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Leukocyte DNA damage after reduced and conventional absorbed radiation doses using 3rd generation dual-source CT technology

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ABSTRACT

Purpose: Computed tomography (CT) scans are an important source of ionizing irradiation (IR) in medicine that can induce a variety of DNA damage in human tissues. With technological improvements CT scans at reduced absorbed doses became feasible presumably lowering genotoxic side effects.
Materials and methods: For measuring DNA damage we performed γH2AX foci microscopy in peripheral blood mononuclear cells (PBMC) after exposure to reduced and conventional absorbed radiation doses using 3rd generation dual-source CT (DSCT) technology.
Results: CT scans performed at reduced absorbed doses of 3 mGy induced significant lower levels ($p < 0.0001$) of DNA damage (0.05 focus per cell ± 0.01 [mean ± standard error of mean]) at 5 min after IR compared to conventional absorbed doses of 15 mGy (0.30 focus per cell ± 0.03). With ongoing DNA repair background γH2AX foci levels (0.05 focus per cell) were approached at 24 h after CT with both protocols.
Conclusion: Our results provide evidence that reduced absorbed doses mediated by adjusted tube current in 3rd generation DSCT induce lower levels of DNA damage in PBMC compared to conventional absorbed doses suggesting a lower genotoxic risk for state-of-the-art tube current reduced CT protocols.

1. Introduction

With increased availability of multi-detector row computed tomography (CT), the use of CT examinations increased rapidly in all age groups in the last decade [1,2]. In parallel, radiation exposure and the resulting biologic effects became topics of ongoing discussion [3]. Although the risk for induction of cancer by a single CT scan is low (about 1:2000 assuming an effective dose of 10 mSv per scan) [4], it may translate into a considerable number of cancer cases in an epidemiologic scale [5]. In addition to theoretical risk calculations two large prospective cohort studies from the UK and Australia provided evidence for a significant increased cancer risk in individuals exposed to CT scans in childhood and adolescence [6,7]. Furthermore, the risk is also increased in individuals undergoing repeated CT scans, which is often required in cancer patients for adequate treatment planning and follow-up, so that individual cumulative radiation doses may exceed >300 mSv [8]. Thus, radiologists, physicists and CT scanner manufacturers seek to keep radiation exposure by CT scans "as low as reasonably achievable" (ALARA principle). Importantly, advances in CT hard- and software including automatic tube voltage selection, automatic tube current selection and iterative image reconstruction (e.g., provided by 3rd generation dual-source CT (DSCT)) allow now for reduction of the applied irradiation (IR) dose and thereby potentially lower genotoxic side effects.

Generally, for dose measurements conventional dosimeters such as ionization chambers are widely-used as they deliver fast and exact dose readings. However, for monitoring biological effects of IR, methods assaying specific molecular changes might be more appropriate. Since many years cytogenetic analysis of dicentric chromosomes has been the gold standard for biological dosimetry, however, sensitivity of this assay is limited to doses >100 mGy

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[9]. For monitoring DNA damage at lower doses >1 mGy, γH2AX immunofluorescence microscopy has evolved as a more sensitive and appropriate method in recent years. This assay relies on detection of the Ser139-phosphorylated form of histone H2AX (also named γH2AX), that accumulates in a region of several megabase pairs around each DNA double-strand break (DSB) [10]. There, multiple γH2AX histones form a platform for other proteins involved in DNA repair, chromatin remodeling and signal transduction [11]. γH2AX foci emerge rapidly after exposure to IR in the nucleus and usability of this assay for monitoring formation and repair of DSB after CT has been demonstrated previously [12–15].

The hypothesis of our study was that lower radiation dose levels, that became clinically feasible with state-of-the-art CT technologies, directly translate into less measurable DNA damage. Therefore, we prospectively compared γH2AX immunofluorescence microscopy based DNA damage measurements in peripheral blood mononuclear cells (PBMC) between a conventional radiation dose as well as a radiation dose reduced CT protocol.

2. Materials and methods

2.1. Peripheral blood samples

After institutional review board approval (Ethics Committee II, Medical Faculty Mannheim, University of Heidelberg) and written informed consent were obtained, peripheral blood samples were withdrawn from 12 healthy volunteers (5 male, 7 female, mean age: 39.3 years, range 25 to 55). PBMC were isolated by Ficoll density gradient centrifugation and cultivated in RPMI-1640 medium supplemented with 10% fetal calf serum, 4 mM glutamine and 1% penicillin/streptomycin. Cells were incubated in a humidified 5% CO_2 atmosphere at 37 °C until exposure to IR by CT.

2.2. In vitro x-ray irradiation

All PBMC samples were scanned in flasks using a 3rd generation DSCT (SOMATOM Force, Siemens Healthcare Sector, Forchheim, Germany). The following imaging parameters were used: 120 kV, effective tube time-current 45 mAs (absorbed doses of 3 mGy), 224 mAs (absorbed doses of 15 mGy) and 746 mAs (absorbed doses of 50 mGy). A rotation time of 0.5 s, a pitch of 35, a collimation of $2 \times 198 \times 0.6$ mm and a scan length of 12 cm were chosen and kept constant for each scan. All samples were positioned in the isocenter of the CT scanner in order to guarantee same IR for each sample. After IR samples were incubated again in a humidified 5% CO_2 atmosphere at 37 °C until analysis of γH2AX foci at indicated time points. All 12 PBMC samples from 12 healthy volunteers were analyzed for γH2AX foci yields at 5 min and 24 h after CT with doses of 3 mGy and 15 mGy, respectively. In 12 PBMC samples γH2AX foci yields were also analyzed at 5 min after CT with a dose of 50 mGy for determination of dose dependence of γH2AX foci formation. In three of 12 PBMC samples γH2AX foci yields were additionally analyzed at 30 min and 5 h after CT with doses of 3 mGy, 15 mGy and 50 mGy, respectively, for determination of DNA repair kinetics.

2.3. Sample processing and γH2AX immunofluorescence staining

PBMC were cytospun (1000 rpm) on glass slides, fixed in paraformaldehyde 4% (10 min) and permeabilized by Triton-X-100 0.1% (10 min). Samples were incubated with a primary mouse monoclonal anti-γH2AX antibody (1:500) (clone JBW301; Merck Millipore, Darmstadt, Germany) overnight at 4 °C. After washing in PBS and blocking in Chemiblocker (Merck Millipore) PBMC were incubated with an Alexa Fluor 488-conjugated goat anti-mouse secondary antibody (1:500) (Invitrogen, Karlsruhe, Germany) for 1 h at room temperature. PBMC were washed again and mounted with Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, US). Images were obtained by an Axioscope A1 fluorescence microscope (Zeiss, Jena, Germany) equipped with a Cool Cube 1 CCD camera (Metasystems, Altlussheim, Germany) and processed by Isis software (Metasystems). Foci analysis was performed by eye during imaging at a 100× objective magnification. At least 100 typical lymphocytic cells were analyzed for each measurement.

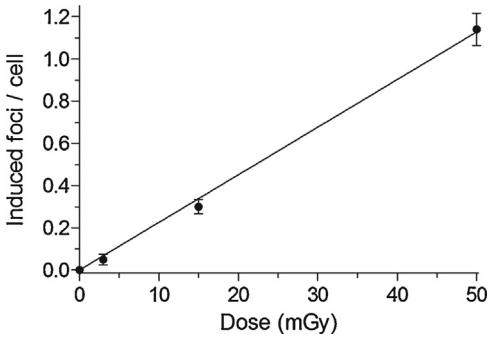

Fig. 1. Linear dose dependency of γH2AX foci formation in peripheral blood mononuclear cells (PBMC) after irradiation. PBMC samples were irradiated each with doses of 3 mGy, 15 mGy and 50 mGy by a 3rd generation dual-source CT. Each data point represents the mean γH2AX foci yield ± standard error of the mean of 12 different PBMC samples at 5 min after irradiation exposure. Mean γH2AX foci yields are corrected by subtracting the mean γH2AX foci yield of the background (0.05 focus per cell).

2.4. Statistical analysis

Statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, US). The paired sample *t*-test was used to test the hypotheses whether there is a significant difference in γH2AX foci yields after absorbed doses of 15 mGy and 3 mGy at 5 min after CT and whether there is no significant difference at 24 h after CT. P-values <0.05 were considered as statistically significant.

3. Results

3.1. Validation of γH2AX foci formation in irradiated PBMC in vitro

γH2AX foci yields were analyzed in PBMC at a dose range from 0 to 50 mGy. γH2AX foci yields increased linearly with dose and the mean increment of γH2AX foci was 1.14 foci per cell at 50 mGy (Fig. 1). A mean background level of 0.05 γH2AX focus per cell was detected in non-irradiated PBMC.

3.2. DNA repair kinetics in PBMC in vitro after IR by CT

DNA repair kinetics were analyzed in three samples of PBMC in a time interval from 0 to 24 h after exposure to IR by CT at absorbed doses of 3 mGy, 15 mGy and 50 mGy, respectively. After induction of DSB by IR, γH2AX foci reached maximum foci yields at 5 min followed by a non-linear biphasic decline with a fast repair component until 5 h and a slow repair component until 24 h after IR (Fig. 2).

3.3. Formation of γH2AX foci in PBMC in vitro after protocols with low absorbed and conventional absorbed doses

12 samples of PBMC from 12 healthy volunteers were scanned separately using protocols with absorbed doses of 3 mGy and 15 mGy. DNA damage was analyzed by γH2AX foci microscopy (Fig. 3) at 5 min and 24 h after IR. The mean increment of γH2AX foci