Radiation Synovectomy Using ^{165}Dy Ferric-Hydroxide and Oxidative DNA Damage in Patients with Different Types of Arthritis

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Radiation synovectomy is an effective treatment for chronic synovitis refractory to pharmacological treatment in patients with rheumatoid or seronegative arthritis. Concerns persist about possible radiation-induced cytogenetic damage after radiation synovectomy leading to recommendations to use this technique only in the elderly. Micronucleus (MN) frequency in lymphocytes and urinary excretion of 8-hydroxy-2′-deoxyguanosine (8OHdG) as an indicator of cellular oxidative DNA base damage are biomarkers of radiation-induced cytogenetic damage. The course of both biomarkers was studied in patients with different types of chronic synovitis undergoing radiation synovectomy with very short-lived ^{165}Dy–ferric-hydroxide (DFH). **Methods:** Radiation synovectomy of the knee was performed in 13 men and 12 women (mean age, 44 ± 15 y) using a mean activity of 9.48 ± 1.65 GBq ^{165}Dy-DFH in 27 consecutive treatments. MN frequency in lymphocytes and urinary excretion of 8OHdG, measured by high-performance liquid chromatography, were assessed before and 4 (MN only) and 20 h after radiation synovectomy. **Results:** Urinary excretion of 8OHdG in patients (in μmol/mol creatinine; pretreatment mean, 3.1 ± 3.4; median, 2.27) was not significantly different from that in healthy volunteers (mean, 2.0 ± 1.2; median, 1.87) and not altered by radiation synovectomy (post-treatment mean, 2.5 ± 1.5; median, 2.04, NS). An increase in 8OHdG levels after radiation synovectomy of more than 1 SD was found in only 1 patient, who experienced leakage to the lymph nodes but who already had elevated urinary 8OHdG levels before treatment. The frequency of MN/500 binucleated cells (BNCs) was slightly lower in patients (pretreatment mean, 4.3 ± 2.6; median, 4.25) than in healthy volunteers (mean, 5.4 ± 2.3; median, 5.3) and did not significantly change after therapy, either (4-h post-treatment mean, 3.9 ± 2.1, median, 3.8; 20-h post-treatment mean, 4.1 ± 2, median 3.8 MN/500 BNC). In 22 of 27 treatments, no leakage to nontarget organs could be monitored, whereas leakage to the local lymph nodes and the liver was detected after 5 treatments. **Conclusion:** Radiation synovectomy using ^{165}Dy-DFH causes no significant radiation burden to most patients as indicated by the absence of adverse changes in levels of biomarkers of cytogenetic damage and a low incidence of leakage. These data suggest that the risk of malignancy may not be elevated.

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Radiation synovectomy is an alternate, locally acting treatment for chronic synovitis refractory to the repetitive, intraarticular application of glucocorticoids and systemic pharmacological treatment in patients with rheumatoid or seronegative arthritis (RA or SA, respectively). A few case reports exist in the literature about malignancies after radiation synovectomy (*1*). Therefore, concerns have persisted about possible radiation-induced cytogenetic damage after radiation synovectomy (*2*). Chromosomal studies of circulating lymphocytes revealed an increased incidence of radiation-induced aberrations in patients undergoing radiation synovectomy with ^{198}Au or ^{90}Y (*3–9*). The immobilization of the treated joint after intraarticular injection (*10*) and the introduction of new preparations of ^{90}Y (*6*) or new radionuclides with short half-lives and relatively large particulate carriers (*11–13*) have significantly reduced the frequency and degree of leakage from the treated joint, decreasing the irradiation of local lymph nodes and lymphocytes. The micronucleus (MN) assay that measures the frequency of MN in lymphocytes is an accepted method of analysis for radiation-induced chromosomal aberrations. MN result from failure of chromosomal fragments or of whole chromosomes to incorporate into daughter nuclei during mitosis (*14*). The relation between the degree and site of leakage and the frequency of MN is not consistent (*15*). Furthermore, levels of biomarkers of cytogenetic damage were analyzed at extended time intervals after radiation synovectomy, with a possible influence of confounding factors, particularly when using a very short-lived radionuclide such as ^{165}Dy (*16,17*).

Urinary excretion of 8-hydroxy-2′-desoxyguanosine (8OHdG) has been described as a sensitive marker of oxidative DNA damage by ionizing radiation linked to the response to radiotherapy and chemotherapy in lung cancer patients (*18*). The levels of 8OHdG have not been assessed

in patients undergoing radionuclide therapy. Measurement of urinary excretion of 8OHdG as a marker of oxidative DNA damage by radiation is a highly sensitive method (*19*). 8OHdG might be more suitable for biological dosimetry than the MN assay, the latter showing in some cases a low proliferation response of lymphocytes isolated from patients with chronic inflammatory disease (*9,14*). 8OHdG reflects total radiation energy absorbed by different cells, whereas MN relate to the DNA damage in lymphocytes. This difference might be of importance since it is not known whether any suggested increase in malignancy risk already derives from irradiation of the highly vascular inflamed synovium or, in the case of leakage of circulating lymphocytes, through the lymph nodes (*3,4*).

We postulated that the urinary excretion of 8OHdG might provide a useful index of total DNA damage (i.e., that which has occurred but has been repaired) in patients undergoing radiation synovectomy. Therefore, this study aimed to investigate the course of biomarkers and their relevance in patients with different types of chronic synovitis of the knee treated with radiation synovectomy with ^{165}Dy–ferric-hydroxide (FDH).

MATERIALS AND METHODS

^{165}Dy-DFH Preparation

^{165}Dy-DFH is a precipitate of ^{165}Dy-hydroxide on Fe(II)Fe(III)hydroxide used as carrier. The improved preparation of the carrier was previously described in detail (*13*).

The carrier was prepared as follows: 90 mg $FeSO_4 \times 7\ H_2O$ and 14 mg $Fe(NO_3)_3 \times 9H_2O$ were dissolved in 0.5 mL 0.1 mol/L H_2SO_4 and diluted with 7.5 mL H_2O. Two mL 0.5 mol/L NaOH were slowly added while shaking the vial. The 10-mL vial was sealed and heated for 30 min at 90°C. The color of the suspension changed from green to deep black during heating. The suspension was filtered through an 8-μm Nuclepore polycarbonate membrane (Millipore Inc., Bedford, MA) before use.

A 3-mg sample of natural Dy_2O_3 (Aldrich 20.318–1; Aldrich, Vienna, Austria) was dissolved in concentrated HNO_3 and evaporated in a quartz ampoule. The resulting $Dy(NO_3)_3$ was neutron-irradiated in the 9-MW nuclear reactor of the Austrian Research Center Seibersdorf. The radiochemical preparation was performed under remote handling conditions in a lead-shielded cell. The target was dissolved in 1 mL 0.1 mol/L H_2SO_4 and transferred into a 10-mL septum-closed vial. To precipitate the ^{165}Dy-DFH, a mixture of 1.5 mL 0.5 mol/L NaOH and 0.2 mL ferromagnetic "black" Fe(II)Fe(III)hydroxide suspension (0.4 mg Fe) was added, treated in an ultrasonic bath, and heated for 5 min at 90°C. After resuspension the ^{165}Dy-DFH particles were immobilized at the vial wall by applying an electromagnet. The solution was removed and discarded. The ^{165}Dy particles were washed with 0.01 mol/L NaOH, resuspended in 0.01 mol/L NaOH/0.9% NaCl solution (pH 10–11), resuspended in the same solution, and autoclaved at 120°C for 30 min.

Study Protocol and Patients

The use of radiation synovectomy was approved by the Ethical Committee of the University of Vienna, and all patients gave their written informed consent to participate. The study included 25 patients (13 men, 12 women; mean age, 44 ± 15 y; range, 19–69 y) with RA (n = 6), psoriatic arthritis ([PA] n = 2), reactive arthritis ([ReA] n = 2), osteoarthritis ([OA] n = 3), pigmented villonodular synovitis ([PVNS] n = 4), and SA (n = 5). Of the remaining 3 patients, 1 had adult-onset Still's disease, another had arthritis associated with inflammatory bowel disease (IBD), and another had ankylosing spondylitis. Patients were eligible for radiation synovectomy if suffering from persistent synovitis of the knee refractory to systemic and local pharmacological treatment, but without any evidence of infection, trauma, or joint instability. Local management involved glucocorticoids that were injected intraarticularly. Systemic pharmacotherapy was defined as the use of nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and disease-modifying antirheumatoid drugs for at least 3 mo. The latter were taken by patients with autoimmune disease (RA, Still's disease, IBD). NSAIDs were taken by all patients. All patients had undergone multiple arthrocenteses of the affected knee with cytological analysis for confirmation of diagnosed chronic synovitis. Patients with SA, ReA, and OA were treated when arthritis scintigraphy with ^{99m}Tc-human immunoglobulin (HIG) had indicated synovitis. HIG scintigraphy was used as the imaging modality, because this method yields high accuracy in detecting and grading inflammatory joint disease (*20*).

Twelve patients (including all patients with PVNS) had undergone diagnostic arthroscopy at least 6 wk before radiation synovectomy. Patients with RA were included after excluding a state of acute polyarticular exacerbation of the disease. Radiographs taken before inclusion were classified according to Larsen et al. (*21*).

The use of any accepted form of contraception was obligatory for any fertile women treated with ^{165}Dy-DFH. Exclusion criteria for treatment with ^{165}Dy-DFH were pregnancy, any life-threatening or infectious disease, or the presence of a major Baker cyst of the respective joint.

Each patient was admitted to the application room at calibration time. Arthrocentesis was performed in this room, under strictly aseptical conditions, with the patient in a supine position. ^{165}Dy-DFH was injected into the joint space using a 1.2-mm gauge needle. The knee was then moved once or twice through a flexion/extension arc and immobilized in extension. The patient was confined to rest for 5–6 h to minimize movement. Both knee joints were treated with an interval of at least 2 mo in 2 patients.

Estimation of Activity for Therapy

Earlier experience showed a target dose of about 100 Gy to be necessary for the therapeutic effect of radiation synovectomy. The activity to be applied to obtain a dose of 100 Gy was derived from the equation for estimation of radiation for the evaluation of the dose D resulting from β-emitting sources distributed in a volume V (*13*). The contribution of γ-rays to the energy dose is less than 0.1% and therefore negligible. Injected activities (Table 1) were sometimes lower than calculated activities, because of the time gap between calibration and application and small amounts of radioactivity remaining in the syringe.

Biokinetics and Biodistribution: Determination of Leakage

γ Camera Imaging. Imaging of the ^{165}Dy distribution was performed using a large-field-of-view γ camera adjusted to 94.7 keV ^{165}Dy rays (3.6%). A low-energy collimator was used. Data were stored by a computer for subsequent analysis and review. Patients were scanned in an anterior view 1 and 3 h after intraarticular injection of ^{165}Dy-DFH.

Whole-Body Counter Measurements. Whole-body counter measurements were performed as published (*13*). In summary, the

TABLE 1
Patient and Volunteer Characteristics

	Patients	Healthy volunteers
Sex (M/F)	13/12	MN, 18/25; 8OHdG, 41/41
Age (y)	44 ± 15	MN, 38 ± 11; 8OHdG, 38 ± 12
Activity applied (GBq)	9.48 ± 1.65	
Larsen stage	2 (0–4)	
Smokers	8	
MN before therapy*	4.3 ± 2.6; 4.3 (1.4–9.9)	5.4 ± 2.3; 5.3 (1.3–11.7)
MN 4 h after therapy*	3.9 ± 2.1; 3.6 (1.3–7.3)	
MN 20 h after therapy*	4.1 ± 2.0; 3.8 (0.7–7.9)	
8OHdG before therapy†	3.1 ± 3.4; 2.3 (0.8–13.7)	2.0 ± 1.2; 1.9 (0.1–5.8)
8OHdG 20 h after therapy†	2.5 ± 1.5; 2.0 (1.0–6.1)	
Leakage to lymph nodes	3 (twice in 1 patient)	
Leakage to liver	2	

*MN given as MN/500 binucleated cells (BNCs).
†8OHdG given as μmol/mol creatinine.
Values are given as frequencies or mean ± SD and/or median with range.

clinical whole-body counter is a shadow-shield construction consisting of the shielding and the transport construction. The latter carries the guide rails of the movable patient bed, the shielding of the measurement area, and the holding device of detectors and collimators. Two-slit collimators of 10-cm thickness can be moved in front of the detectors. A slit width of 2.5 mm was used for all measurements.

Activity profiles were measured with 4 NaI crystal detectors that are arranged in pairs above and below the bed. Within 10 min, 345 scanning steps of 1 cm each were performed. Some hours after application, the activity was localized from activity profiles that were obtained with a clinical whole-body counter. The scan length is displayed as the longitudinal axis of the body (x axis), whereas the counts per step (1 step = 1 cm) are plotted on the y axis. From the count ratio of the upper to the lower and from the left to the right detectors, a localization of activity point sources can be obtained orthogonally to the scan direction. In all coordinates spatial resolution is 1 cm. Under the measurement conditions described earlier, the efficiency was calculated to be 5.5 MBq. The linearity of the efficiency is guaranteed below an activity of 1.8 GBq.

Patients were scanned 2, 4, and, in case of leakage, 6 h after intraarticular injection of ^{165}Dy-DFH.

Measurement of Blood Activity. Blood activity levels were determined from 8-mL blood samples taken before and 1 and 3 h after therapy, using a γ counter calibrated for ^{165}Dy.

Arthritis Scintigraphy with ^{99m}Tc-HIG

Ten patients with SA, OA, or ReA underwent scintigraphy with ^{99m}Tc-HIG to visualize the intensity of inflammation. One milligram of modified polyclonal HIG (Technescan HIG; Mallincrodt Medical BV, Petten, The Netherlands) was labeled with ^{99m}Tc-pertechnetate according to the instructions of the manufacturer. Ten patients were injected intravenously with 740 MBq. Imaging was performed 4 and, if clinically necessary, 24 h after injection using a low-energy, general-purpose collimator. Anterior spot images of the respective joints were obtained at a preset time of 7 min. The images were collected in a 256 × 256 matrix for quantitative analysis and visually interpreted (Fig. 1).

Determination of Biomarkers for Genotoxicity

Cell Culture and Cytokinesis Block MN Assay. The MN frequency in lymphocytes was calculated from 22 venous blood samples taken immediately before and 4 and 20 h after treatment. Blood samples from 43 healthy volunteers (18 men, 25 women) of comparable age (38 ± 11 y) were analyzed according to the same protocol. The test protocol for cytokinesis block MN assay was adapted from Fenech (*22*) with some modifications. Lymphocytes were isolated from 5 mL whole blood by a density centrifugation with Ficoll–Paque (Pharmacia Biotech, Pitscataway, NJ) and washed twice with 3 mL pH 7.2 phosphate-buffered saline. Isolated lymphocytes were suspended in 5.0 mL chromosome medium 1A supplemented with phytohemagglutinin (Gibco BRL; Life Technologies, Paisley, UK) and incubated for 72 h at 37°C. At 44 h, Cytochalasin B (Sigma Chemical, St. Louis, MO) was added at a final concentration of 3 μg/mL. At the end of the incubation period, the cells were pelleted by centrifugation, followed by hypotonic treatment (0.075 mol/L KCl) for 8 min to cause cell swelling. The cells were pelleted again, fixed with methanol:glacial acetic acid (1:1), dropped on slides, air-dried, and stained with 4′,6-diamidino-2-phenylindole ([DAPI] Serva, Heidelberg, Germany). The slides were coded and at least 2000 binucleated cells (BNCs) with well-preserved cytoplasm were scored and evaluated for the incidence of MN. More than 1 MN in a BNC was still scored as 1 event. The criteria for identification of MN were: (a) DAPI positive, (b) the same intensity of stain as 1 of the main nuclei, (c) diameter <20% of the smaller of the main nuclei, (d) a round and well-defined shape, and (e) in the same plane of focus.

Urinary Excretion of 8OHdG

The urinary excretion of 8OHdG was measured by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (*23*). In brief, morning urine specimens underwent a clean-up procedure by a 2-step solid phase extraction with Bond Elut C_{18}/OH cartridges (Varian, Harbor City, CA) using 50 mmol/L KH_2PO_4, pH 7.5 (buffer A), with 15% and 20% methanol as eluents. To remove methanol, the samples were evaporated and subsequently filled with buffer A to give a final volume of 1.5 mL. An 80-μL aliquot of the prepared eluate was injected into the HPLC system. Quantification of 8OHdG in urine was performed by peak-height measurement and results are expressed as 8OHdG-to-creatinine ratios (μmol/mol). Levels of 8OHdG were also analyzed in spontaneous urine of 82 healthy nonsmokers (41 men, 41 women; mean age, 38 ± 11 y) serving as controls.

Cytological Evaluation of Knee-Joint Effusions

All effusions obtained on the day of treatment or the days after were analyzed blindly. Significant amounts of effusions (>10 mL) were drawn from 15 patients on the day of treatment and from 4 patients within a few days after radiation synovectomy. It is well known that some patients experience adverse effects (e.g., swelling and pain) after radiation synovectomy. In our experience and that of

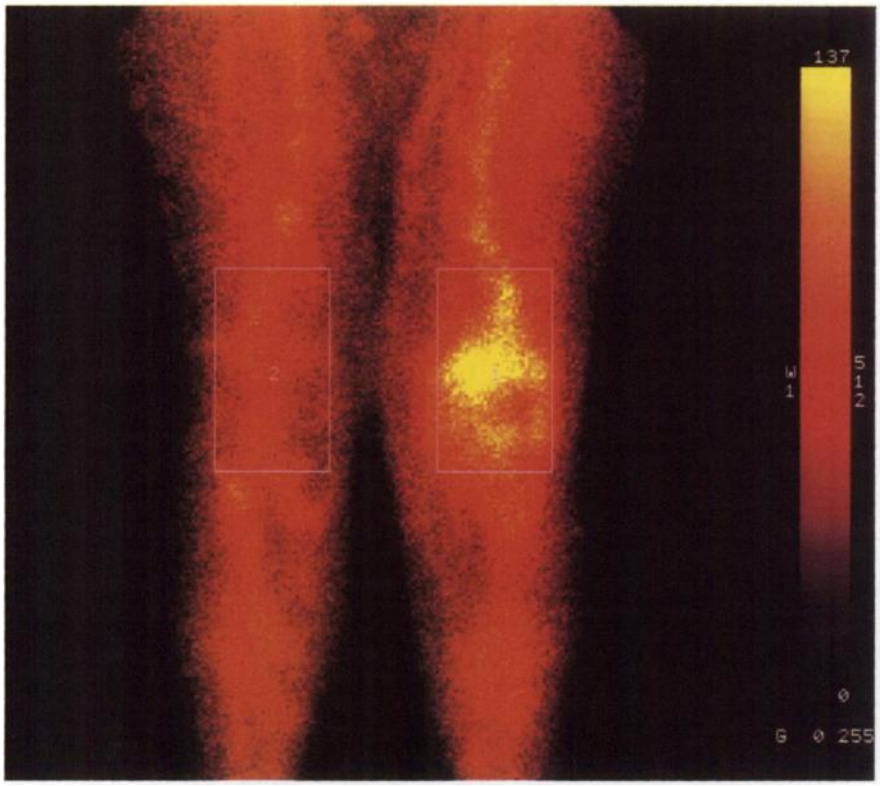

FIGURE 1. ^{99m}Tc-HIG scintigraphy (4 h after intravenous injection) in patient with seronegative arthritis of left knee joint before radiation synovectomy. Active synovitis of left knee joint is visualized by markedly increased retention of tracer, whereas there is no evidence of any inflammatory process in right knee joint.

others (*24*), these symptoms are most likely caused by a transient inflammatory response. We hypothesized that, in the event of an acute inflammatory response, both increased local blood flow and vascular permeability could induce an increase in oxidative DNA damage or MN frequency in circulating lymphocytes. For that reason, post-treatment effusions were drawn when clinically required for palliation of symptoms and analyzed.

Microscopic Evaluation. Effusions were examined twice by 1 of the authors, who was unaware of the clinical and cytogenetic findings. Cytological analysis was performed after Giemsa staining using a magnification of ×400 and the following microscopic features were assessed semiquantitatively: fibrin exudation and mononuclear and polymorphonuclear cell infiltration.

In Vitro Experiments for the Determination of Free ^{165}Dy-DFH in Effusions

A 5-mL sample of synovial fluid, obtained immediately before radiation synovectomy by arthrocentesis of the treated knee, was incubated with 0.5 mL ^{165}Dy-DFH (740 MBq ^{165}Dy-DFH, containing 0.05 mg of the natural Dy_2O_3). The amount of free ^{165}Dy (<0.45 μm) was determined by the use of a Millipore filter (Millipore) after homogenization at 37°C for 2 and 4 h. For comparison, 0.5 mL ^{165}Dy-DFH were incubated with 5 mL 0.9% saline solution. The mean pH value of effusions drawn from 7 patients immediately before treatment was 8.4 ± 0.33. The amount of free ^{165}Dy with a diameter <0.45 μm was 0.96% ± 0.36% after 2 h and 1.5% ± 0.33% compared with 0.03% for ^{165}Dy-DFH incubated with a reference solution of 0.9% saline.

Statistical Analysis. Data are given as frequencies or mean ± SD. Medians with range are added for biomarkers of cytogenetic damage, because these were not distributed normally. Nonparametric tests (Wilcoxon, Friedman) were used for comparisons of pretreatment and posttreatment values. Correlation analyses were used to relate the levels of biomarkers before and after radiation synovectomy. Statistical difference was assumed if the null hypothesis could be rejected at the 0.05 probability level.

RESULTS

Patient characteristics and results are given in Table 1.

Biokinetics and Biodistribution: Determination of Leakage

γ Camera Imaging. Imaging of the ^{165}Dy-DFH distribution revealed some leakage to the local lymph nodes in 1 patient.

Whole-Body Counter Measurements. Whole-body counter measurements revealed some leakage to the liver in 2 patients and the lymph nodes in 2 patients (Table 2). Maximum nontarget organ doses to the lymph nodes and liver were 6.92 and 0.2 Gy, respectively, as calculated by MIRDOSE3 (Oak Ridge National Laboratory, Oak Ridge, TN). Injected activities were homogeneously distributed intra-articularly in all treated patients.

Measurement of Blood Activity. In most patients any leakage to the blood was not measurable. In 9 patients leakage to the blood was detected with a median of 0.12% of the applied activity (range, 0.001%–0.95%).

Arthritis Scintigraphy with ^{99m}Tc-HIG

Arthritis scintigraphy with ^{99m}Tc-HIG revealed an increased retention of the tracer in all investigated knees of patients undergoing radiation synovectomy for arthritis (Fig. 1).

TABLE 2
Characteristics of Patients with Leakage of ^{165}Dy-DFH

Patient no.	Sex	Age (y)	Smoker	Diagnosis	Site of leakage	Exposure* (Gy)	MN†			8OHdG‡	
							T_0	T_1	T_2	T_0	T_2
1	F	44	Yes	SA	Liver	0.2	4.4	3.7	7.3	NA	NA
2	F	50	No	Still's disease	Lymph nodes	5.15	NG	NG	NG	5.4	7.1
						4.31	NG	NG	NG	5.4	5.5
3	F	58	No	PVNS	Liver	0.07	2.5	2.2	2.7	2.5	1.4
4	F	58	No	SA	Lymph nodes	6.92	7.2	6.6	4.1	NA	NA

*Radiation exposure calculated according to MIRDOSE3.
†MN given as MN/500 BNC.
‡80 HdG given as μmol/mol creatinine.
T_0 = before therapy; T_1 = 4 h after therapy; T_2 = 20 h after therapy; NA = not available; NG = lymphocytes not grown.
Values given as frequencies, mean ± SD, and/or median with range.

MN Frequency in Peripheral Blood Lymphocytes

There was no significant difference in the mean frequencies of MN/500 BNCs in patients (4.3 ± 2.6; median, 4.25) and healthy volunteers (5.4 ± 2.3; median, 5.3), although MN frequency tended to be lower in patients. MN frequency did not significantly change after radiation synovectomy (Table 1). An increase in the frequency of MN of >1 SD after radiation synovectomy was found in 3 patients, of whom 2 already had elevated baseline levels (cutoff level, 4 MN/500 BNC). One of these patients revealed some leakage to the liver (patient 1, Table 2). Biomarker levels of patients with autoimmune disease are shown in Table 3.

Urinary Excretion of 80 HdG

There was no significant difference in urinary 8OHdG (μmol/mol creatinine) levels in patients (pretreatment mean, 3.1 ± 3.4; median, 2.27) and healthy volunteers (2.0 ± 1.2; median 1.87). Urinary excretion of 8OHdG was not significantly altered by radiation synovectomy (Table 1). An increase in urinary excretion of 8OHdG (μmol/mol creatinine) after radiation synovectomy of more than 1 SD was shown in only 1 patient (patient 2, Table 2), with elevated baseline 8OHdG levels far outside the normal range. This female patient was treated twice and revealed some leakage of activity to the local lymph nodes after both treatments but with no further increase in urinary 8OHdG excretion. Patients with autoimmune disease exhibited increased levels of 8OHdG (Table 2).

TABLE 3
Biomarkers of Cytogenetic Damage in 9 Patients with Autoimmune Disease

Sex (M/F)	4/5
Age (y)	53 ± 9
Activity applied (GBq)	9.57 ± 1.35
Larsen stage	2 (1–3)
Smokers	3
C-reactive protein (mg/dL)	5.4 (1.9–13)
MN before therapy*	5.2 ± 2.9
MN 4 h after therapy*	4.9 ± 1.7
MN 20 h after therapy*	4.8 ± 1.7
8OHdG before therapy†	4.5 ± 2.9
8OHdG 20 h after therapy†	3.9 ± 2.2
Leakage to lymph nodes	3 (twice in 1 patient)
Leakage to liver	0

*MN given as MN/500 BNC.
†80 HdG given as μmol/mol creatinine.
Values given as frequencies, mean ± SD, and/or median with range.

Correlation Analysis

No relations were found among applied activity, age, and biomarker levels in our study. Pre- and post-treatment levels of biomarkers were significantly related to each other (8OHdG: pre- versus post-treatment levels, $r = 0.627$, $P < 0.001$; MN frequency: pre- versus 4-h post-treatment levels, $r = 0.546$, $P = 0.003$; pre- versus 20-h post-treatment levels, $r = 0.420$, $P = 0.029$).

Adverse Effects

Post-treatment effusions and local tenderness were observed in 7 patients within a few days after treatment with a maximum duration of 6 d. Arthrocentesis was performed in 4 patients to resolve the symptoms quickly.

Cytological Evaluation of Effusions of the Knee Joint

All 15 pretreatment effusions analyzed revealed signs of chronic inflammation characterized by the predominance of lymphocytes. Post-treatment effusions were obtained within 48 h after tracer injection from those 4 patients requiring arthrocentesis for palliation of symptoms. One patient each had PA, SA, RA, and arthritis associated with IBD. Microscopic evaluation revealed signs of an acute inflammatory response with an excess of neutrophils as compared with the cytological pretreatment pattern.

DISCUSSION

This study demonstrates that both MN frequency and urinary excretion of 8OHdG do not significantly change in

patients undergoing radiation synovectomy with ^{165}Dy-DFH. The assessments of MN frequency as a biomarker of cytogenetic damage might have some limitations in chronic inflammatory disease. The sensitivity of the MN assay might be limited even more when patients were treated with irradiation, as it was reported in some cases that lymphocytes had not responded to growth stimuli (*16,25*). Indeed, MN frequency could not be determined in 3 patients, all of whom were treated with a combination therapy of immunosuppressive drugs. It is unclear whether the underlying disease or immunosuppressive treatment or a combination of both is responsible for this effect. Urinary 8OHdG excretion was assessed as an alternative biomarker for biological dosimetry. The results suggest that the total radiation burden absorbed by cells is not significantly altered by radiation synovectomy with ^{165}Dy-DFH. However, the dose–response curve of biomarkers to radiation shows marked interindividual variations that characterize a variable susceptibility to cytogenetic factors (e.g., radiation, cancer, inflammation, or smoking) (*26*). We found a trend toward an increased urinary excretion of 8OHdG in patients as compared with healthy volunteers. In agreement with previous studies, the increase was the most pronounced in patients with autoimmune disease (e.g., RA) (*27*). Inflammation can accelerate the development of cancer (*28*), but the link between inflammation and cancer is by no means a simple one. On the one hand, one might hypothesize that persistently elevated biomarker levels reflect the fact that patients with RA are exposed to an increased risk of cancer and mortality, independent of whether they were taking antirheumatoid drugs (*29–31*). On the other hand, cancers do not develop with certainty at the most intense site of oxidative stress (i.e., the synovium), where excessive cell proliferation is present in patients with RA. High levels of MN and 8OHdG were seen in another patient with chronic inflammation, suffering from arthritis associated with IBD. This is in agreement with recent reports about an increased oxidative stress and decreased antioxidant defenses in IBD (*32*).

Significant leakage was rarely observed in this study and not relevant except in 2 patients. Doses to nontarget organs were far below those reported for other radionuclides, thus confirming earlier studies (*13,17*). However, it is a major task to identify causes of leakage other than early mobilization (*1*), because leakage might increase the risk of malignancies, particularly when localized in the lymph nodes. Recently, we postulated that joint effusions could promote the dissociation of the ^{165}Dy-DFH complex (*13*). In vitro studies revealed that the amount of free ^{165}Dy was significantly increased after incubation with the patients' effusions as compared to the addition of physiologic saline solution. Nevertheless, the amount of free ^{165}Dy contributed negligibly to nontarget organ exposure as clearly demonstrated by very low levels of radioactivity in the blood of patients in whom effusions were analyzed. Leakage to the blood was not related to any leakage to the lymph nodes or the liver, either. Translated into clinical practice, we suggest the complete removal of any effusion from the treated joint and to inject physiologic saline solution if required for activity distribution.

Any local adverse reaction (e.g., swelling or pain) was infrequently observed and temporary in this study. Local adverse reactions were not associated with changes in biomarker levels, although an acute inflammatory response with a marked shift in the cytological pattern of the joint effusions was seen within a few hours after radiation synovectomy in 4 patients.

Limitations

Biomarkers of cytogenetic damage show marked interindividual variations as a result of various exogenous and endogenous factors. However, the use of a protocol that assessed levels of biomarkers just before and 4 and 20 h after treatment reduced the probability that other factors might have influenced the results.

CONCLUSION

This study demonstrates that radiation synovectomy using ^{165}Dy-DFH does not cause any significant oxidative DNA damage, which suggests that the risk of malignancy may not be elevated. The underlying disease should be considered when interpreting the levels of biomarkers of cytogenetic damage in terms of malignancy risk in patients with chronic synovitis undergoing radiation synovectomy.

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REFERENCES

1. Litpon JH, Messner HA. Chronic myeloid leukemia in a women with Still's disease treated with ^{198}Au synoviorthesis. *J Rheumatol.* 1991;18:734–735.
2. Siegel ME, Siegel HJ, Luck JV. Radiosynovectomy's clinical applications and cost effectiveness: a review. *Semin Nucl Med.* 1997;27:364–371.
3. De La Chapelle A, Oka M, Rekonen A, Ruotsi A. Chromosome damage after intra-articular injections of radioactive yttrium. *Ann Rheum Dis.* 1972;31:568–572.
4. Stevenson AC, Bedford J, Dolphin GW, et al. Cytogenetic and scanning study of patients receiving intra-articular injections of gold-198 and yttrium-90. *Ann Rheum Dis.* 1973;32:112–123.
5. Jalava S. Irradiation synovectomy in experimental arthritis and in human rheumatoid arthritis. *Scand J Rheumatol.* 1974;2(suppl):1–32.
6. Gumpel JM, Beer TC, Crawley JCW, Farran HEA. Yttrium-90 in persistent synovitis of the knee—a single centre comparison: the retention and extra-articular spread of four Y-90 radiocolloids. *Br J Radiol.* 1975;48:377–381.
7. Doyle DV, Glass JS, Gow PJ, Daker M, Grahame R. A clinical and prospective chromosomal study of yttrium-90 synovectomy. *Rheumatol Rehab.* 1977;16:217–222.
8. Lloyd DC, Reeder EJ. Chromosome aberrations and intra-articular yttrium-90. *Lancet.* 1978;1:617.
9. Daker M. Prospective study on chromosomal analyses in 30 patients with ^{90}Y synovectomy. *Therapiewoche.* 1979;29:492–496.
10. Williams PL, Crawley JCL, Freeman AM, Lloyd DC, Gumpel JM. Feasibility of outpatient management after intra-articular yttrium-90: comparisons of two regimens. *Br Med J.* 1981;282:13–14.
11. Hnatowich DJ, Kramer RI, Sledge CB, Noble J, Shortkoff S. Dysprosium-165 ferric hydroxide macroaggregates for radiation synovectomy. *J Nucl Med.* 1977;19:303–308.

12. Sledge CB, Zuckerman JD, Zalutsky MR, et al. Treatment of rheumatoid synovitis of the knee with intra-articular injection of dysprosium 165-ferric hydroxide macroaggregates. *Arthritis Rheum.* 1986;29:153–159.
13. Pirich C, Prüfert U, Havlik E, et al. Monitoring of biodistribution and biokinetics of dysprosium-165 ferric-hydroxide with a shadow-shield whole-body counter. *Eur J Nucl Med.* 1997;24:398–402.
14. Carrano A, Heddle JA. The fate of chromosome aberrations. *J Theor Biol.* 1973;38:289–304.
15. Noble J, Jones AG, Davies MA, Sledge CB, Kramer RI, Livni E. Leakage of radioactive particle systems from synovial joint fluid studied with a gamma camera. *J Bone Joint Surg.* 1983;65A:381–389.
16. Prosser JS, Izard BE, Brown JK, et al. Induction of micronuclei in peripheral blood lymphocytes of patients treated for rheumatoid or osteoarthritis of the knee with dysprosium-165 hydroxide macroaggregates or yttrium-90 silicate. *Cytobiosis.* 1993;73:7–15.
17. Edmonds J, Smart R, Laurent R, et al. A comparative study of the safety and efficacy of dysprosium-165 hydroxide macro-aggregate and yttrium-90 silicate colloid in radiation synovectomy: a multicenter double-blind trial. *Br J Rheumatol.* 1994;333:947–953.
18. Erhola M, Toyokuni S, Okada K, et al. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2′-desoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett.* 1997;409:287–291.
19. Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. *Free Radical Res.* 1986;1:163–172.
20. Pons F, Moya F, Herranz R, et al. Detection and quantitative analysis of joint inflammation with ^{99m}Tc-polyclonal human immunoglobulin. *Nucl Med Commun.* 1993;14:225–231.
21. Larsen A, Dale K, Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. *Acta Radiol Diagn.* 1977;18:481–491.
22. Fenech M. The cytokinesis-block micronucleus assay in nucleated cells. *Prog Clin Biol Res.* 1990;340B:195–206.
23. Germadnik D, Rüdiger HW. Assay for the determination of urinary 8-hydroxy-2′-deoxyguanosine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci App.* 1997;689:399–403.
24. Clunie G, Lui D, Cullum I, Ell PJ, Edwards JCW. Clinical outcome after one year following samarium-153 particulate hydroxyapatite radiation synovectomy. *Scand J Rheumatol.* 1996;25:360–366.
25. Gantenberg HW, Wuttke K, Streffer C, Müller WU. Micronuclei in human lymphocytes irradiated in vitro or in vivo. *Radiat Res.* 1991;128:276–281.
26. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender, and body mass-index. *Carcinogenesis.* 1992;13:2241–2247.
27. Lunec J, Herbert K, Blount S, Griffiths HR, Emery P. 8-hydroxydeoxyguanosine: a marker of oxidative DNA damage in systemic lupus erythematosus. *FEBS Lett.* 1994;348:131–138.
28. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J.* 1995;313:17–29.
29. Wolfe F, Mitchell DM, Sibley JT, et al. The mortality of rheumatoid arthritis. *Arthritis Rheum.* 1994;37:481–494.
30. Myllykangas-Luosujarvi R, Aho K, Isomaki H. Mortality from cancer in patients with rheumatoid arthritis. *Scand J Rheumatol.* 1995;24:76–78.
31. Tugwell P, Pincus T, Yogum D, et al. Methotrexate-Cyclosporine Combination Study Group: combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. *N Engl J Med.* 1995;333:137–141.
32. Lih-Brody L, Powell SR, Collier KP, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci.* 1996;41:2078–2086.