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Assessment of DNA double-strand breaks induced by intravascular iodinated contrast media following in vitro irradiation and in vivo, during paediatric cardiac catheterization

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Paediatric cardiac catheterizations may result in the administration of substantial amounts of iodinated contrast media and ionizing radiation. The aim of this work was to investigate the effect of iodinated contrast media in combination with in vitro and in vivo X-ray radiation on lymphocyte DNA. Six concentrations of iodine (15, 17.5, 30, 35, 45, and 52.5 mg of iodine per mL blood) represented volumes of iodinated contrast media used in the clinical setting. Blood obtained from healthy volunteers was mixed with iodinated contrast media and exposed to radiation doses commonly used in paediatric cardiac catheterizations (0 mGy, 70 mGy, 140 mGy, 250 mGy and 450 mGy). Control samples contained no iodine. For in vivo experimentation, pre and post blood samples were collected from children undergoing cardiac catheterization, receiving iodine concentrations of up to 51 mg of iodine per mL blood and radiation doses of up to 400 mGy. Fluorescence microscopy was performed to assess γH2AX-foci induction, which corresponded to the number of DNA double-strand breaks. The presence of iodine in vitro resulted in significant increases of DNA double-strand breaks beyond that induced by radiation for ≥17.5 mg/mL iodine to blood. The in vivo effects of contrast media on children undergoing cardiac catheterization resulted in a 19% increase in DNA double-strand breaks in children receiving an average concentration of 19 mg/mL iodine to blood. A larger investigation is required to provide further information of the potential benefit of lowering the amount of iodinated contrast media received during X-ray radiation investigations. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: contrast media; ionizing radiation; DNA breaks; double-stranded; DNA damage; paediatrics; cardiac catheterization

1. INTRODUCTION

The average annual per-capita effective radiation dose from medicine worldwide (approximately 0.6 mSv of the total 3.0 mSv received from all sources) has doubled in the past 10–15 years (1). In their lifetime, members of the UK population receive an average effective dose of 200 mSv resulting in a fatal cancer risk of 1% (2). Children are of particular concern because they are estimated to be 2-3 (3), and potentially up to 8-10 (4) times more radiosensitive compared to adults. The frequency of paediatric cardiac catheterizations (PCC) has increased rapidly in recent years (5) resulting in substantial radiation exposures (6,7), which can be greater than those received from computed tomography (CT) (8). Although more epidemiological evidence is still required, 50 mGy of ionizing radiation is thought to triple the risk of leukemia in children (9). Ionizing radiation is known to cause DNA lesions, of which double-strand breaks (DSBs) are of greatest concern because of their contribution to genetic instability and association with the potential development of carcinogenesis (10). Radiation induced DNA DSBs develop following disruption of the DNA phosphate backbone either by direct DNA radical production or by radicals formed through water radiolysis (11). The most widely used and most sensitive biomarker for detecting DNA DSBs as a result of low dose ionizing radiation exposure is the phosphorylated histone variant γH2AX (12,13).

Immunofluorescence microscopy enables the identification of γH2AX-foci, which represent a 1:1 relationship with the number of DNA DSBs (14).

Examinations involving the use of iodinated contrast media (ICM) have increased rapidly in recent decades. It has been estimated that approximately eight million litres of ICM are administered worldwide each year (15). Administration of ICM is commonly used to visualize cardiovascular anatomy during

procedures such as CT and cardiac catheterizations. The presence of ICM in the blood is essential because it enables the differentiation of cardiovascular structures and surrounding soft tissue on radiological images. Manufacturers of ICM provide recommended safe dose levels for administration to patients (16,17) however during complex cardiac catheterizations these levels can be exceeded. The potential detrimental effects of ICM are well established. These include allergic reactions, hyperthyroidism and contrast-induced nephropathy (18–20). However the influence of ICM upon DNA damage requires further assessment because of the uncertainty of evidence in the literature. Two studies investigating contrast enhanced CT have found a significant increase in the amount of in vivo DNA damage in patients who received ICM compared to those who did not (21,22). Similar findings have been found in vitro (23) whilst other authors have not observed clinically relevant changes (24,25). The purpose of this study was to examine differences in DNA DSBs between several concentrations of iodine and radiation doses similar to those that may occur in PCC.

2. MATERIALS AND METHODS

2.1. In vitro irradiation

Ethical approval was obtained from the Ulster University, Jordanstown. Informed written consent was obtained from three healthy volunteers. Each volunteer provided 35 mLs of blood, drawn up from the antecubital vein into vials containing di-potassium ethylene diamine tetra-acetic acid to prevent coagulation. The 35 mL blood sample from each volunteer was divided into 35 individual 1 mL samples and groups of seven samples were assigned to five radiation dose groups of 0 mGy, 70 mGy, 140 mGy, 250 mGy and 450 mGy. For each radiation dose the 7 x 1 mL samples were assigned to seven iodine concentrations of 0, 15, 17.5, 30, 35, 45 and 52.5 mg iodine per mL blood (Table 1). This was achieved by pipetting the relevant blood volumes into eppendorf tubes containing a predetermined volume of ICM (Omnipaque 300, GE healthcare). A non-ionic ICM was chosen for this investigation because it is standard practice in cardiovascular imaging (26). The in vitro iodine concentrations investigated represented a range of concentrations investigated in previous studies (21–25) and also corresponded to the clinical in vivo concentrations observed in this study. A Carestream DRX-Evolution digital radiography system was used to irradiate the blood samples. Irradiated eppendorf tubes were placed in the centre of a 3.5 x 3.5 cm X-ray field and irradiated individually using a tube potential of 120 kVp and tube currents of 160, 320, 560 and 1000 mA to achieve the desired radiation dose.

2.2. In vivo irradiation

The in vivo irradiations were performed as part of our ongoing clinical work, which aims to potentially lower radiation dose in paediatric cardiac catheterization. Approval for this part of the study was obtained from the Office for Research Committees Northern Ireland and the Belfast Health and Social Care Trust. A total of 69 consecutive participants (36 males, 33 females: mean age 5.8 years ± 4.3: age range 0–16 years) who were scheduled to undergo paediatric cardiac catheterization between April 2014 and April 2015 provided informed written consent to participate. The exclusion criteria was as follows: unwilling to provide consent, known cancer, history of radiotherapy or chemotherapy, viral infections, blood disorders, exposure to toxins, history of antioxidant supplementation, physical exercise two hours prior to participation. Imaging was performed using a Philips Allura Xper FD10/10 bi-plane cardiovascular system (Philips Healthcare, Amsterdam, Netherlands). A 1 mL blood sample pre and post cardiac catheterization was obtained from each participant. 57 patients (33 males, 24 females: mean age 5.4 ± 4.2) received an average of 19 mg iodine per mL blood (Iomeron 350, Bracco Imaging, Milan, Italy) and an average radiation dose of 116 mGy. 12 patients underwent unenhanced imaging (four males, eight females: mean age 7.8 ± 4.17) and received an average radiation dose of 16.6 mGy. The radiation dose in the form of air kerma was recorded from the X-ray systems display panel. Each individual's weight and sex was used in combination with the amount of ICM administered to determine the in vivo concentrations of iodine.

2.3. Lymphocyte separation

All in vitro blood samples were incubated at 37 ℃ for 30 minutes in order to capture optimal DNA damage repair by allowing time for DNA damage signaling (27). The in vivo blood samples were stored in a blood transport cooler at 4 ℃ and transported immediately to our laboratory for immediate analysis. All blood samples were added to 1 mL phosphate buffered solution (PBS) and carefully layered using a pipette onto lymphocyte separation medium Histopaque 1077 (Sigma-Aldrich, Dorset, UK). Centrifugation was performed at 2000 rpm for 30 minutes at 21 ℃ and stopped without the use of a brake mechanism. The lymphocyte layer was aspirated with a pipette, placed in 5 mLs of PBS and centrifuged at 1070 rpm for five minutes. The resulting lymphocyte cell pellet was washed with PBS and spotted onto superfrost plus slides (Menzel-Glaser, Thermo Fisher Inc., Braunschweig, Germany) and left to dry for 30 minutes. Cells were then fixed with 2% formaldehyde for 10 minutes.

2.4. Immunofluorescence and microscopy

Fixed cells were permeabilized using 0.5% Triton-X100 PBS for 10 minutes and then washed with PBS. Cells were blocked in 5% horse serum and 0.1% Triton-X100 PBS for 30 minutes, washed with PBS and incubated with 1:1000 mouse anti-H2AX phosphorylated monoclonal antiphospho histone H2AX (ser 139) clone (Millipore, Herfortshire, UK) in the dark for one hour at room temperature. After washing slides with 0.1% Triton-X100 PBS cells

Table 1. Summary of the volumes of Omnipaque 300 and blood mixed to achieve the seven iodine concentrations prior to irradiation

mg iodine per mL blood	0	15	17.5	30	35	45	52.5
Omnipaque 300 (μl)	0	50	58	83	116	149	175
Blood (μl)	1000	950	942	917	884	851	825

were incubated with Alexa Fluor 488 goat anti-mouse IgG, IgA, IgM (H&L) (Life technologies, Paisley, UK) in the dark for one hour at room temperature. As a secondary assay, the p53 binding protein (53BP1), which accumulates at γH2AX was also stained by adding pAb anti-53BP1 antibody (Novus Biological Ltd, Cambridge, UK) and Alexa Fluor 568 goat anti-rabbit IgG (H&L) (Life Technologies, Paisley, UK). Slides were finally washed with PBS, mounted with vectashield hardset DAPI (Vector Laboratories, Peterborough, UK) and protected with cover slips. The numbers of γH2AX-foci were counted using an Olympus BX3 epiflurescence microscope with x63 objective. 100 lymphocyte cells were scored for each of the 35 in vitro samples, and due to dispersal of irradiated lymphocytes in the circulating blood 300 cells were scored for in vivo samples.

2.5. Statistical analysis

Data is presented as the mean and standard error of the allocated in vitro radiation and concentration of iodine for all cells counted from the three volunteers. Dunnet's t-test was used to compare non-irradiated samples with and without iodine. One-way ANOVA was used to compare differences in γH2AX-foci for baseline samples, radiation doses and concentrations of iodine. Statistical Package for Social Science 21 (SPSS Inc., Chicago, IL, USA) was used for the data analysis. After Bonferroni correction for inequalities, differences were considered statistically significant when p was < 0.05. An ANCOVA test was performed for γH2AX-foci numbers for in vivo contrast enhanced and unenhanced examinations. The radiation dose was used as the covariant affecting γH2AX-foci induction.

3. RESULTS

3.1. Lymphocytes irradiated in vitro

3.1.1. Baseline comparison

The baseline γH2AX-foci observed in the three healthy participants in non-irradiated samples without iodine are demonstrated in Fig. 1. Individual mean baseline levels ranged from 0.01-0.03 γH2AX-foci per cell and were not statistically significant from each other $(p > 0.05)$.
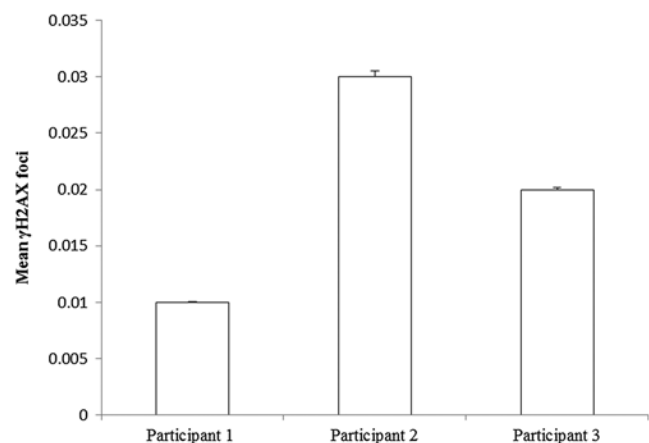
3.1.2. Non-irradiated samples

For the non-irradiated samples mean γH2AX-foci were ≤ 0.03 (Table 2). Compared to the control sample without iodine, no significant difference in mean γH2AX-foci was observed for any of the concentrations of iodine $(p > 0.05)$.

3.1.3. Irradiated samples

An increase in the mean number of γH2AX-foci in irradiated cells was readily observed compared to non-irradiated cells (Fig. 2). In those samples without iodine a considerable increase in mean γH2AX-foci was observed as radiation dose increased (Fig. 3). The mean γH2AX-foci clearly increased as iodine concentration increased for all irradiated samples (Fig. 4). Compared to 0 mg iodine per mL blood, the increase in mean γH2AX-foci was not significant for 15 mg iodine per mL blood, $(p > 0.05)$. However the increase in mean γH2AX-foci was significant for iodine concentrations ≥ 17.5 mg iodine per mL blood $(p < 0.05)$. The effects of increasing radiation dose and concentration of iodine demonstrated a linear increase in mean γH2AX-foci (R^2 0.96). Iodine contributed to a greater percentage of mean γH2AX-foci induction at lower radiation doses (Table 3). This was demonstrated for the lymphocytes exposed to 70 mGy whereby the presence of iodine increased γH2AX-foci by 44-111% whilst for 450 mGy the mean increase in γH2AX-foci was 11-59%. The individual response for each participant demonstrated a similar response and an increasing trend in mean γH2AX-foci induction as iodine concentration increased. The results of our 250 mGy dataset for each participant are demonstrated in Fig. 5.

3.2. Lymphocytes irradiated in vivo

Pre irradiation samples contained no more than an average of 0.07 γH2AX-foci. Participants who received ICM during paediatric cardiac catheterization were found to have 47% greater mean γH2AX-foci (0.9±0.07) compared to those who did not receive ICM (0.48±0.04). Following an adjustment using the ANCOVA test to account for the effect of radiation dose upon DNA DSB induction, ICM was found to have caused an average increase of 19% γH2AX-foci (0.086±0.08) compared to those who did not receive ICM (0.7±0.019) (Table 4).

4. DISCUSSION

The γH2AX assay has proven to be a reliable, reproducible and sensitive biomarker for the detection of DNA DSBs in human

Figure 1. Individual baseline γH2AX-foci in non-irradiated blood samples without iodine for each participant. Differences in mean γH2AX-foci were not significant $(p > 0.05)$.

Table 2. Comparison of γH2AX-foci in blood samples containing concentrations of iodine in non-irradiated blood samples

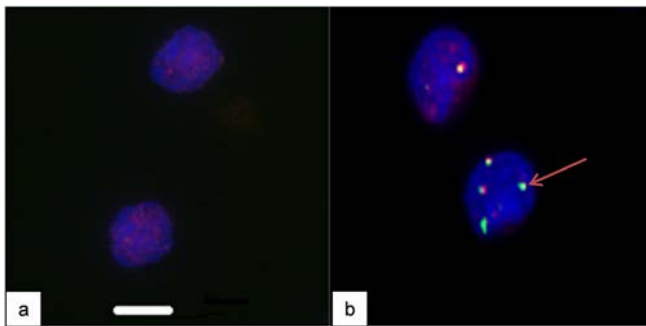| mg iodine per mL blood	Mean foci and Standard error	Dunnet's t-test (p-value)
0	0.02 ±0.0011	(Control group)
15	0.03 ±0.0011	0.831
17.5	0.01 ±0.0017	0.969
30	0.02 ±0.0005	0.600
35	0.00 ±0.0015	0.831
45	0.00 ±0.0001	0.372
52.5	0.00 ±0.0003	0.203

Figure 2. Example images after immunofluorescence of DNA in peripheral blood lymphocytes, scale 20 μm: (a) 0 mGy and (b) 450 mGy (arrows indicate individual γH2AX-foci).
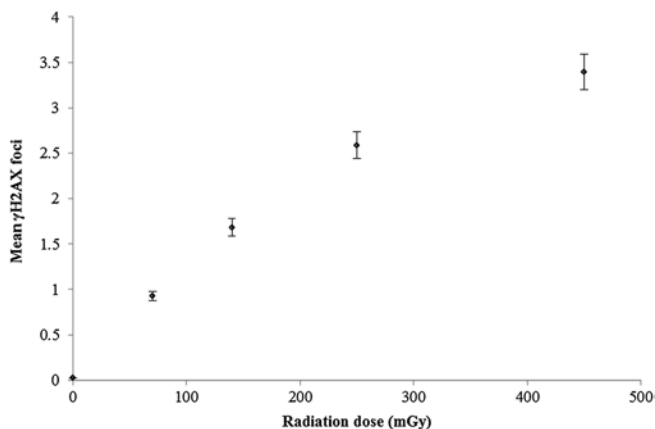

Figure 3. Mean γH2AX foci induction with standard error for 0 – 450 mGy without the presence of iodine.

Table 3. The percentage increase in γH2AX-foci in irradiated samples containing iodine concentrations compared to samples irradiated without the presence of iodine

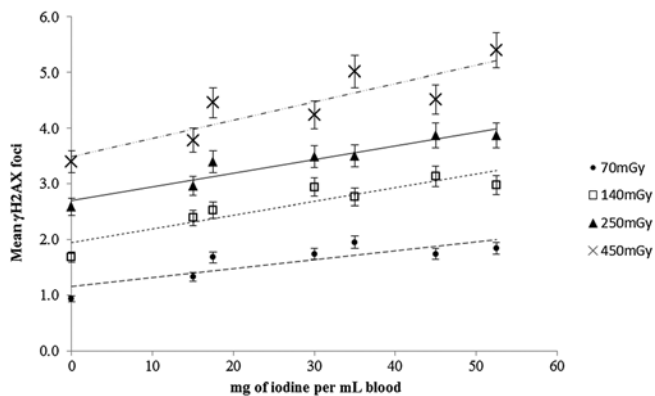| Radiation dose (mGy)	mg iodine per ml blood	Increase in mean foci (%)
70	15	44
	17.5	82
	30	89
	35	111
	45	89
	52.5	99
140	15	42
	17.5	50
	30	75
	35	64
	45	86
	52.5	76
250	15	14
	17.5	31
	30	35
	35	35
	45	49
	52.5	49
450	15	11
	17.5	36
	30	25
	35	48
	45	33
	52.5	59

Figure 4. Mean γH2AX-foci induction with standard error for 70 - 450mGy with 0 - 52.5 mg iodine per mL blood for three healthy volunteers. Compared to the control samples of 0mGy in Table 2, a significant difference was observed for 70mGy, 140mGy, 250mGy and 450mGy ($p < 0.05$). Compared to the control sample with 0 mg iodine per mL blood, one-way ANOVA with bonferoni correction for inequalities demonstrated no significant difference for 15 mg iodine per mL blood ($p > 0.05$) and a significant difference for 17.5 – 52.5 mg iodine per mL blood ($p < 0.05$). Multiple R-squared for radiation dose and iodine was 0.96.

Figure 5. Mean γH2AX foci induction with standard error for 250 mGy with 0 - 52.5 mg iodine per mL blood for participants 1-3.

lymphocyte cells following exposure to ionizing radiation (21,24,27). This study has assessed γH2AX-foci in lymphocytes following in vitro irradiation (0-450 mGy) of venous whole blood with and without the presence of iodine. The potential enhancement effect of ICM in vivo was also investigated in children undergoing cardiac catheterization. Detection of γH2AX-foci has enabled the quantification of the number of DNA DSBs, a potentially carcinogenic lesion. The in vitro radiation doses and iodine concentrations investigated were similar to our in vivo observations and also enabled comparison with previous published studies (21–25,28). A total of six iodine concentrations were investigated in vitro and represents a greater number of iodine concentrations previously investigated. This study also

Table 4. Mean in vivo γH2AX foci induction for patients undergoing paediatric cardiac catheterization with and with iodinated contrast media

	ICM group (n 57)	Non-ICM group (n 12)
Mean γH2AX foci induction	0.09±0.07	0.48±0.04
Mean γH2AX-foci induction with ANCOVA test	0.7±0.019	0.86±0.008

investigated the in vivo effect of ICM in the presence of ionizing radiation in children undergoing medical imaging.

It has been established that as radiation dose increases a linear increase in the number of DNA DSBs occurs (27,29). Yet there is uncertainty in the literature regarding the additional effect from the presence of ICM in blood during irradiation. As found in previous studies (21–25), the presence of iodine did not result in a significant difference in γH2AX-foci in non-irradiated blood samples owing to its few pharmacological and cellular effects (30). However our study has found a significant increase in mean γH2AX-foci for ≥17.5 mg iodine per mL blood during irradiation, beyond that caused by ionizing radiation. The in vitro increase in mean γH2AX-foci, as a result of the presence of iodine during irradiation was highly significant and up to 111%. For comparison, the in vitro increase in mean in γH2AX-foci, caused by increasing the radiation dose from 70 to 200 mGy in our study, was 118-181%. Therefore the amount of DNA DSBs caused by the presence of iodine in vitro was comparable to what would occur by increasing the radiation dose. Additionally our in vivo observations demonstrated an average increase of 19% more DNA DSBs in children undergoing cardiac catheterization who received an average of 19 mg iodine per mL blood.

The main paradigm of ionizing radiation damage is thought to occur as a consequence of the photoelectric effect; the emission of a k-shell electron following the absorption of energy from an X-ray photon. Photoelectrons deposit their energy to orbital electrons in absorbing atoms (31). This event may damage the chemical bonds of DNA directly, or indirectly by causing the radiolysis of water; the disassociation of water molecules into its constituent atoms. The result of which is the production of highly reactive free radicals that have a high degree of reactivity with their surrounding environment, because they can either donate an electron, or accept an electron from other molecules (32). Clusters of free radicals, formed adjacent to DNA are significant because they react with the covalent and hydrogen bonds of DNA strands. Compared to the chemical elements carbon (z=6), hydrogen (z=1), oxygen (z=8) and nitrogen (z=7), which make up more than 95% of the human body, iodine has a much greater atomic number (z=53). Therefore it is plausible that the presence of iodine, containing a much greater number of electrons, results in an increase in the photoelectric effect and the consequent amount of free radical production.[†] The reason that the amount of observed DNA DSBs was significant in our study is likely because of the K-edge absorption of iodine (34 keV), which is also in the energy range of photons used in medical imaging. Additionally the increase in photoelectrons, caused by the presence of iodine, is also thought to contribute to the Auger effect (33). The Auger

effect is a process that occurs when an outer shell electron fills an inner shell vacancy shell. This event may be accompanied by either the emission of a photon that has energy equivalent to the energy gap between the outer and inner electrons, or this energy can instead be transferred to another outer shell electron that is then ejected from the atom (34). The result of additional photon generation and ejected electrons may then be responsible for creating more free radicals and further DNA DSBs.

Our in vitro results have demonstrated that DNA DSBs increase as a result of the presence of ICM during irradiation in an average cell population from three healthy individuals. These results are in broad agreement with previously published in vitro results (21–23). The in vitro percentage increase in mean γH2AX-foci in our study for 35-45 mg iodine per mL blood (33-111%) was comparable to the in vitro findings observed 30 minutes post irradiation in vitro by Pathe et al. (22) (36-95%) who investigated 40 mg iodine per mL blood. The percentage increase observed by Grudzenski et al. (21) for 33 mg iodine per mL blood at 500 mGy (60%) was also comparable to the 48% that we observed in vitro using 35 mg iodine per mL blood at 450 mGy.

Jost et al. (25) investigated two iodine concentrations of 5 mg and 50 mg per mL of blood and a radiation dose range of 25 mGy-1 Gy, finding consistent evidence of additional DNA DSBs for 50 mg iodine per mL blood and not for 5 mg iodine per mL blood. Jost et al. (25) concluded that 50 mg per mL blood is beyond a typical iodine concentration administered in diagnostic CT examinations but did not investigate other concentrations of iodine. Joubert et al. (28) did not find additional DNA damage in the presence of 10 mg iodine per mL blood during irradiation of endothelial cells. The findings by Jost et al. (25) and Joubert et al. (28) are consistent with the findings of our in vitro results, demonstrating no additional DNA damage below 17.5 mg iodine per mL blood.

The percentage in vitro increase in mean γH2AX-foci for the presence of 15 mg iodine per mL blood (11%) was less than that found by Deinzer et al. (23) (38%) for a similar radiation dose. Furthermore our in vitro results for 15 mg iodine per mL blood were not statistically significant for 70-450 mGy whilst Deizner et al. (23) found significant enhancement with 7.5 and 15 mg iodine per mL blood using 20 and 500 mGy. It could be postulated that these differences may be due to the use of multiple volunteers in this study. However work assessing intra and inter variability of baseline and radiation induced γH2AX-foci in 32 healthy adults, demonstrated that genetic or environmental factors do not appear to significantly modify baseline and radiation-induced γH2AX-foci yields (35). One reason for the differences in the results from this study and (23), may be because the Deinzer et al. (23) study mixed ICM with PBS and blood prior to irradiation whereas this study irradiated ICM and blood prior to adding PBS. The presence of PBS in addition to blood during irradiation may have exaggerated DNA damage due to the production of more free radicals from radiolysis due to the presence of more water molecules.

Beels et al. (24) investigated 0-20 mg iodine per mL blood using 0-50 mGy, and did not find evidence of DNA damage enhancement. Radiation doses of 50 mGy may therefore require a larger concentration of iodine (>20 mg mL blood) to produce an enhancement effect. Beels et al. (24) comment that the iodine concentrations and radiation doses they investigated are within the normal limits of CT examinations. Our study considers PCC procedures, which can involve the use of greater volumes and concentrations of ICM compared to CT. For imaging of an anatomical structure such as the left ventricle, an injection of a large bolus of ICM would result in concentrations of iodine >50 mg

[†]Correction added on 21 December 2015, after first online publication: "k-shell" was removed from this sentence.

per mL blood during irradiation for an average patient (36). Although more difficult to estimate, it is likely that comparable iodine concentrations may be present during imaging of the coronary arteries during these procedures. PCC also involves a large proportion of continuous fluoroscopic imaging of the cardiac region therefore consideration should be given to any remnant iodine concentrations, which may occur in the circulating blood. According to ICM manufacturer guidance notes the maximum amount of ICM administered during cardiac procedures should not exceed 5 mLs/kg. A typical volume of blood for an average adult, 5 mLs/kg of ICM corresponds to approximately 20-25 mg iodine per mL blood. It is known that doses of >20-25 mg iodine per mL blood, are frequently exceeded in adult catheterizations during repair of the left anterior descending artery (37). Therefore, the greatest concentrations of iodine investigated by Jost et al. (25) are clinically relevant to complex interventional procedures, which may on occasions exceed ICM dose limits in PCC.

Our study demonstrated a greater magnitude of DNA damage by the presence of iodine at the lowest observed radiation doses. This meant that iodine contributed to a greater percentage of the total number of DNA DSBs than ionizing radiation at lower radiation doses. Pathe et al. (22) and Deinzer et al. (23) also made this observation. The mechanism for this is unclear. It may be that the presence of iodine reaches a threshold effect, which is independent of increasing radiation dose. Regardless of the reason, the results indicate that for a given iodine concentration, an increase in radiation dose is not likely to exaggerate the DNA damage caused by the presence of iodine.

The mean baseline levels for the three volunteers in this investigation (0.02) were typical of the numbers expected in healthy volunteers (22,29). Similarly to this study, previous investigations also used manual foci scoring. Although 100 cells were scored in this study, baseline levels compared well with Deinzer et al. (23) who scored 200 cells per sample and Pathe et al. (22) who scored either 40 cells or until 40 foci per sample were detected. Joubert et al. (28) and Jost et al. (25) did not specify the number of cells that were counted.

Our in vivo observations demonstrate that children can receive considerable levels of ICM, up to 65 mg iodine per mL blood during cardiac catheterizations. Therefore, the greatest concentrations of iodine investigated by Beels et al. (24) and Jost et al. (25) are clinically relevant to complex interventional procedures. The average 19% increase in DNA DSBs, in those patients that received an average of 19 mg iodine per mL blood during cardiac catheterization, is in agreement with out in vitro findings. Our in vivo results support previous evidence of DNA damage enhancement from contrast enhanced CT (21,22,38). The average amount of DNA damage caused by the presence of ICM was much lower in our study than Pathe et al. (22) (58%) and Piechowiak et al. (38) (107%) but were more similar to Grudzenski et al. (21) (30%). The reason that the enhancement effect caused by ICM was lower in our study was because cardiac catheterizations take place over a longer period time (up to two hours) and therefore iodine would have begun to accumulate in the renal system rather than remain completely in the blood circulation. Our in vivo findings have helped demonstrate the clinical relevance of ICM. The established linear response of DSB to ionizing radiation, as well as ionizing radiation and cancer risk (39), suggest that the presence of iodine during in vivo irradiations may increase the risk of cancer induction by 19%. Consequently these findings are relevant to clinicians who perform contrast-enhanced procedures involving higher amounts of ionising radiation in children. Our current work and

data from elsewhere (40) suggest that average radiation induced cancer risk to children undergoing cardiac catheterization is approximately 1 in 1000. Children may also receive multiple procedures during childhood resulting in a cumulative cancer risk that may be as high as 6.5% (41).

A number of limitations exist within this study. The linear no threshold theory states that there is no safe radiation dose and that cancer risk increases linearly with radiation exposure (39). It is also established that there is a linear increase in the generation of DSBs with increasing radiation exposure (42). However our study has not reliably quantified the additional cancer risk as a result of the additional DSBs caused by the presence of iodine in the blood during irradiation. It must also be acknowledged that the DNA DSBs observed in this study captured the repair process of a DSB 30 minutes post irradiation when γH2AX accumulation is most prevalent. DSBs are primarily repaired by two cellular pathways known as non-homologous end joining (NHEJ) and homologous recombination (HR) (43) and γH2AX is known to disperse once a DSB is repaired (44). This study therefore did not determine whether non-repair or misrepair occurred for any of the observed DNA damage. The presence of γH2AX-foci >20 hours following ionizing radiation exposure is thought to represent aberrant chromatin structure by illegitimate strand rejoining (45). Consequently it may be of interest to investigate DNA DSB repair in as a result of the presence of ICM during irradiation in future studies over a 24 hour period. Although this study also attempted to assess DNA DSBs in vivo, the half-life of ICM in the vascular circulation and its rapid distribution into extra cellular fluid may result in a reduction of iodine concentrations in the circulating blood. This may result in gradual decrease in concentrations of iodine in the field of view during lengthy cardiac imaging as opposed to an accumulation of iodine. At present the effects of fractionated high doses of ionizing radiation, such as those used in radiotherapy, have been proven to cause cellular hypersensitivity (46,47). Cardiovascular fluoroscopy procedures would also involve fractionated radiation exposures in combination with ICM however evidence of hypersensitivity in low to moderate doses associated with diagnostic imaging is less clear compared to high doses associated with radiotherapy (48,49). This should be considered in future investigation on the effects of ICM. This study used one type of ICM because a variation in amounts of DNA damage as a result of differing manufacturing brands has been strongly disproven (23). The additional DNA damage caused by the presence of iodine in lymphocytes cells does not portray the affect upon DNA in cells elsewhere in the body. Peripheral blood lymphocyte cells were used because an abundance of them are easily obtained, they are non-cycling and have very low background levels of DNA DSBs, giving excellent sensitivity. With regards to the effects of other circulating cells, there has been little work done in this area. This is likely because red blood cells and platelets do not have DNA. Neutrophils do not appear to form γH2AX-foci at the same intensity following irradiation making it difficult to quantify any additional damage with contrast medium (50). The dispersal of iodine to all areas of the body may warrant future investigations focusing upon the effects of other regions of the body.

Future consideration should be given to the excretion time of ICM patients. The plasma half-life of ICM in patients with normal renal function is reported to range from 30 minutes (30) up to two hours (51) whilst renal impairment can result in a half-life of ICM of ≥ 30 hours (52). Remnant iodine concentrations in the blood circulation may render an individual more susceptible to DNA damage enhancement if they were to undergo further

medical radiation exposure. At present there has been no investigation into a link between ICM and increased risk of cancer, which may be an interesting area of future research. In particular it may be of interest to investigate a link between iodine enhanced DNA damage and risk of renal cell carcinoma caused by the presence of contrast-enhanced renal examinations. Our study did not determine the effects of ICM in the presence of radiation on bone marrow, therefore a future study could consider the influence of ICM upon progenitor cells. It may be of interest to assess for chromosome aberrations in white blood cells one - two years post irradiation with ICM to determine whether the bone marrow received additional damage.

5. CONCLUSION

The in vitro findings in our study have demonstrated that significant additional DNA damage occurs from irradiation of 70-450 mGy in the presence of ≥ 17.5 mg iodine per mL blood beyond that of the radiation induced damage alone. Our in vivo observations have demonstrated the clinical relevance to adult and paediatric patients undergoing interventional cardiovascular procedures of the enhancement effect of ICM. Iodine during irradiation was found to cause a comparable amount of DNA damage to that caused by increasing the radiation dose. Intravenous urography, micturating cystourethrography and contrast enhanced CT of the abdomen may also be of concern. The administration of ICM should be taken into consideration and investigation should focus on the potential benefit of optimizing the dosage of ICM. Future investigation could explore the potential additional cancer risk caused by ICM.

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