The influence of x-ray contrast agents in computed tomography on the induction of dicentrics and γ-H2AX foci in lymphocytes of human blood samples

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The influence of x-ray contrast agents in computed tomography on the induction of dicentrics and \( \gamma \)-H2AX foci in lymphocytes of human blood samples

G Jost\(^1\), S Gollier\(^1\), H Pietsch\(^1\), P Lengsfeld\(^1\), M Voth\(^1\), T E Schmid\(^2\), F Eckardt-Schupp\(^3\) and E Schmid\(^4\)

\(^1\) Bayer Schering Pharma AG, 13353 Berlin, Germany
\(^2\) Department of Radiation Oncology, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany
\(^3\) Institute of Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany
\(^4\) Institute for Cell Biology, Center for Integrated Protein Science, University of Munich, 80336 München, Germany

E-mail: Ernst.Schmid@lrz.uni-muenchen.de

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Abstract

The aim of this study was to investigate and quantify two biomarkers for radiation exposure (dicentrics and \( \gamma \)-H2AX foci) in human lymphocytes after CT scans in the presence of an iodinated contrast agent. Blood samples from a healthy donor were exposed to CT scans in the absence or presence of iotrolan 300 at iodine concentrations of 5 or 50 mg ml\(^{-1}\) blood. The samples were exposed to 0.025, 0.05, 0.1 and 1 Gy in a tissue equivalent body phantom. Chromosome aberration scoring and automated microscopic analysis of \( \gamma \)-H2AX foci were performed in parts of the same samples. The theoretical physical dose enhancement factor (DEF) was calculated on the basis of the mass energy-absorption coefficients of iodine and blood and the photon energy spectrum of the CT tube. No significant differences in the yields of dicentrics and \( \gamma \)-H2AX foci were observed in the absence or presence of 5 mg iodine ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. At an iodine concentration of 50 mg ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. At an iodine concentration of 50 mg ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. At an iodine concentration of 50 mg ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. At an iodine concentration of 50 mg ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. At an iodine concentration of 50 mg ml\(^{-1}\) blood up to 0.1 Gy, whereas at 1 Gy the yields were elevated for both biomarkers. 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1. Introduction

Compared with the extensive results on in vivo and in vitro induction of chromosome aberrations or micronuclei in human lymphocytes by diagnostic x-ray radiation qualities in combination with iodinated contrast agents (Adams et al 1977, Cochran et al 1980, Hadnagy et al 1982, Norman et al 1978, Parvez et al 1987, Sinues et al 1991), relatively little information has been reported on similar biological effects of computed tomography (CT) scans in vivo and in vitro. This situation is remarkable, because in the past two decades the increasing use of CT scans in clinical practice has made CT a significant contributor to the total collective dose from all medical x-ray examinations. Moreover, due to the faster rotational times and the greater volume coverage of multi-slice CT scanners, the number of clinical CT examinations has continued to increase in the last years. Brenner and Hall (2007) focused in a recent review on the increasing number of CT scans, the associated radiation doses and the consequent cancer risks in adults and particularly in children.

In the energy range of CT radiation, the photoelectric interaction and the Compton scattering account for the locally absorbed radiation dose. In contrast to the Compton effect, the photoelectric interaction strongly depends on the atomic number of the absorber. Therewith elements with a high-order number, as x-ray contrast agents, absorb radiation more strongly than the light elements in biological tissue. Consequently, a contrast enhancement in x-ray imaging is physically always linked with the emission of secondary radiation (photoelectrons, x-ray fluorescence, Auger electrons) based on the photoelectric effect. This phenomenon leads in turn to an increase in energy absorption in the short distance surrounding of the enhancing substances. The physical effect of photoelectric dose enhancement in the presence of iodine-containing contrast agents was first described by Callisen et al (1979), who present a quantitative estimation of the dose enhancement on the basis of the energy absorption coefficients of iodine and water. Both coefficients are strongly energy dependent (Corde et al 2004), which demonstrates the importance of the x-ray tube spectrum and the filtering used for the imaging procedure. Boudou et al (2007) calculated a dose enhancement of 1.51 for a iodine concentration of 5 mg ml$^{-1}$ and 80 keV monochromatic synchrotron radiation using Monte Carlo simulations. For a local iodine concentration of 50 mg ml$^{-1}$, a concentration which is far away from any diagnostic CT applications, and a 120 kV CT tube spectrum a dose enhancement factor (DEF) between 4 and 6 was calculated by Verhaegen et al (2005). The variance originates from self-absorbing effects due to the highly concentrated contrast agents itself and different spatial positions within the used head phantom. In contrast to the theoretical estimations, the dosimetry is very challenging, due to the short range of the secondary radiation (approximately 2–20 μm) (Callisen et al 1979). Although several experimental approaches, like film dosimetry (Morris et al 2006) or gel dosimetry (Boudou et al 2007, Jost et al 2009), can be found in the literature no measurements with standard certified dosimetry techniques were presented until now. However, the physical description of the photoelectric dose enhancement does not necessarily predict the biological relevance of a local short-distance dose enhancement in the range of diagnostic x-ray doses. The present study focuses therefore on the biological consequences of an iodine-based contrast agent-related dose enhancement in human blood samples.

In general, the quantification of dicentrics is the recommended method for estimating the biological effect of exposures to low dose levels of ionizing radiation (‘biological dosimetry’, IAEA 1986), because clinically relevant biological damage is rather reflected by chromosomal damages. However, in recent studies, Löbrich et al (2005) as well as Rothkamm et al (2007) have demonstrated that the assessment of γ-H2AX foci formation in human lymphocytes can also serve as a relevant biomarker for CT examinations. In order to compare the radiosensitivity
of both biomarkers, we have recently performed experiments using CT scan-exposed blood samples (Golfier et al. 2009). In fact, our results indicated that scoring of dicentrics and γ-H2AX foci formation are similarly sensitive methods to quantify a radiation-induced biological damage at dose levels from 0.025 Gy up to 1 Gy.

The aim of this study was to investigate and quantify both biological endpoints in human lymphocytes after CT scans in the presence of an iodinated contrast agent. These experimental conditions should provide further information on the quantitative evaluation of biological effects induced by CT scans.

2. Materials and methods

2.1. Physical DEF

The theoretically expected photoelectric dose enhancement was calculated on the basis of the mass energy-absorption coefficients ($\mu_{en}/\rho$) and the spectral distribution of photons emitted from the x-ray tube (Callisen et al. 1979, Jost et al. 2008). The $\mu_{en}/\rho$ coefficients of blood and of a mixture of blood and contrast agent are based on the data of the NIST standard reference database (http://physics.nist.gov/PhysRefData/XrayMassCoef/cover.html). A 120 kV CT x-ray tube spectrum was provided by the manufacturer. The DEF was calculated as

$$\text{DEF} = \int_{E=10\text{keV}}^{120\text{keV}} \left( w \left( \frac{\mu_{en}}{\rho} \right)_{\text{CM}} + (1-w) \left( \frac{\mu_{en}}{\rho} \right)_{\text{Blood}} \right) \cdot N(E) \, dE,$$

where $N(E)$ represents the relative photon rate at the energy $E$ and $w$ the weight fraction of contrast agents. The latter depends on the contrast agent concentration ($w/v$) and the density of the contrast agent–blood mixture.

2.2. Blood collection

Fresh whole blood was drawn from the same healthy male donor, as already used in our previous CT irradiation experiments (Golfier et al. 2009). Two blood samples were mixed with iodotrolan 300 (ISOVIST, Bayer Schering Pharma AG, Berlin, Germany) to obtain either an iodine concentration of 5 or 50 mg ml$^{-1}$ blood. Another two blood samples remain unchanged, one served as reference and was irradiated without contrast media and the other served as control and was kept unirradiated during the same time period. The blood samples were contained in cylindrical polypropylene vials, with a volume of 2 ml (Nunc GmbH & Co.KG, Langenselbold, Germany). We used one sample vial for each biomarker and radiation dose. For the detection of the chromosome aberrations only iodine-containing blood samples were used. The reference and control samples were already investigated in our previous study using CT scan-exposed blood samples under identical experimental conditions (Golfier et al. 2009). The blood samples were kept at room temperature, RT (20–22 °C), when exposed to CT scans. This was already accepted for our earlier irradiation studies with photons at the electron storage ring BESSY II (Berlin, Germany) (Krumrey et al. 2004) or at the x-ray calibration facility of the Physikalisch-Technische Bundesanstalt (PTB, Braunschweig, Germany) (Buermann et al. 2005).

2.3. Irradiation conditions

The irradiation conditions are as previously described in detail (Golfier et al. 2009). In brief, the blood samples were placed in appropriate slots of a tissue equivalent body phantom (QRM,
Möhrendorf, Germany) to achieve realistic measurement conditions including beam hardening and scattering effects. The x-ray irradiation was performed by using a 64-slice CT scanner (Sensation 64, Siemens Medical, Erlangen, Germany). The used measurement parameters are 120 kV, 150 mAs and a rotational time of 1 s. The total beam collimation was 28.8 mm (24 × 1.2 mm) and the irradiation was performed without table feed. A scan time of 5 s equals a dose of 0.025 Gy; higher doses were realized by scaling up the irradiation time. The measurement of the radiation doses was performed before the irradiation of the blood samples using a UNIDOS dosimeter and an ionization chamber (Type 31 010, PTW, Freiburg, Germany) calibrated to water energy dose. For dosimetry this chamber was placed at the slot of the phantom, using the corresponding phantom inserts. These measurements were validated by dose simulations using the Monte Carlo-based CT dose simulation software ImpactMC (Vamp GmbH, Erlangen, Germany). The differences in the measurements are <5%.

After radiation exposure, blood was processed for the detection of either chromosome aberrations or γ-H2AX foci in lymphocytes. Whereas the γ-H2AX foci formation test has been started immediately after irradiation at the laboratories in Berlin (Germany), the corresponding chromosome aberration test could not be started before a 3 h transport to the laboratories in Munich (Germany). However, it is well known that this delay between radiation exposure and start of blood cultures has no influence on the counting process of chromosome aberrations, e.g. for the induction of dicentrics which involves an interaction (or exchange) between DNA double-strand breaks of two chromosomes. This fact is an important prerequisite for the analysis of chromosome aberrations as a valuable dose-assessment method, i.e. biological dosimetry following known or suspected radiation over-exposure (International Atomic Energy Agency (IAEA 1986)).

2.4. Blood culture conditions and chromosome analysis

After the 3 h transport blood cultures were established. Cultures contained 0.5 ml whole blood, 4.5 ml RPMI-1640 medium supplemented with 15% fetal calf serum, 1% glutamine, antibiotics and 2.5% phytohemagglutinin. They were incubated for 46 h in a humidified 5% CO₂ atmosphere at 37 °C. Colcemid (0.03 μg ml⁻¹) was present during the entire incubation period. This colcemid method which was originally suggested by Kanda et al (1994) has been optimized and standardized in our laboratory more than one decade ago as an alternative cell cycle-control technique to the conventional method using fluorescence plus Giemsa staining. In this way, it was not only ensured that chromosome aberrations could exclusively be scored in the first mitosis following CT scans, but it was also possible to analyze all cells that ever reached mitosis before fixation. Chromosome preparation and Giemsa staining were carried out according to standardized laboratory procedures (IAEA 1986). All object slides were coded. Only complete cell nuclei were analyzed for structural chromosome aberrations, i.e. dicentrics, centric rings, excess acentric fragments, chromatid breaks or exchanges. Excess acentric fragments were recorded as all fragments minus one per dicentric or centric ring.

2.5. Lymphocyte separation and γ-H2AX foci analysis

For γ-H2AX foci formation blood samples were diluted 1:2 with prewarmed RPMI medium and incubated for 45 min in a humidified 5% CO₂ atmosphere at 37 °C. Lymphocytes were isolated according to the manufacturer’s instructions (Greiner bio-one, Germany). Briefly, 6 ml diluted blood samples were carefully layered over 3 ml Ficoll–Paque Plus (Amersham Biosciences). After centrifugation at 800 × g for 15 min at RT, the lymphocytes were collected from the interface, washed twice in TBS (tris-buffered saline) and suspended in 1 ml TBS.
For immunohistochemistry, 500 μl cell solutions were fixed in 2.7% paraformaldehyde for 45 min, washed with TBS, centrifuged on slides and stored at −20 °C until analysis. All slides were coded. The fixed cells on slides were washed twice with TNT, permeabilized with TNT Triton-X (0.5%) for 10 min, washed twice in TNT and blocked in TNT containing 5% goat serum for 45 min. The samples were incubated overnight at 4 °C with a mouse anti-γ-H2AX antibody (clone JBW301: Upstate, Charlottesville, VA), rinsed with TNT, incubated at RT with a biotin-conjugated goat anti-mouse antibody (Dianova, Hamburg, Germany) for 2 h, rinsed with TNT and incubated at RT with CY3-conjugated streptavidin (Dianova, Hamburg, Germany) for 1 h. Finally, the cells were washed and embedded in Immuno-Mount (Shandon, Pittsburgh, USA). Scoring of focus numbers per cell was performed on a confocal microscope LSM510 (Zeiss, Jena, Germany) applying 1 μm slices z-stage acquisition through the cells. The foci were automated counted at an objective magnification of x63 by using the NIH ImageJ (Abramoff et al. 2004) software with a 3D particle counter macro (Carpenter et al. 2006).

2.6. Statistics
The determined counts (dicentrics, γ-H2AX foci, respectively) of two samples were compared by calculating the ratio of lambdas, which represents the location parameter of the Poisson distribution. Applying a chi-square test for significance, a difference at the p-value <0.05 was considered statistically significant. Calculations were performed using the SAS procedure PROC GENMOD of SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results
A theoretical physical radiation dose enhancement by a factor of 1.56 and 6.30 was calculated in the presence of 5 and 50 mg iodine ml⁻¹ blood, respectively. Table 1 shows the results of the CT scan-induced chromosome aberration yields in human lymphocytes in the absence or presence of both iodine concentrations. For induction of dicentrics in the presence of 5 mg iodine ml⁻¹ blood no significant increases of dicentric yields were found in the dose range from 0.025 to 0.1 Gy compared to the reference data obtained without contrast medium (figure 1(A)). However, at the dose of 1 Gy, an increase of the dicentric yield by a factor of 1.74 ± 0.18 was determined, which is in principle in agreement with the calculated physical DEF of 1.56. In the presence of 50 mg iodine ml⁻¹ blood, a significant increase of dicentric yields was observed for all radiation doses except 0.025 Gy with respect to the reference data determined in the absence of the contrast medium. The respective enhancement factor of 9.5 ± 1.4 exceeds the calculated physical factor of 6.3 by about 50%.

In the present study, the background frequency of (3.1 ± 2.2) × 10⁻⁴ dicentrics per cell (total number of cells 6385) is in accordance with the overall background frequency of (2.8 ± 1.2) × 10⁻⁴ dicentrics per cell (total number of cells 18 000) from the present donor (Schmid et al. 2008). In addition, this frequency is not significantly different from the mean value of (4.1 ± 0.7) × 10⁻⁴ dicentrics per cell obtained from 141 control individuals (total number of cells 92 550) examined in our laboratory (Bauchinger and Schmid 1998). As shown in table 1, elevated yields of dicentrics and excess acentric fragments have been found in cells correlated with increasing radiation doses. Owing to the low frequencies of centric rings, a similar dose and concentration-dependent increase could only be observed at the two highest dose levels. This different finding in the yields of dicentrics and centric rings in human lymphocytes following radiation exposure to low doses is not surprising, because we noted earlier (Bauchinger and Schmid 1998) that a value of approximately 10 exists for the yield ratio of dicentrics and centric rings, independently of ionizing radiation qualities. In
contrast, there was no recognizable dependence of the yield of chromatid-type aberrations, both on radiation dose and contrast agent concentration. The intercellular distributions of the dicentric yields are also given in table 1. At any dose level, both in the absence and presence of iodine, the distributions of the dicentric yield data show regular dispersion, i.e. a Poisson distribution, as seen from the ratio of variance and mean, $\sigma^2/\mu$, and from the test quantity $u$, which approximates to a unit normal deviation.

The corresponding data on $\gamma$-H2AX foci formation in human lymphocytes following CT scans in the absence or presence of both iodine concentrations are presented in table 2. The number of foci per cell in the presence of 5 mg iodine ml$^{-1}$ blood compared to the data obtained without contrast agent shows no consistent results (figure 1(B)); for 0.025 Gy a significantly
higher number of foci per cell, for 0.050 Gy a significantly lower number of foci and for 0.1 Gy no statistically significant difference was detected. At the high dose of 1 Gy, the number of foci per cell was increased by a factor of 1.35 ± 0.08. In the presence of 50 mg iodine ml\(^{-1}\) blood, an increase of \(\gamma\)-H2AX foci data was obtained at all radiation doses with respect to the reference data determined in the absence of the contrast agent (figure 1(B)). However, the averaged enhancement factor of 2.3 ± 0.5 is remarkably lower than the calculated physical factor of 6.3. In contrast to the findings for the dicentric data, the intercellular distribution of the yield data for the \(\gamma\)-H2AX foci formation is overdispersed at 11 out of 12 irradiation levels (table 2) independently of the absence or presence of the contrast medium as \(\sigma^2/\nu\) differs significantly from value 1. There is no overall increase in the dispersion coefficient \(\sigma^2/\nu\) with increasing dose or contrast agent concentration.

### 4. Discussion

In the present study, the analyses of chromosome aberrations and \(\gamma\)-H2AX foci formation were employed to measure biological effects in blood samples induced by contrast enhanced CT scans. Based on the fact that a contrast enhancement in x-ray imaging is physically always linked with the emission of secondary radiation the DEF was calculated. As stated in equation (1), the DEF increases linearly with the iodine weight fraction. It is notable that the latter does not linearly depend on the iodine concentration expressed in weight per volume, e.g. mg iodine ml\(^{-1}\) blood. The calculated DEF values are in principle in agreement with previous findings using similar iodine concentrations and photon energies (Verhaegen \textit{et al} 2005, Boudou \textit{et al} 2007). However, in the present DEF calculations beam hardening and scattering effects were not considered. Both affect the local photon energy spectrum and consequently the local dose enhancement. For the calculation of spatially resolved dose distributions in the presence of contrast agents, highly sophisticated Monte Carlo-based dose simulations should be used (Deak \textit{et al} 2008).

The findings of the present investigations actually illustrate that a dose enhancement in iodinated contrast agent-containing blood samples can be determined with both biological

### Table 2. Intercellular distribution of \(\gamma\)-H2AX foci in human lymphocytes induced by CT doses D in the absence or presence of the contrast agent iotrolan 300 at two iodine concentrations.

<table>
<thead>
<tr>
<th>Iodine Dose (mg ml(^{-1}))</th>
<th>Cells scored</th>
<th>(\gamma)-H2AX foci/cell</th>
<th>Intercellular distribution of (\gamma)-H2AX foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0) (1) (2) (3) (4) (5) (6) (7) (8) (9) (\geq 10)</td>
</tr>
</tbody>
</table>
|                             |             |                          | 49 \(0.469\) 29 \(1.4\) 18 \(1.1\) 1 | 0.90 \(0.1\) \(0.3\) \(0.5\) \(0.8\) \(1.0\) | 0.02 \(0.05\) \(0.10\) \(0.50\) \(1.00\) | 0.02 \(0.05\) \(0.10\) |}
|                             |             |                          | 0.025 \(0.580\) 0.21 \(1.1\) 5 | 2.52 \(4.44\) \(5.11\) \(6.22\) \(8.44\) |}
|                             |             |                          | 0.050 \(1.03\) 0.36 \(2.1\) 7 | 2.38 \(3.51\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 0.100 \(1.50\) 0.72 \(3.6\) 17 | 2.38 \(3.51\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 1.000 \(4.69\) 25 \(1.1\) 14 | 2.38 \(3.51\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 5 \(0.25\) 259 \(0.726\) 138 | 1.26 \(2.93\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 5 \(0.10\) 170 \(1.54\) 59 | 2.38 \(3.51\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 5 \(1.00\) 185 \(6.35\) 23 | 1.26 \(2.93\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 0.025 \(0.65\) 0.892 | 3.17 \(19.99\) \(27.02\) \(37.02\) \(47.02\) |}
|                             |             |                          | 0.050 \(0.155\) 2.232 | 1.36 \(3.17\) \(4.54\) \(6.35\) \(8.44\) |}
|                             |             |                          | 0.100 \(0.469\) 3.983 | 2.36 \(20.73\) \(20.73\) \(20.73\) |}
|                             |             |                          | 1.000 \(2.13\) 11.709 | 2.58 \(16.26\) \(16.26\) \(16.26\) |}
endpoints. However, a substantial difference between 5 and 50 mg iodine ml\(^{-1}\) blood and between the low doses (up to 0.1 Gy) and the high dose (1 Gy) was observed for the two biomarkers. At the lower iodine concentration and doses up to 0.1 Gy, the determined difference in radiation-induced yields for both biomarkers is considerably lower than estimated from the calculated physical dose enhancement. The dicentric yields do not differ significantly from those of the reference samples, whereas the \(\gamma\)-H2AX foci data show inconsistent results suggesting that scoring chromosome aberrations may be a more robust method for low-dose biological dosimetry. In contrast, at the dose of 1 Gy, the relative increase of radiation-induced yields for both biomarkers is consistent with the estimated physical enhancement. However, it has to be pointed out that this high dose level, which was used in the present study as a positive control, is well outside the maximum clinical imaging dose (2–20 mSv) (Mettler et al. 2008).

Considerable differences in the radiation-induced yields of the two biological endpoints were observed in the samples containing 50 mg iodine ml\(^{-1}\) blood, i.e. a concentration which is well outside the local iodine concentration in CT. Within the complete dose range from 0.025 to 1.0 Gy, the averaged enhancement factor of 9.5 \(\pm\) 1.4 obtained for the dicentric data even exceeds the calculated physical factor of 6.3, whereas the averaged enhancement factor of 2.3 \(\pm\) 0.5 obtained for the \(\gamma\)-H2AX foci data is clearly below the calculated enhancement value. The potential additional biological effect at high contrast doses may be explained by the high amount of secondary radiation possessing a higher relative biological effectiveness (RBE) due to the large proportion of low-energy electrons (Zellmer et al. 1998, Regulla et al. 2002). In fact, we noted earlier (Regulla et al. 2001, 2002) that the yield of dicentrics in human lymphocytes exposed to the photoelectrons and Auger electrons emerging from the surface of an irradiated gold foil under backscatter conditions reflects not only a localized dose enhancement caused by the release of secondary electrons from the material, but also an increase of the expected RBE owing to the slowed down spectrum of these particles.

The results of the CT scan-induced \(\gamma\)-H2AX foci observed in the samples containing 50 mg iodine ml\(^{-1}\) blood are at variance with the corresponding dicentric data. This finding is surprising because our recent comparison of the radiosensitivity of both biomarkers in CT scan-exposed blood samples in the absence of the contrast medium indicated that scoring of dicentrics and \(\gamma\)-H2AX foci formation are similarly sensitive methods to quantify a radiation-induced biological damage at dose levels from 0.025 Gy up to 1 Gy (Golfier et al. 2009). The reason for the present observation of an averaged biological enhancement even lower than the calculated physical enhancement value determined by the analysis of \(\gamma\)-H2AX foci has not been clarified until now and will be investigated in further studies. Possibly the applied quantification technique for enumerating \(\gamma\)-H2AX foci formation may play a role. Automated analysis as presently used for the quantification of the \(\gamma\)-H2AX foci has significant advantages over manual counting which was applied in our previous study (Golfier et al. 2009). The automated technique can be performed in a consistent and reproducible manner and should not be compromised by investigator-introduced biases and artifacts (Böcker and Iliakis 2006). However, it should be taken into account that this method seems to be highly dependent on the respective threshold or gating values used. For example, based on the observation that fewer numbers of \(\gamma\)-H2AX foci could be detected with increasing threshold values, Qvarnström et al. (2004) and Kataoka et al. (2006) concluded that the threshold level has an important impact on their quantification. Thus, it cannot be excluded that the present \(\gamma\)-H2AX data may be influenced by the automated quantification method. Especially, at the high dose of 1 Gy a potential overlap of adjacent foci may not be accurately separated resulting in an underestimation of foci counts. Evidence for such an explanation could be that, e.g. at the high dose of 1 Gy, the manually analyzing of \(\gamma\)-H2AX foci (Golfier et al. 2009) revealed about
two times higher yields than automated scoring (present study), although in both investigations blood from the same donor has been used. In contrast, at lower doses up to 0.1 Gy the number of γ-H2AX foci counts agrees well for both quantification methods (manually, automated, respectively). In addition, an influence of the time point of enumerating γ-H2AX foci following CT scans cannot be excluded, especially at the high iodine concentration of 50 mg ml$^{-1}$. The γ-H2AX foci labels the damage immediately after the induction of DNA double-strand breaks and induces the cellular repair machinery, whereas the dicentric chromosome is an unreparable resting damage. For example, Rothkamm et al (2007) showed both in vivo and in vitro, biphasic repair kinetics of double-strand breaks with fast earlier and slow later decline components of γ-H2AX foci formation in human lymphocytes after CT scans. Thus, the choice of a certain time point for enumerating γ-H2AX foci decides on covering mainly the fast or slow component of the repair kinetics. However, since for the present experiments, the repair mechanisms were not the main point of interest, a time level of 60 min following CT scans has been used. As clinically the permanent biological damage is the crucial question, the quantification of dicentrics is currently the recommended method for estimating an exposure to very low dose levels of radiation (‘biological dosimetry’).

In the present study, the individual radiation response of blood lymphocytes from a healthy male donor was investigated. For the detection of chromosome aberrations only iodine containing samples were analyzed. The reference data, i.e. the dicentric yields from the samples without a contrast agent, were taken from our previous study (Golfier et al 2009) on analyzing the dose response of CT scan-exposed blood samples from the same donor under identically experimental conditions. As the high reproducibility of scoring of dicentrics was shown in several studies (for example, Krumrey et al 2004, Buermann et al 2005, Schmid et al 2008), the reference data were not investigated again in the present investigation. The background frequency of dicentrics determined in the former study agrees well with the total background frequencies averaged from all studies with blood from the same donor. The high reproducibility is further indicated by the comparable low standard error of the total background frequency.

In general, the present results regarding dose enhancing by contrast agent demonstrate that in the dosing regime covering standard contrast-enhanced CT procedures (radiation doses and contrast doses), no biological dose-enhancing effect is present. This result is consistent with earlier data reported by Löbrich et al (2005) who could not observe any difference in the γ-H2AX foci levels in lymphocytes of patients and corresponding data obtained in the absence of a contrast agent. Only at therapeutic radiation doses, a dose-enhancing effect can be detected. This measured effect as obtained by scoring of dicentrics is, however, above the range of theoretical expectations of the physical enhancement effect indicating an additional biological effect due to the emitted photoelectrons.

5. Conclusions

In this work, CT scan-induced dicentrics and γ-H2AX foci formation in blood samples of a healthy donor were investigated in the presence and absence of an iodinated x-ray contrast agent. Based on the results of the experiments, it can be concluded that scoring of dicentrics and quantification of γ-H2AX foci are appropriate methods to detect radiation-induced biological damage after CT scans. CT scans in the presence of 5 mg iodine ml$^{-1}$ blood, representing clinically used concentrations of x-ray contrast agents, did not result in biological dose-enhancing effects, except for minor effect at a therapeutic dose of 1 Gy. However, with 50 mg iodine ml$^{-1}$ blood, permanent damage could be detected across the complete dose range. At
1 Gy, a clear biological dose-enhancing effect could be detected by scoring dicentrics with a dose enhancement above the theoretical expectations based on the physical dose enhancement.

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