/[^{131}I]hippuran clearance was reduced to 48–68%. In the Hodgkin's disease patients (HD, n = 7) given 40 Gy in 4 weeks to 30–50% of the left kidney, the ^{99m}Tc-DTPA filtration and the relative ^{99m}Tc-DMSA uptake in the left kidney was reduced to 75 ± 4% and 81 ± 3%, respectively. The absolute ^{99m}Tc-DMSA changes were 78 ± 10% and 135 ± 13%, respectively. No significant renal functional alterations were observed in patients with either ovarian carcinoma (n = 7) or seminoma (n = 7). These data suggest a significant, compensatory response of the non-irradiated or low-dose irradiated kidney which, however, appears to be incomplete after contralateral, whole-volume, high-dose irradiation. Such compensatory response might be overestimated when considering only relative or absolute changes in radioactivity uptake.
Eur J Cancer, Vol. 29A, No. 16, pp. 2239–2243, 1993.

INTRODUCTION

OVER THE last 40 years many investigators have reported on early and late radiation effects in the kidney [1–7]. The loss of renal function largely depends on the total radiation dose [5], the irradiated volume [8–10] and on the dose per fraction [11, 12]. It has also been shown clinically and experimentally that unilateral kidney irradiation causes shrinkage of this kidney with contralateral renal enlargement [9, 13]. In addition, some experimental data have demonstrated that following removal of the contralateral unirradiated kidney, the irradiated kidney has retained some reserve functional capacity [13, 14]. The radiation response of the kidney, therefore, seems to be influenced by the presence and physiological status of its contralateral counterpart.

In a previous paper [10], we reported on a prospective analysis of radiation nephropathy in 26 patients irradiated to various doses and volumes on their kidneys for intra-abdominal malignant disease. A continuously progressive deterioration in renal function was found from 6 months after irradiation onwards, which seemed to be both dose- and volume-dependent. All patients now have a minimal follow-up of 7 years. This paper presents a progress report on the radiation-induced renal functional changes in these patients, with particular emphasis on the compensatory response of the non- or low-dose irradiated kidney.

Correspondence to L. Dewit.
L. Dewit and M. Verheij are at the Department of Radiotherapy; R.A.Valdés Olmos is at the Department of Nuclear Medicine of The Netherlands Cancer Institute (Antoni van Leeuwenhoek Huis), Plesmanlaan 121, 1066 CX Amsterdam; and L. Arisz is at the Department of Internal Medicine, Division of Nephrology, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
Revised and accepted 22 July 1993.

MATERIALS AND METHODS

Patients

Between June 1983 and June 1986, 39 patients were entered in the study. 13 were excluded from analysis for various reasons, such as tumour recurrence or incomplete follow-up data due to patients' non-compliance. Patients were divided into four groups according to their tumour type, and thus the related radiation treatment technique (Table 1). Patients with gastric non-Hodgkin's lymphoma (gNHL) stage I–IIE (group I) received whole abdominal irradiation to a dose of 20 Gy at 1.5 Gy per fraction with a gastric boost of 20 Gy at the same fraction size, which included the entire left kidney. The right kidney was shielded in the posterior field by a kidney-shaped 50% transmission block, which reduced the absorbed dose to 12–13 Gy in 3 weeks at 1 Gy per fraction. A second group of patients with Hodgkin's disease (HD) stage I–IIA (group II) was irradiated on the para-aortic lymph nodes and the spleen to a total dose of 40 Gy in 4 weeks at 2 Gy per fraction. The radiation field included approximately 30–50% of the left kidney and less than 30% of the right kidney. Patients with ovarian carcinoma (OC) stage I–IIIA (group III) received total abdominal irradiation to a total dose of 25 Gy in 3.5 weeks. In this group, both kidneys were shielded posteriorly by a 50% transmission block, which reduced the absorbed dose to the entire kidneys to 17–18 Gy at 1 Gy per fraction. Finally, patients with testicular seminoma (ST) stage I–II (group IV)

Table 1.

Group (n)	Total dose to the kidneys (Gy)		Dose per fraction to the kidneys (Gy)		Irradiated volume of the kidneys (%)	
	Left	Right	Left	Right	Left	Right
I (5)	40	12–13	1.5	1.0	100	100
II (7)	40	40	2.0	2.0	30–50	< 30
III (7)	17–18	17–18	1.0	1.0	100	100
IV (7)	25–35	25–35	2.0	2.0	20–30	20–30

Group I, NHL of stomach, stage I–IIE, radiation therapy (XRT) total abdomen plus gastric boost. Group II, Hodgkin's disease stage I–IIA, XRT para-aortic nodes and spleen. Group III, ovarian carcinoma, stage I–IIIA, XRT total abdomen plus pelvic boost. Group IV, testicular seminoma, stage I–II, XRT para-aortic nodes.

were irradiated on the para-aortic lymph nodes to a total dose of 25–35 Gy in 2.5–3.5 weeks at 2 Gy per fraction, including the medial third of both kidneys. All patients were treated by radiation therapy exclusively.

Assessment of renal function

Glomerular function was determined by ^{99m}Tc-diethylene-triamine-penta-acetic acid (^{99m}Tc-DTPA) renography and creatinine clearance. In addition, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured as standard clearances during continuous infusion of [^{125}I]iothalamate/[^{131}I]hippuran [15]. Tubular function was analysed by ^{99m}Tc-dimercapto-succinyl acid (^{99m}Tc-DMSA) scintigraphy.

For the renography, patients received 185 MBq ^{99m}Tc-DTPA in an intravenous (i.v.) bolus injection. Images were made on a 128 × 128 matrix by a dual-head gamma camera (Siemens Rota 2, Siemens B.V. The Hague, The Netherlands) at a rate of one image per second in the first min, and one image every 30 s thereafter for 29 min. Anterior and posterior images were conjugated in order to minimise the influence of absorption within the patient. The left-to-right ratio in activity between the second and third min after bolus injection calculated from the generated time–activity curve was used as an index of the division of the glomerular filtration between the two kidneys (divided function) [16, 17]. Changes in the divided function were calculated as the relative decrease in left-to-right ratio, compared with the pretreatment value.

For the scintigraphy, 74 MBq ^{99m}Tc-DMSA were given intravenously. The remaining activity in the syringe was measured to calculate the net injected dose. Five hours later, 5-min anterior and posterior images were recorded on a 256 × 256 matrix and conjugated, using the same equipment. This was repeated after placing a standard with known activity on the patient, in order to estimate the absolute uptake of the radiopharmaceutical agent in both kidneys. The relative uptake was calculated by taking the background corrected individual uptake divided by the sum of uptake in both kidneys. The absolute uptake was calculated by relating the uptake in each kidney to the standard [18]. Changes in tubular function were assessed by calculating the differences in relative and absolute uptake, compared with the pretreatment value. All tests were performed with patients in the supine position and after proper hydration.

Single photon emission computed tomography analyses

To quantify radiation-induced changes in the renal volume, single photon emission computed tomography (SPECT) measurements were performed in the gNHL patients following each ^{99m}Tc-DMSA scintigraphy [19]. The SPECT method, used in lung function analysis [20, 21], was applied to the kidney, with some modifications. The dual-head rotating gamma camera recorded 2 × 30 images (6° apart) of 30 s each on a 64 × 64 matrix. The tomographic reconstruction into transaxial 1 pixel slices took place according to the filtered back-projecting method (Medical Data Systems, Ann Arbor, Michigan, U.S.A.). A computer program was used to calculate kidney volumes using a combination of a gradient search and a threshold operation [22]. The actual pixel size and centre of rotation value were established by calibration tests. For volume measurements, the number of pixels in all sections multiplied by the slice thickness (0.63 cm) was summed up. The accuracy of this analysis was checked in a phantom with a known volume and was within 2% (E. Damen, The Netherlands Cancer Institute). Changes in renal volume were related to the pretreatment values.

All tests, except the iothalamate/hippuran clearance, were carried out before radiation treatment, and at 6-month intervals during the first 5 years of follow-up, and once a year thereafter. The iothalamate/hippuran clearance test was introduced later on in the follow-up as a complementary and more accurate index of overall kidney function. It was performed only in patient groups I and II.

Blood pressure

Blood pressure was measured at 6-month intervals at the outpatient clinic. It was always measured with the patient in the sitting position. A persistently increased blood pressure was defined as a systolic blood pressure ≥ 145 mmHg and a diastolic blood pressure > 90 mmHg or an increase ≥ 10% of the latter compared with the pretreatment value at the third consecutive, independently measured reading. It was always confirmed by a number of independent readings at home by the general practitioner.

Statistical analysis

Statistical analyses of the data were performed by standard procedures using paired Student's t-tests. Differences were considered significant when P values were < 0.05.

RESULTS

Renal functional changes

In the 5 patients with gNHL (group I), the ^{99m}Tc-DTPA filtration and ^{99m}Tc-DMSA uptake declined progressively down to 25 ± 10% (mean ± 1 S.E.M.; $P < 0.001$) and 31 ± 11% ($P < 0.005$), respectively, after 6–9 years (Fig. 1a). In the 7 HD patients (group II) these functions were reduced to 75 ± 4% ($P < 0.005$) and 81 ± 3% ($P < 0.005$), respectively (Fig. 1b).

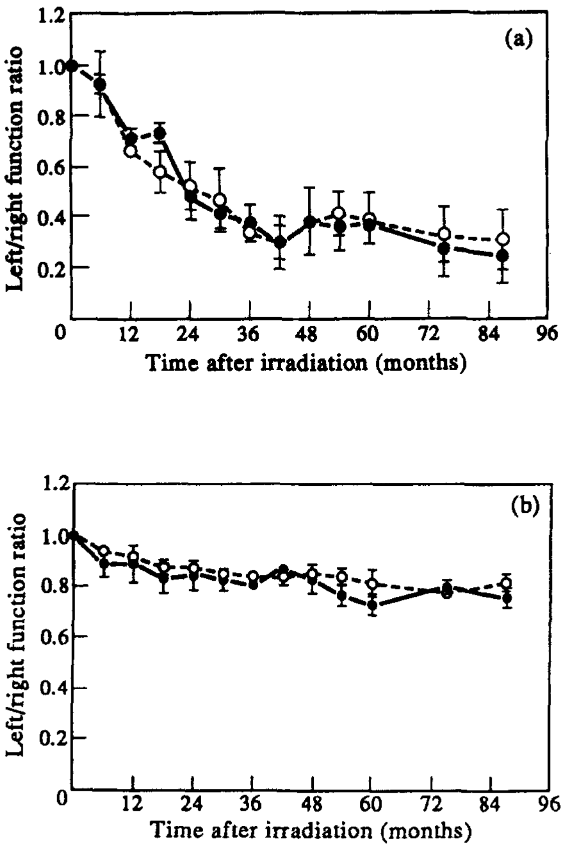

Fig. 1. Relative change in glomerular (●) and tubular function (○) as a function of time in gNHL patients (a) and HD patients (b). Error bars are 1 S.E.M.

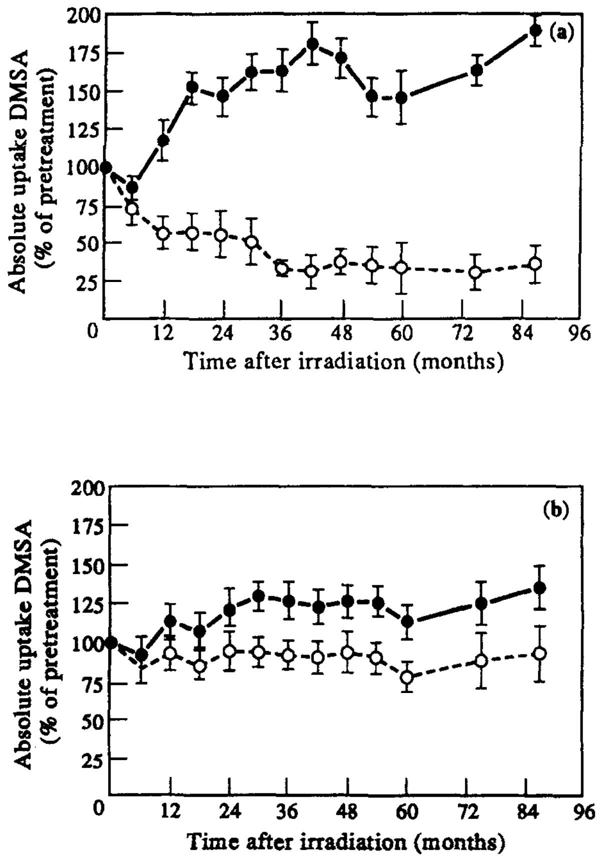

Fig. 2. Change in absolute ^{99m}Tc-DMSA uptake as a function of time in the left (○) and right kidney (●) of gNHL patients (a) and HD patients (b). Error bars are 1 S.E.M.

Separate kidney function was obtained by analysing the changes in absolute renal ^{99m}Tc-DMSA uptake in the left and right kidney. Absolute ^{99m}Tc-DMSA uptake in the left kidney in the gNHL patients decreased progressively to 33 ± 12% ($P < 0.01$) of pretreatment value, whereas the uptake in the right kidney increased to 187 ± 11% ($P < 0.025$; (Fig. 2a). The changes in the HD patients were less dramatic with a slightly decreased uptake in the left kidney, down to 78 ± 10% ($P < 0.025$), and an increase in the right kidney to 135 ± 13% ($P < 0.025$) of pretreatment value (Fig. 2b).

Creatinine clearance decreased significantly in group I from 110 ± 16 ml/min (mean ± 1 S.E.M.) before treatment to 75 ± 13 ml/min ($P < 0.001$) 6–9 years after radiation treatment. In group II, it decreased slightly from 131 ± 8 to 101 ± 10 ml/min. The [^{125}I]iothalamate clearance and [^{131}I]hippuran clearance in the group I patients were 59 ± 4 ml/min (normal range 100–120) and 243 ± 20 ml/min (normal range 500–600), respectively. In group II, the GFR/ERPF values fell within the normal range (data not shown).

No significant changes in renal function or blood pressure were observed in patients with ovarian carcinoma (group III) and seminoma testis (group IV). Therefore, these data are not presented.

Renal volume changes

The volume changes of the kidneys were assessed in the patients irradiated for gNHL (group I) using the SPECT data. It was found that the left kidney shrank to 30 ± 13% ($P < 0.005$) of its pretreatment value, whereas the right kidney showed a moderate increase in volume to 119 ± 7% ($P < 0.005$) (Fig. 3).

Blood pressure measurements

A persistently increased blood pressure was observed in 3 out of 5 patients in group I and in 3 out of 7 patients in group II after a mean time of 16.4 months (range 12–19) postirradiation. 4 of these 6 patients received anti-hypertensive medication.

DISCUSSION

With a minimal follow-up of 7 years, both the glomerular and tubular function decreased in the patients with gNHL and, to a

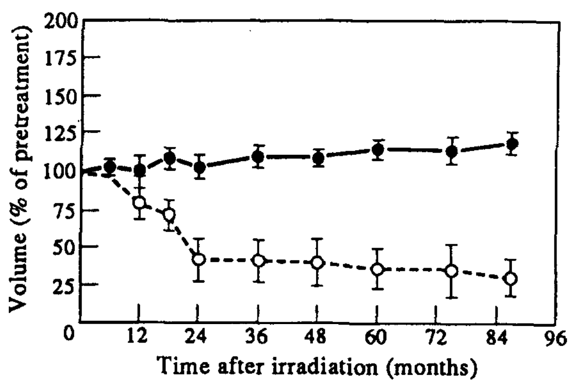

Fig. 3. Relative volume change, as determined with SPECT, of left (○) and right (●) kidney as a function of time in gNHL patients. Error bars are 1 S.E.M.

lesser extent, in patients with HD. The extent of this reduction is in accordance with the results of other investigators [2, 3, 8, 9]. The progressive nature of the renal functional impairment fits in with the concept of late radiation effects in flexible type (F-type) tissues in general [23, 24] and in the kidney in particular [25, 26]. It is, therefore, virtually impossible to pinpoint a specific time point at which the late radiation effects in the kidney are stabilised. The progressive nature of the damage stresses the need for a long-term follow-up of these patients.

The low-dose irradiated right kidney in the gNHL patients appeared to develop a substantial and sustained compensatory hyperfunction. The extent of the hyperactivity estimated was, however, strongly dependent on the assay used, since the volumetric changes with SPECT showed a much smaller increase in the right kidney than the absolute ^{99m}Tc-DMSA uptake. It is likely that the volumetric changes more closely reflect the changes in renal functional mass than do the scintigraphic alterations. There are a number of arguments in favour of this point of view. For instance, renal weight changes in mice, after unilateral kidney irradiation followed by contralateral nephrectomy, have been shown to correlate closely with the number and the size of the remaining tubular cells [13]. In addition, compensatory renal enlargement in the mouse has been shown to be largely due to cellular hypertrophy [13, 26], and, to a lesser extent, due to a hyperplastic cellular response [26–29]. In contrast, scintigraphic analyses in pigs have been shown to largely overestimate renal functional impairment after unilateral kidney irradiation [14]. Single radiation doses in excess of 9 Gy seemed to result in a "non-functioning" kidney with an apparent—presumed functional—recovery after contralateral nephrectomy by 782%, as assessed with [^{131}I]hippuran renography. The weight of these kidneys increased, however, by only 240% at most, 6 months after contralateral nephrectomy [15]. Scintigraphic analyses of renal function after unilateral kidney irradiation, therefore, have to be interpreted with caution. For a paired organ such as the kidney, unequal changes in renal function might, for instance, perturb the distribution and clearance of a bolus i.v. injected radiopharmaceutical agent. The ^{99m}Tc-DMSA scintigraphic analyses in the present study suggest a sustained, near-complete, compensatory response of the low-dose irradiated kidney in the gNHL patients. Overall, renal function in these patients, as assessed with creatinine clearance and iothalamate/hippuran clearance, was substantially reduced. This is in accordance with what would be inferred from the volumetric data. Thus, volumetric analyses appear to reflect more accurately renal functional changes than do scintigraphic assays of individual renal function.

The functional changes observed in the HD patients were much less pronounced than in the gNHL cases. The absolute DMSA activity changes were too small to detect discrepancies with the volumetric changes. Furthermore, creatinine clearance and iothalamate/hippuran clearance were not substantially reduced. However, some clinically relevant radiation damage might have occurred, since 3 of 7 HD patients developed hypertension, presumably of renovascular origin [30].

No significant alterations in renal function were found in the patients with ovarian carcinoma or seminoma. For the ovarian carcinoma patients, this is likely to be due to the fact that equal radiation doses were given to the left and the right kidney, and only to a modest total dose (17–18 Gy). In such cases, external scintigraphic measurements are relatively insensitive in detecting renal functional impairment, even when using the calculated absolute uptake of the radiopharmaceutical agent [19]. In the seminoma patients, the irradiated kidney volume is apparently too small to be detected by external scintigraphy.

In conclusion, unilateral irradiation of the entire left kidney to a total dose of 40 Gy in fractions of 1.5 Gy is associated with a severe and progressive unilateral renal functional impairment, and an incomplete compensatory increased function in the contralateral, low-dose irradiated kidney. The changes in renal function are best quantified by measuring renal volume alterations rather than by analysing absolute uptake of a radiopharmaceutical agent. Continued long-term follow-up is required beyond 7 years after irradiation due to the progressive nature of this process.

1. Avioli LV, Lazor MZ, Cotlove E, Brace KC, Andrews JR. Early effects of radiation on renal function in man. *Am J Med* 1963, **34**, 329–337.
2. Birkhead BM, Dobbs CE, Beard MF, Tyson JW, Fuller EA. Assessment of renal function following irradiation of the intact spleen for Hodgkin's disease. *Radiology* 1979, **103**, 473–475.
3. Le Bourgeois HP, Meigvan M, Parmentier C, Tubiana M. Renal consequences of irradiation of the spleen in lymphoma patients. *Br J Radiol* 1979, 52, 56–60.
4. Kunkler PB, Farr RF, Luxton RW. The limit of renal tolerance to X-rays. *Br J Radiol* 1952, **25**, 190–201.
5. Luxton RW. Radiation nephritis: a long term study of 54 patients. *Lancet* 1961, **II**, 1221–1224.
6. Luxton RW, Kunkler PB. Radiation nephritis. *Acta Radiol Ther Phys Biol* 1964, 2, 169–177.
7. Thompson PL, Mackay IR, Robson GSM, Wall AJ. Late radiation nephritis after gastric X-irradiation for peptic ulcer. *Quart J Med* 1971, **40**, 145–157.
8. Kim TH, Freeman CR, Webster JH. The significance of unilateral radiation nephropathy. *Int J Radiat Oncol Biol Phys* 1980, **6**, 1567–1571.
9. Kim TH, Somerville PJ, Freeman CR. Unilateral radiation nephropathy—the long-term significance. *Int J Radiat Oncol Biol Phys* 1984, **10**, 2053–2059.
10. Dewit L, Anninga JK, Hoefnagel CA, Nooijen WJ. Radiation injury in the human kidney: a prospective analysis using specific scintigraphic and biochemical endpoints. *Int J Radiat Oncol Biol Phy* 1990, **19**, 977–983.
11. Stewart FA, Soranson JA, Alpen EL, Williams MV, Denekamp J. Radiation-induced renal damage. The effects of hyperfractionation. *Radiat Res* 1984, **98**, 407–420.
12. Williams MV, Denekamp J. Radiation induced renal damage in mice: influence of fraction size. *Int J Radiat Oncol Biol Phys* 1984, **10**, 885–893.
13. Michalowski A, Cullen BM, Burgin J, Rogers MA. Development of structural renal damage and its quantitation. *Br J Cancer* 1986, **53** (suppl. VII), 295–297.
14. Robbins MEC, Hopewell JW, Golding SJ. Functional recovery in the irradiated kidney following removal of the contralateral unirradiated kidney. *Radiother Oncol* 1986, **6**, 309–316.
15. Donker AJ, van der Hem GK, Sluiter WJ, Beekhuis H. A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. *Neth J Med* 1977, **20**, 97–103.
16. Gates GF. Glomerular filtration rate: estimation from fractional renal accumulation of ^{99m}Tc-DTPA. *Am J Röntgenol* 1982, **138**, 565–570.
17. Anninga JK, Hoefnagel CA, Dewit L. The role of quantitative 99m-Tc-DTPA renography and 99m-Tc-DMSA scintigraphy in detection and follow up of radiation nephropathy. In Schmidt HAE, Chambron J. *Nuclear Medicine. Quantitative Analysis in Imaging and Function.* Stuttgart, Schattauer, 1990, 417–419.
18. Taylor A. Quantitation of renal function with static imaging agents. *Semin Nucl Med* 1982, 12, 330–344.
19. Groshhar D, Frankel A, Iosilevsky G, *et al.* Quantitation of renal uptake of technetium-99m DMSA using SPECT. *J Nucl Med* 1989, **30**, 246–250.
20. Klumper A, Zwijnenburg A. Dual isotope (^{81}Kr^{m} and ^{99}Tc^{m}) SPECT in lung diagnosis. *Phys Med Biol* 1986, **31**, 751–761.
21. Zwijnenburg A, Klumper A, Roos CM, van der Schoot JB, van

Zandwijk N, Marcuse HR. Lung volume calculations from $^{81}Kr^{m}$ SPECT for the quantification of regional ventilation. *Clin Phys Meas* 1988, 9, 147–154.
22. Michalowski A. Effects of radiation on normal tissues: hypothetical mechanisms and limitations of *in situ* assays of clonogenity. *Radiat Environ Biophys* 1981, 19, 157–172.
23. Wheldon TE, Michalowski AS, Kirk J. The effect of irradiation on function in self-renewing normal tissues with differing proliferative organisation. *Br J Radiol* 1982, 55, 759–766.
24. Lebesque JV, Hart AAM, Stewart FA. Reirradiation at long time intervals in mouse kidney: a comparison between experimental results and predictions of the F type tissue model. *Int J Radiat Biol* 1988, 53, 417–428.
25. Stewart FA, Lebesque JV, Hart AAM. Progressive development of radiation damage in mouse kidneys and the consequences for reirradiation tolerance. *Int J Radiat Biol* 1988, 53, 405–415.
26. Johnson HA, Roman JMV. Compensatory renal enlargement. Hypertrophy versus hyperplasia. *Am J Pathol* 1966, 49, 1–13.
27. Withers HR, Mason KA, Thames HD. Late radiation response of kidney assayed by tubule-cell survival. *Br J Radiol* 1988, 59, 587–597.
28. Soranson J, Denekamp J. Precipitation of latent renal injury by unilateral nephrectomy. *Br J Cancer* 1986, 53 (suppl. VII), 268–272.
29. Otsuka M, Meistrich M. Radiation-induced proliferation in contralateral unirradiated kidneys. *Radiat Res* 1993, 134, 247–250.
30. Verheij M, Dewit L, Valdés Olmos RA, Arisz L. Captopril ^{99m}Tc-DTPA renography in patients with radiation-induced renovascular hypertension. In Schmidt MAC, Höfer R, eds. *Nuclear Medicine.* Stuttgart, Schattauer, 1992, 545–548.

Acknowledgements—The authors wish to thank Prof. H. Bartelink for his continuous support, Dr F.A. Stewart for her fruitful comments and Dr E. Damen for his advice with the SPECT analyses. Mrs M. Langendijk is acknowledged for skilful performing of the iothalamate/hippuran clearances. This study was partially financially supported by the Dutch Cancer Society (Koningin Wilhelmina Fonds), grant NKI 90-15.

Eur J Cancer, Vol. 29A, No. 16, pp. 2243–2248, 1993.
Printed in Great Britain
0959–8049/93 $6.00 + 0.00

Evidence of Post-translational Regulation of P-Glycoprotein Associated with the Expression of a Distinctive Multiple Drug-resistant Phenotype in Chinese Hamster Ovary Cells

Siobhán McClean and Bridget T. Hill

Chinese hamster ovary (CHO) cells following exposure to fractionated X-irradiation *in vitro* dominantly expressed a distinctive multiple drug-resistant phenotype, characterised by resistance to vinca alkaloids, epipodophyllotoxins and colchicine, but not to anthracyclines, together with overexpression of P-glycoprotein (Pgp), but without any concomitant elevation in Pgp mRNA (*J Natl Cancer Inst* 1990, 82, 607–612; 1992, 85, 48–53). To investigate the mechanism of this Pgp overexpression, Pgp stability was examined in an X-irradiation pretreated subline and compared with that of two colchicine-selected drug-resistant CHO sublines. These studies revealed a slower turnover of Pgp in the X-irradiated cells ($T_{1/2} \geq 40$ h) relative to the drug-selected sublines ($T_{1/2} = 17$ h), indicating that Pgp overexpression appears to be differently regulated in these independently-derived resistant sublines. These data add support to our proposal that the development of drug resistance following X-irradiation may arise by a mechanism distinct from that operating after drug selection.
Eur J Cancer, Vol. 29A, No. 16, pp. 2243–2248, 1993.

INTRODUCTION

THE DEVELOPMENT of drug resistance in patients treated with chemotherapy alone or combined with radiotherapy represents a major obstacle to the successful treatment of cancer [1, 2]. *In vitro* multidrug resistance (MDR) is characterised by resistance to many functionally and structurally varied antitumour agents, and overexpression of the 170 kDa membrane protein, P-glycoprotein (Pgp) [3, 4]. Pgp expression is considered to be regulated by a number of mechanisms, including gene amplification [5], transcriptional activation [6] and translational or post-translational modifications [7]. For example, Chinese hamster ovary (CHO) cells, selected clonally with increasing concentrations of colchicine, exhibited an increase in Pgp gene copy number, Pgp mRNA and protein, at every step in the selection procedure [5, 8]. In contrast, Shen *et al.* [6] observed that in colchicine-selected human KB leukaemia sublines, elevation in Pgp mRNA could occur prior to *mdr1* gene amplification. In a series of human ovarian SKOV-3 carcinoma cell lines, derived by selection with increasing concentrations of vincristine or vinblastine, initially Pgp mRNA and Pgp levels increased without any amplification of the *mdr1* gene, but at higher levels of selection, amplification of the DNA occurred, followed at a later stage by increased overexpression of Pgp without any further increase in Pgp mRNA [7].

Recently, we reported that the expression of a stable multiple

Correspondence to B.T. Hill.
The authors are at the Cellular Chemotherapy Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, U.K.
Revised 29 July 1993; accepted 5 Aug. 1993.