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CHROMOSOME ABERRATION ANALYSIS IN PERIPHERAL LYMPHOCYTES OF GULF WAR AND BALKANS WAR VETERANS

H. Schröder†‡, A. Heimers†‡, R. Frentzel-Beyme†‡, A. Schott§ and W. Hoffmann†‡
†Center of Environmental Research and Technology (UFT),
University of Bremen, Leobener Strasse, 28359 Bremen, Germany
‡Bremen Institute for Prevention Research, Social Medicine,
and Epidemiology (BIPS), Linzer Strasse 8, 28359 Bremen, Germany
§World Depleted Uranium Center Berlin (WoDUC),
and decoding office, Harnackstrasse 18, 14195 Berlin, Germany

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Abstract — Chromosome aberrations and sister chromatid exchanges (SCEs) were determined in standard peripheral lymphocyte metaphase preparations of 13 British Gulf War veterans, two veterans of the recent war in the Balkans and one veteran of both wars. All 16 volunteers suspect exposures to depleted uranium (DU) while deployed at the two different theatres of war in 1990 and later on. The Bremen laboratory control served as a reference in this study. Compared with this control there was a statistically significant increase in the frequency of dicentric chromosomes (dic) and centric ring chromosomes (cR) in the veterans' group, indicating a previous exposure to ionising radiation. The statistically significant overdispersion of dic and cR indicates non-uniform irradiation as would be expected after non-uniform exposure and/or exposure to radiation with a high linear energy transfer (LET). The frequency of SCEs was decreased when compared with the laboratory control.

INTRODUCTION

A significant proportion of Gulf War veterans developed one or several mostly non-specific complaints. Typical symptoms include headache, chronic fatigue, depression, impaired short-term memory and other cognitive defects, sleep disturbances, agitation, respiratory and gastrointestinal disorders, muscle and joint pains, diseases of the skin and intermittent fever. These heterogeneous symptoms have been referred to as the 'Gulf War syndrome'.

A variety of studies in the USA[1–3], the UK[4–6] and Denmark[7] have shown that men and women who served in the Gulf War in 1990 to 1991 suffer from considerably more ill health than their non-deployed peers. Besides epidemiological studies, some surveys on haematological parameters revealed differences in veterans' blood samples compared with their controls, such as findings of amplicons in sera of Gulf War veterans that were homologous to regions of human chromosome 22q11.2, suggesting that genetic alterations in this region may have played a role in the pathogenesis of Gulf War syndrome[8], a significantly decreased number of immune competent cells[9] and a decreased capacity to detoxify organophosphate insecticides and chemical warfare nerve gases observed in serum samples[10].

Many hypotheses have been put forward to explain the aetiological basis for the reported complaints of the veterans, including exposure to infectious agents, chemical warfare agents, vaccines and environmental pollutants. To date none of these hypotheses has been substantiated[2,11,12].

The notion that depleted uranium (DU) may have caused at least some of the reported health problems gained some momentum with the emergence of what has recently been called the 'Balkans syndrome'. According to media reports at least 17 Bosnia and Kosovo veterans from various European troops have recently died of specific cancers, 15 of them of leukaemia. According to anecdotal reports in May 2002, Hodgkin's lymphoma is more widespread among Italian soldiers who were in the Balkans compared with the national population and even more frequent than in soldiers from other countries who served in the same area. However, NATO's top medical committee stated in January 2001 that no increase in disease incidence or mortality among soldiers deployed to the Balkans could be confirmed[13]. Later in 2001 a press release of the European Commission summarised the opinion of a group of independent scientific experts on the possible health effects of DU '… On the basis of the information available to date, the experts have concluded that radiological exposure to depleted uranium could not result in a detectable effect on human health. …' (Ref. 14, p. 1). Somewhat contrary to this expert opinion two more recently published reports of the Royal Society[15,16] discuss a small link between DU and cancer[17]. The Royal Society in 2001 concluded '… DU is radioactive and

Contact author E-mail: heisch@uni-bremen.de

poisonous. Exposure to sufficiently high levels might be expected to increase the incidence of some cancers, notably lung cancer, and possibly leukaemia, and may damage the kidneys. …'(Ref. 15, p. 1). To allow better health risk assessments in April 2001 the World Health Organization recommended further research in key areas[18].

The ability of DU uranyl chloride to transform immortalised human osteoblastic cells (HOS) to the tumorigenic phenotype was recently reported by Miller *et al*[19] using an *in vitro* HOS cellular system. Using the same cellular model Miller *et al*[20] have also demonstrated that exposure to DU causes genomic instability in the progeny of DU-exposed cells. The endpoints used to determine this instability were delayed reproductive death and *de novo* formation of micronuclei in surviving cells. In *in vivo* studies Hahn *et al*[21] demonstrated that male Wistar rats developed localised proliferative reactions and soft tissue sarcomas when implanted with DU fragments. Although kidneys and bone were found to be the primary reservoirs for redistributed uranium from surgically implanted DU pellets in gastrocnemius muscles of Sprague–Dawley rats, Pellmar *et al*[22] reported an unexpected accumulation of uranium in brain, lymph nodes and testicles of these animals and pointed to the potential for unanticipated physiological consequences of exposure to DU through this route.

The Gulf War marked the first battlefield use of armour-piercing munitions and reinforced tank armour that incorporated DU, and the Balkans War marked another one. Typical compounds of DU are approximately 99.8% ^{238}U, radioactive half-life $\cong 4.5 \times 10^9$ years, and 0.2% ^{235}U, radioactive half-life 700 million years. DU is a radioactive, pyrophoric, heavy metal, which is 1.7 times as dense as lead. While concerns have been raised about whether DU exposure could have caused some of the symptoms many of the veterans experience, a scientific understanding of the effect of DU on health is still evolving[23].

Routes of individual exposure to DU are inhalation, ingestion, dermal contact or injury (e.g. uranium shrapnel). The solubility and particle size of the DU and the route and duration of exposure determine its radiochemical toxicity[24]. Soluble uranium will be excreted preferentially through the kidneys which explains its occurrence in urine and its nephrotoxicity.

About 10 years after their deployment in the Gulf War, veterans with retained uranium shrapnel (embedded fragments) still excrete measurable levels of uranium with the urine[25,26]. Incorporated DU causes exposure to ionising radiation, predominantly alpha radiation of about 4 MeV, and to a lesser extent beta and weak gamma radiations with a total specific activity of 12,580 Bq g^{-1}.

Quantitative analysis of dicentric chromosomes and centric ring chromosomes is both sensitive and specific in evaluating previous exposure to ionising radiation[27]. Results can be interpreted either as evidence for the presence or absence of exposure to ionising radiation, for example in cases of exposure to incorporated radionuclides, or can be used for derivation of a 'corresponding whole body dose' in cases of acute exposure to externally applied ionising radiation. Sister chromatid exchanges (SCEs) are reciprocal interchanges of the two chromatid arms within a single chromosome. SCE are most efficiently induced by substances that form covalent adducts with the DNA, or interfere with DNA precursor metabolism or DNA repair. The SCE assay is used quite extensively in genetic chemotoxicology studies[28].

The objective of the present study was to investigate veterans of the Gulf War and of the Balkans War with suspected exposure to DU and to chemicals for unstable rearrangement aberrations, i.e. dicentric chromosomes (dic) and centric ring chromosomes (cR) and for SCE. These are validated and reliable biomarkers that have been applied to individuals and in population studies over decades. It should be noted that the investigated individuals are self-selected, report a variety of health complaints and may not be representative of all deployed personnel.

MATERIALS AND METHODS

Sixteen British veterans, two females and 14 males, volunteered to participate in the present pilot study after a call during a meeting in March 2001 of the National Gulf Veterans' and Families' Association. All had been deployed in the Gulf War in 1990 to 1991 or/and in the Balkans War in 1995 to 1996 and 1999 (Table 1) and have suffered from various medical complaints since then. All men and women described situations during their active service which were probably associated with exposure to DU but none of the volunteers is known to have retained fragments of DU ammunition. Exposure to DU was probably through inhalation of uranium oxide in its aerosol form. Eight of the participants reported to have been tested for uranium in the urine in 2000 to 2001. The original measurement data had not been handed out to them and were not available to us.

The volunteers were selected by one of the authors (A.S.) considering the following exclusion criteria:

- previous radiotherapy and/or medical use of cytotoxic drugs;
- greater than average exposure to diagnostic medical radiation;
- heavy smokers (more than 20 cigarettes a day over more than 10 years);
- previous work in the nuclear industry or other badge-monitored occupation.

Status with respect to exclusion criteria, other relevant exposures and potential confounders was assessed in a detailed structured questionnaire developed in our laboratory (available from the authors upon request).

In this study we did not have an appropriate control group comparable to the study group with respect to all of the multiple warfare agents the investigated veterans had been exposed to (including multivaccinations) and feeling ill with comparable complaints but in a documented absence of DU contamination. Therefore our own (Bremen, Germany) laboratory control group was chosen to evaluate the findings of the study.

Our laboratory control group comprises a casual sample of 40 healthy volunteers (13 females and 27 males) and 34,791 cells. With a frequency of dicentric chromosomes and centric ring chromosomes of (0.46 ± 0.11) × 10^{-3} (mean ± SEM) our control is in accordance with other published 'historical' control values[27,29].

The average age in the study group was 40.1 (range 29–57) and 35.1 in the control (range 17–57). Frequencies of the observed chromatid aberrations were compared with a subgroup of our laboratory control comprising 10 male donors (age range 35–57) and a mean frequency of 4.47 ± 0.67 per cell in 10,065 metaphases[30].

The observed frequencies of SCE were compared with the same subgroup comprising 486 second division metaphases with a mean SCE frequency of 6.16 ± 0.31 per metaphase[31]. Two peripheral whole blood samples (2 × 5.5 ml S-Monovetten® LH, Sarstedt, Nümbrecht, Germany) were taken from each donor by venipuncture: the first eight samples were taken in March 2001 during a meeting of the National Gulf Veterans' and Families' Association in the UK, and another eight samples in December 2001 in the office of the World Depleted Uranium Center Berlin during a visit of veterans to Berlin. The native samples were coded and dispatched to Bremen immediately.

Table 1. The study group.

Subject	Sex	Age at time of venipuncture	Veteran of the war in		Current smoker/ non-smoker	Diagnostic radiography/nuclear medicine[c]
1	Male	41 years[a]	Gulf	1990/1991	Non-smoker	1995: teeth 1998: kidney/bladder
2	Male	37 years[a]	Gulf	1990/1991	Non-smoker since 1997	2001: 2 × teeth
3	Male	44 years[a]	Gulf	1990/1991	Non-smoker since 1997	1995 + 1997: SPECT (skull) 1997 + 1999: PET (skull)
4	Male	41 years[a]	Balkans	1995/1996	Smoker	1997: CAT (skull) 2000: teeth
5	Male	36 years[a]	Gulf	1990/1991	Non-smoker	2000: teeth
6	Male	42 years[a]	Gulf	1990/1991	Non-smoker	1994: chest
7	Male	30 years[a]	Balkans	1999	Non-smoker	1998: chest 2001: chest
8	Male	32 years[a]	Gulf	1990/1991	Non-smoker	1993 + 1994: abdomen elbows, knees
9	Female	57 years[b]	Gulf	1990/1991	Non-smoker	1995: skull, chest 1996: mammography 1997: skull, chest
10	Female	38 years[b]	Gulf	1990/1991	Non-smoker	1999: chest
11	Male	49 years[b]	Gulf	1990/1991	Non-smoker	1995: abdomen, hand, leg 1997: kidney
12	Male	34 years[b]	Gulf and Balkans	1990/1991 1997	Non-smoker	None
13	Male	46 years[b]	Gulf	1990/1991	Non-smoker	1998: chest, abdomen 1999: teeth, extremities
14	Male	31 years[b]	Gulf	1990/1991	Non-smoker	1998: spine 1999: skull, neck
15	Male	29 years[b]	Gulf	1990/1991	Non-smoker since 1994	1991: small + large intestine 1999: chest, abdomen 2000: teeth 2001: spine
16	Male	54 years[b]	Gulf	1990/1991	Non-smoker since 1975	1993: chest 1996: chest 1999: chest

[a]March 2001.
[b]December 2001.
[c]Diagnostic X rays, computed axial tomography (CAT scans), fluroscopy and nuclear medicine exams including single photon emission computerised tomography (SPECT) and positron emission tomography (PET) over the past 10 years according to information provided in the questionnaires.

Upon arrival in Bremen (the day after venipuncture) whole blood cultures were set up according to a standard cell cycle controlling method[32]. For internal control two blood samples from a male donor from our laboratory staff were coded and treated simultaneously with the samples of the study group. Three microscopic slides were prepared from each culture and coded individually. After air-drying and fluorescence-plus-Giemsa staining all slides were sealed with a coverslip. The retrieval of metaphases on the slides was faciliated by an automated, computerised system, which includes a data management tool (MetaSystems, Altlussheim, Germany).

A minimum of 1000 complete, unambiguous and readily scorable first division metaphases from each donor were analysed for all types of structural aberrations, and 50 second division metaphases were scored for the SCE analysis. For analysis of exchanges all chromosomes were assigned to their respective groups according to the International System for Human Cytogenetic Nomenclature[33]. The positions of all metaphases were documented and those with suspected chromosomal aberrations were karyotyped routinely for verification.

Upon completion of the analyses the code was broken and the slides were re-assigned to the individual donors. For a pooled analysis, donors were later grouped according to their deployment in the wars (see Table 3).

The upper and lower 95% confidence intervals (CI) were calculated based on a Poisson distribution. Fisher's exact test was applied to analyse differences in the chromosome aberration frequencies. Two-sided exact *p*-values were calculated under the assumption of a binomial distribution. The dispersion index (ratio variance/mean) was used to test for extra Poisson variability. Values <1 indicate underdispersion while values >1 indicate overdispersion. Overdispersion is expected after exposure to densely ionising radiation. The *U*-test was applied to test for overdispersion, which is significant ($p < 0.05$) if variance/mean >1.96.

The Mann–Whitney test was chosen for the statistical analysis of the observed chromatid aberrations and was also applied to calculate differences in the SCE frequencies of the investigated group and our laboratory control (level of significance is $p < 0.05$).

RESULTS

The results of the chromosome aberration analysis are given in Table 2. The average aberration yield (dic and cR) of the veterans' group is $(2.60 \pm 0.4) \times 10^{-3}$ (95% CI 2.2×10^{-3} to 4.9×10^{-3}) per 1000 metaphases and $(0.46 \pm 0.1) \times 10^{-3}$ (95% CI 0.3×10^{-3} to 0.8×10^{-3}) per 1000 metaphases in our laboratory control group. Numerically, this indicates a 5.2-fold elevation among the volunteers compared with the controls. The difference is statistically significant ($p < 0.001$). Among the volunteers, no sample was without chromosomal aberrations. According to the two-sided Fisher's exact test the frequencies of dic and cR in 8 of the 16 cases investigated differ significantly from our laboratory control in individual contrasts (individuals 1, 2, 3, 5, 8, 12, 13 and 16) ($p \leq 0.05$). Stratification according to deployment did not alter the results (Table 3).

Two peculiarities were observed during analysis. One tetraploid first division metaphase of subject 1 (Gulf War veteran) contained a multicentric ring and two acentric fragments. This was the first time that an aberrant cell like this had been observed in our laboratory. The absence of four chromosomes was confirmed by karyotyping. Whether or not these chromosomes had actually formed the multicentric ring could not be determined in the absence of banding. This metaphase was not included in the analysis due to tetraploidy and the unclear chromosomal origin of the aberration. Secondly, in the metaphase preparations of subject 5 (Gulf War veteran) one cell contained a pulverised G or Y chromosome (pvz(G/Y)), together with a partly pulverised C-group chromosome, most probably pvz(11q).

Findings for acentric fragments and double minutes are summarised in Table 2. Compared with our laboratory control the number of acentric fragments observed in the veterans' group is significantly elevated ($p < 0.05$). Column 8 of Table 2 also gives the number of observed chromatid aberrations; their specification is shown in Table 4. When compared with the subgroup of our laboratory control (4.5 ± 0.7) chromatid aberrations are significantly elevated in the veteran's group (9.6 ± 0.8).

The intercellular distribution of dic and cR is given in Table 5 and shows overdispersion for the group as a whole which is due to the results of the individuals 1, 3 and 8.

Results of the SCE analysis are given in Table 6. Only 8 of the 16 metaphase preparations yielded a sufficient number of second division metaphases for SCE analysis. The Mann–Whitney test revealed a significantly lower frequency of SCEs in the study group (4.2 ± 0.5) when compared with the subgroup of our laboratory control (6.2 ± 0.3).

DISCUSSION

A considerable number of the veterans of the Gulf War and of the Balkans War suspect exposures to DU during their deployment. A multitude of investigations have been undertaken and are continuing with respect to various symptoms of the Gulf War syndrome. To our knowledge, McDiarmid *et al*[26] were the first investigators to apply chromosome aberration analysis to a group of veterans of the Gulf War with retained DU shrapnel. Since in their results all 'chromosomal aberrations' are pooled without any further specification, a meaningful comparison with our results is not possible. The observed number of dicentric chromosomes and

centric ring chromosomes in 16 blood samples of approximately 1000 metaphases each ranged from one to seven and the resulting aberration frequencies of the combined group of veterans was significantly different from our laboratory control. Significant aberration frequencies were also observed on the individual level for 8 of the 16 individuals. The elevation is independent of the war to which our volunteers had been deployed. Results for acentric fragments and chromatid aberrations are consistent with the chromosome-type aberrations.

These results strongly indicate previous exposure to

Table 2. Results of the chromosome aberration analysis.

Subject	Number of cells analysed	Number of doc+cR	Mean frequency per cell	CI 95%−	CI 95%+	Number of excess ace+min	Number of ctd aberrations
1[a]	1001	4[d],[e]	0.0040	0.0011	0.0102	7[e]	12
2[a]	1002	3[e]	0.0030	0.0006	0.0087	5	10
3[a]	1001	7[e]	0.0070	0.0028	0.0144	5	13
4[b]	1003	2	0.0020	0.0002	0.0072	12[e]	15
5[a]	1001	3[e],[f]	0.0030	0.0006	0.0088	3	11
6[a]	1003	2	0.0020	0.0002	0.0072	7[e]	12
7[b]	1002	2	0.0020	0.0002	0.0072	0	5
8[a]	1000	4[e]	0.0040	0.0011	0.0102	1	8
9[a]	1005	1	0.0010	0.00003	0.0056	4	9
10[a]	1000	1	0.0010	0.00003	0.0056	4	9
11[a]	1002	2	0.0020	0.0006	0.0072	1	5
12[c]	1002	3[e]	0.0030	0.0006	0.0088	6	5
13[a]	999	3[e]	0.0030	0.0006	0.0088	7[e]	2
14[a]	1001	1	0.0010	0.00003	0.0056	5	12
15[a]	1001	1	0.0010	0.00003	0.0056	5	11
16[a]	1001	3[e]	0.0030	0.0006	0.0031	2	14
Total	16024	42[g]	0.0026	0.0019	0.0035	74[e]	153
Control	34791	16	0.0005	0.0003	0.0008	106	(45, subgroup)

Abbreviations: dic, dicentric chromosome; cR, centric ring chromosome; Cl 95%−, lower 95% confidence interval (according to the Poisson distribution); Cl 95%+, upper 95% confidence interval (according to the Poisson distribution); ace+min, acentric fragment and double minutes; ctd, chromatid type aberration, including breaks, gaps and quadriradials (quarad, counted as two breaks).
[a]Gulf War veteran.
[b]Balkans War veteran.
[c]Gulf and Balkans War veteran.
[d]One tetraploid metaphase is not included containing a multicentric ring.
[e]Elevation is significantly different from laboratory control, $p \leq 0.05$.
[f]One metaphase is not included containing a pulverised chromosome, pvz(G/Y) and a partly pulverised C-group chromosome.
[g]Elevation is significantly different from laboratory control, $p < 0.001$.

Table 3. Results of the chromosome aberration analysis according to the individual's deployment in the war theatres.

Group	Number of cells analysed	Number of dic+cR	Mean	95% CI−	95% CI+	Number of excess ace+min	Number of ctd aberrations
Gulf, subjects (all except 4, 7,12)	13017	35*	0.0027	0.0019	0.0037	56*	128
Balkans (subjects 4, 7)	2005	4*	0.0020	0.0005	0.0051	12*	20
Gulf and Balkans (subject 12)	1002	3*	0.0030	0.0006	0.0088	6	5

Abbreviations: dic, dicentric chromosome; cR, centric ring chromosome; 95%CI−, lower 95% confidence interval (according to the Poisson distribution); 95%CI+, upper 95% confidence interval (according to the Poisson distribution); ace+min, acentric fragment and double minutes; ctd, chromatid aberrations including breaks and gaps.
*Elevation is significantly different from laboratory control, $p \leq 0.05$.

ionising radiation for the group as a whole, and for at least 50% of the individual members. A high degree of specifity of the chromosome aberration assay is generally accepted in the scientific literature. The assay, however, cannot differentiate between different sources of exposure to ionising radiation. At the moment information on the history with respect to diagnostic and therapeutic radiation exposure from medical sources of all of the individuals in the group is limited. Using questionnaire information solicited by one of the authors (A.S.) the highest dose due to diagnostic radiation in the past for any of the volunteers would be in the range of some 5–50 mSv, attributable mainly to single photon emission computed tomography (SPECT), positron emission tomography (PET) and computed axial tomography (CAT scan). Although an element of underestimation is presumed with respect to medical radiation exposure it appears unlikely that this might explain the increase in the aberration frequencies. None of the individuals investigated had documented shrapnel injuries. It can therefore be excluded that retained fragments of DU in body tissues could be the cause of the observed significant increase of dic and cR.

The intercellular distribution of dic and cR indicates significant overdispersion on the group level which is due to the results of three veterans who had been deployed in the Gulf War. Overdispersion of dic and cR is a known consequence of non-uniform irradiation, e.g. partial body exposure or incorporated radionuclides, and/or irradiation with high linear energy transfer (LET) radiation. The very rare finding of a multicentric ring in one tetraploid metaphase and the finding of one metaphase containing pulverised chromosome material may be considered as a further indication of high-LET radiation. It is noteworthy that we have previously observed pulverised chromosomes in Concorde pilots exposed to cosmic radiation, which has an important contribution from high-LET radiation[31]. Doubling of metaphase chromosome sets and pulverisation was frequently observed in our laboratory after *in vitro* treatment of blood samples with the alpha-emitting radionuclide ^{210}Po, and an increase in chromatid type aberrations was found in groups of people from a high ^{222}Ra area in Germany (unpublished results).

Considering the limited potential for exposure of veterans to ionising radiation during their deployment in the Gulf War more than a decade ago, and considering the known decline of dicentric chromosomes following acute exposure to ionising radiation, our cytogenetic findings are unexpected. Assuming a contamination due to ingested soluble oxides of DU about 10 years ago which would have been excreted with the urine within some few days, and further assuming a half-life of dicentric chromosomes of about 3.5 y the doses deduced from the actual aberration yield would be implausible. We therefore dismiss the hypothesis of a single acute event 10 years ago which might have led to the positive findings in our dicentric assay. Major bias in the analysis is an unlikely explanation since all volunteers and controls were analysed blindly by the same experienced scorers.

However, there is no doubt that the veterans were exposed to a large number of different agents on the battlefield besides DU dust, e.g. combustion products from oil-well fires, diesel exhaust products, medical drugs (including pyridostigmine bromide, which is used as a prophylactic agent against chemical warfare nerve gas), immunisations, organophosphates, carbamates, pyrethroids and other pesticides as well as insect repel-

Table 5. Intercellular distribution of dicentric chromosomes and centric ring chromosomes.

Subject	Distribution			Variance/ mean	*U*
	0	1	2		
1	998	2	1	1.497	12.85*
2	999	3	0	0.998	—
3	995	5	1	1.280	6.76*
4	1001	2	0	0.999	—
5	998	3	0	0.998	—
6	1001	2	0	0.999	—
7	1000	2	0	0.999	—
8	997	2	1	1.497	12.85*
9	1004	1	0	—	—
10	999	1	0	—	—
11	1000	2	0	0.999	—
12	999	3	0	0.998	—
13	996	3	0	0.998	—
14	1000	1	0	—	—
15	1000	1	0	—	—
16	998	3	0	0.998	—
Total	15985	36	3	1.140	12.71*
Control	34775	16	0	1.000	—

*Overdispersion is significant, $p \leq 0.05$.

Table 4. Chromatid aberrations (ctd) — specification.

	Number of cells analysed	Number of breaks and fragments	Number of ctd gaps	Number of quadriradials*	Total number of ctd aberrations
Veterans	16024	39 (25.5%)	98 (64%)	8 (10.5%)	153
Control	10065	17 (37.8%)	26 (57.8%)	1 (4.4%)	45

*Each counted as two chromatid (ctd) breaks.

lents in unknown mixtures and concentrations[8]. Hence, in this rather exceptional complex exposure situation there is ample potential for synergism between chemicals and radiation with respect to their impact on DNA breaks. Obviously, appropriate *in vitro* simulation is impracticable. However, as dicentric chromosomes are reliable indicators of ionising radiation our findings contradict official releases from the IAEA, the WHO, the MOD and the DOE, stating that the radiotoxicity of DU would be negligible. Computer simulations have also calculated only a small radiological risk associated with the use of DU weapons[34].

Hence, at present there can only be speculation about the mechanisms behind the observed cytogenetic effects, especially considering the relatively low specific radioactivity of DU. Recent research has shown that DU particles were still detectable through modern air sampling techniques 2 years after the end of the war in Kosovo[35]. In addition, these results indicate that DU dust was widely dispersed into the environment following the explosion of DU rounds[36]. Moreover, in two selected soil samples from Kosovo, Danesi *et al*[37] found hundreds of thousands of DU particles in a few milligrams of contaminated soil, indicating that there may be 'spots' at different sites hit by DU rounds. Most of the particles analysed had a diameter of <5 μm and 50% of them were <1.5 μm. Studies of uranium in rat lungs show that insoluble forms of uranium are poorly transported to blood and are retained in the lung to a far greater extent than soluble forms[38], and human autopsies have shown that tracheobronchial and other pulmonary related lymph nodes had unexpectedly high concentations of retained actinides[39]. Bramhall[40] has calculated chronic alpha-irradiation to local lung tissue from inhaled uranium 10 times the natural background rate assuming a particle size of 0.5 μm in diameter from uranium oxide, and communicates that larger particles would deliver higher doses. Recently, Miller *et al*[41] have demonstrated in *in vitro* experiments that DU is able to catalyse reactions of hydrogen peroxide and ascorbate, generating oxidative DNA damage that can induce carcinogenic lesions that had actually been observed in former experiments with mice[19].

Table 6. Results of the sister chromatid exchange (SCE) analysis.

Subject	Number of cells	Number of SCE	Range SCE/cell	SCE/cell (mean ± SEM)
1	50	209	0–9	4.18 ± 0.31
2	50	246	1–10	4.92 ± 0.29
3	50	223	0–10	4.46 ± 0.36
4	35	135	1–9	3.86 ± 0.37
5	47	139	0–9	2.96 ± 0.28
6	50	246	0–11	4.92 ± 0.35
8	50	219	0–10	4.38 ± 0.31
9	50	198	0–9	3.96 ± 0.30
Total	382	1615	0–11	4.23 ± 0.12
Control	486	2990	0–24	6.16 ± 0.31

Key: Number of cells = number of analysed cells; number of SCE = number of SCE (total of SCE and colour switches); mean ± SEM = mean of SCE/cell ± standard error of the mean; SEM, standard error of the mean; SCE, sister chromatid exchange.

These results add plausibility to our cytogenetic findings: if the members of our study group had inhaled insoluble (e.g. ceramic) small-sized particles of DU these might have been deposited and concentrated in their deeper lung and could have delivered considerable doses to the local tissues and the tracheobronchial lymph nodes. Local doses from DU radioactivity may then persist (chronic) and would accumulate to considerable local doses which may have contributed to the cytogenetic damage in the peripheral blood lymphocytes of those individuals.

Since the SCE assay is accepted to measure genotoxic exposure almost exclusively with respect to chemical toxicants[42] we have applied this analysis additionally to the chromosome aberration analyses as a measure of potential chemical hazards that might have affected the veterans of our study group. McDiarmid *et al*[43] observed elevated frequencies of SCE in a group of soldiers during and after their deployment in Kuwait (Gulf War) in 1991 and a decline of these cytogenetic markers with time (months) afterwards. The authors discuss potential sources of cytotoxicity, especially oil-well fires. These authors also reported associations of elevated SCE frequencies with elevated urine uranium levels of US Gulf War veterans with retained metal shrapnel from DU ammunition[44]. The results of the present SCE analysis show a lower frequency of SCE compared with a subgroup of our laboratory control. However, as only 8 out of the 16 blood samples yielded a sufficient number of second division metaphases for analysis and in only 6 cases could 50 cells be analysed for SCE this result should be interpreted with caution. Given the limited stability of SCE in living cells, these findings may reflect the actual absence of chemical hazards to our volunteers rather than the absence of exposures to cytotoxic agents years ago during their deployment in the wars.

Based on the internal consistency of the results presented we recommend further investigations to evaluate the cytogenetic findings of this pilot study. Due to the small size and heterogeneity of the study group our findings should be interpreted with due caution. However, the results raise some concern with respect to potential biological hazards from DU exposure. These should be addressed in a larger scale cytogenetic study using state-of-the-art exposure assessment including other biomarkers and appropriately matched controls.

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