HIROSAKI UNIVERSITY

Radiation Emergency Medicine 2014 Vol. 3 No. 2 19-23

REM
Radiation Emergency Medicine

Review

Multidisciplinary Biodosimetric Tools for a Large-scale Radiological Emergency – the MULTIBIODOSE Project

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Received 4 March 2014; revised 25 April 2014; accepted 28 April 2014

Large scale radiological emergencies pose a particular problem for emergency preparedness because of potentially large numbers of worried well and the necessity to carry out triage in a timely manner. Here, an indispensable tool is biological dosimetry. Although a number of biodosimetric tools exist, not many have been tested and adapted for a large scale emergency scenario. In the framework of an EU-funded project MULTIBIODOSE we tested a variety of biodosimetric tools and adapted them to different mass casualty scenarios. The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario, as well as speed of performance. The project was completed in April 2013. Its major conclusions are presented.

Key words: radiation emergency, biological dosimetry, triage, radiation protection

1. Introduction

The increasing use of ionizing radiation in industry and medicine enhances the risk of radiation accidents. At the same time, there is concern about the use of radiation sources for the purpose of terrorist attacks. A possible consequence of either scenario is the potential exposure

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to radiation of a large number of people[1]. Large scale radiological emergencies pose a particular problem for emergency preparedness because of the necessity to quickly identify the non-exposed (worried well), the moderately exposed that do not require immediate medical attention and those exposed that must be taken care of without delay. It can easily be imagined that, due to stress, many people will show symptoms like nausea or diarrhoea that can be misinterpreted as signs of severe radiation exposure. Others may show no symptoms at all despite the fact that they were exposed. Therefore, an indispensable element of preparedness for large scale emergency is the availability of biological dosimetry that can help to quickly triage people according to the absorbed dose[2].

A number of biodosimeters or markers of exposure exist[3, 4], but they are usually time consuming and therefore not suitable for large-scale emergency scenarios, where the primary aim is speed of performance and not precision of dose estimate. Moreover, these methods differ in their specificity to various exposure scenarios and in the stability of the signal. In view of this, different biodosimetric tools should be applied after an emergency so that the dose information can be made available with optimal speed and precision.

Between May 2010 and April 2013 the European Commission funded the collaborative research project MULTIBIODOSE, in which a variety of biodosimetric tools were analysed, validated and adapted to different mass casualty scenarios. Emphasis was placed on harmonising the tools in the partner institutions in order to create a network of competent laboratories with a capacity high enough to cope with a mass radiation emergency event in a timely manner. The partners included representatives from radiation protection authorities, health protection authorities, independent research institutes and universities. The major results and conclusions of the project are presented here. More information about the project can be found at www.multibiodose.eu.

2. The biodosimetric tools and their characteristics

The following seven dosimetric methods were tested and validated for their suitability as tools to triage exposed individuals in case of a large-scale radiological emergency:

1. Manual and automated dicentric (Dic) assay
2. Automated micronucleus (MN) assay
3. Gamma-H2AX assay
4. Electron paramagnetic resonance spectroscopy (EPR)
5. Optically stimulated luminescence (OSL)
6. Skin speckle assay (SSA)
7. Serum protein assay (SPA)

The assays were tested for their ability to categorise an exposed person according to three exposure levels: below 1 Gy, between 1 and 2 Gy, above 2 Gy. They were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance. Moreover, some of them were well established as biodosimetric tools and only needed to be adapted to a mass casualty scenario, while other assays required further validation.

The dicentric assay in peripheral blood lymphocytes is regarded as the gold standard for biological dosimetry[5] and an ISO standard exists for it. Indeed, it was invented more than 50 years ago and rich experience exists as to its use in radiation accidents[6]. The micronucleus assay in human peripheral blood lymphocytes can be regarded as a variant of the dicentric assay, because micronuclei arise as consequence of chromosomal aberrations[7]. The assay is currently in the process of ISO standardisation. Its advantage over the dicentric assay is that large numbers of cells can be scored within a shorter time than that required for dicentrics. However, MN are not specific for ionizing radiation and the spontaneous frequency is much higher than that of dicentrics. Inherent to both the Dic and MN assay is the need to culture lymphocytes under in vitro conditions for 2-3 days so that the chromosomal damage can be visualised. The analysis of dicentric chromosomes and micronuclei can be carried out manually or automatically, with the help of dedicated image acquisition and analysis tools[8]. In MULTIBIODOSE dicentric chromosomes were analysed both manually and automatically, while micronuclei were only analysed automatically.

The gamma-H2AX assay is based on analysing the formation of DNA repair protein clusters called gamma-H2AX "foci" in peripheral blood lymphocytes of an exposed person[9, 10]. The assay does not require culturing of cells so that the results can be obtained within a few hours. However, foci disappear as DNA damage is repaired and the signal can only be detected during the first 1-2 days after exposure. Similarly as dicentrics and micronuclei, foci can be analysed automatically and manually. In MULTIBIODOSE both analysis modes were used.

EPR spectroscopy is a technique allowing studying radiation-induced free radicals or defects in biological or inert materials[11]. Mineral glass from LCD or touch screens of portable electronic devices such as smart phones are suitable materials. Consequently, these can be used for individual dose assessment. The main advantages of EPR are its high radiation specificity of radio-induced signals, signal linearity in the high dose range (> 1 Gy) and, above all, long term signal stability (up to several years). Its detection threshold in smart phone glass displays is 1 Gy. The method is currently in the process

Table 1. General characteristics of the assays. Given for most low LET radiation qualities such as gamma and X-rays

Assay	Time span after exposure during which the assay can yield usable results			Exposure scenario that can be detected by each method alone			Specific for ionising radiation	Sensitivity of the assay (dose range in Gy)
	Days	Weeks	Months	Acute	Pro-tracted	Partial body		
Dic manual	√	√	√	√	√	√	√	0.1 - 5
Dic automated	√	√	√	√	√	√	√	0.1 - 5
MN	√	√	√	√	√	√		0.3 - 5
Gamma H2AX	√			√				0.2 - 5
EPR (ped*)	√	√	√	√	√		√	1 - >10
OSL (ped*)	√	√		√	√		√	0.01 - >10

*ped: portable electronic devices

Table 2. Approximated duration of sample analysis using different methods. The table does not provide precise time estimates, but rather a comparative overview of the characteristic of each method. The times for analysing dicentric chromosomes are given for 50 (manual) or 150 (automated) mitotic cells, and micronuclei for 1000 binucleated cells

	Time per step per sample Time in hours			
	Culture of cells	Preparation of slides/samples	Analysis	Total
Dic manual	48	4	0.5	52.5
Dic automated	48	4	0.2	52.2
MN	72	4	0.2	76.2
Gamma H2AX	0	2	0.1	2.1
EPR (ped*)	0	0.2	0.2	0.4
OSL (ped*)	0	0.3	0.06	0.36

*ped: portable electronic devices

of ISO standardisation. OSL can be used to assess the dose of ionizing radiation by measuring light emitted from irradiated objects following optical stimulation[12]. Electronic elements such as aluminium oxide-coated resistors used in mobile phones have luminescent properties and can be used as individual dosimeters. The advantage of OSL is its very high specificity and sensitivity to radiation (from several mGy to several Gy). There is a signal loss of 50% in the first 10 days after irradiation and fading correction must be applied.

The skin speckle assay and the serum protein assay were two new tools that have hitherto not been validated as biological dosimeters. SSA is based on analysing radiation-induced speckle patterns in the skin and SPA on radiation-induced changes in the level of selected serum proteins. SSA was tested on skin of pigs and SPA in blood samples collected from breast cancer patients undergoing external beam radiotherapy. The obtained results suggested that at least one month must pass between radiation exposure and analysis before a radiation-induced signal can be detected at the level of SSA. The results of the SPA showed that, due to strong inter-individual variability, more analyses are required to validate the assay. In view of this SPA and SSA were not included in the battery of MULTIBIODOSE tools.

General characteristics of the assays are given in Table 1. The best signal stability is given for the dicentric and micronucleus assays along with EPR. A high specificity for radiation is only given for the dicentric assay and EPR and OSL. The sensitivity of the assays differs somewhat. However, for the purpose of triage, where it generally suffices to correctly categorise a person into a dose group of < 1 Gy, 1-2 Gy and > 2 Gy, all assays are equally suitable. It should be mentioned that the precision of dose estimate of the biological assays (i.e. based on peripheral blood lymphocytes) depends on the number of analysed cells. When the analysis is carried out manually, this step is a major time factor. When the analysis is carried out automatically, a sufficiently high number of cells (usually around 150) can be carried out within a reasonable time. Thus, for the automated Dic and MN assays carried out on a large number of samples, the bottleneck is not the analysis of slides by a human scorer, but the capacity to culture and harvest blood lymphocyte samples and the availability of specialised microscopy equipment for automated image capture.

3. The performance of the assays

Detailed information about the performance of the assays was published elsewhere[13-21]. In order to compare the assays with respect to their time requirements, the durations of the different steps along with the total time required to obtain a result are summarised in Table 2. It can be seen that EPR, OSL and gamma H2AX foci allow obtaining results within one day. Dic and MN take much

Table 3. Approximated duration (in days) between the time point of sample arrival to the laboratory and the completion of dose estimation results, calculated for different numbers of samples analysed by one or five laboratories. The aim of this table is not to provide precise time estimates, but rather to give a comparative overview of the characteristic of each method

	Total time to analyse samples*					
	Time in days for					
	1 sample	50 samples	100 samples		1,000 samples	
	1 lab	1 lab	1 lab	5 labs	1 lab	5 labs
Dic manual	2.5	6	9	5	65	16
Dic automated	2.5	4	5	3	24	7
MM	3.5	4	5	4	20	6
Gamma H2AX	< 1	1	1	1	3	3
EPR (ped)	< 1	1	4	1	40	14
OSL (ped)	< 1	1	4	1	40	14

*does not include time for shipment of samples. Calculation made for one person per lab working 8 hours per day. In case of automatic scoring the machine works 24h /day. ped: portable electronic devices.

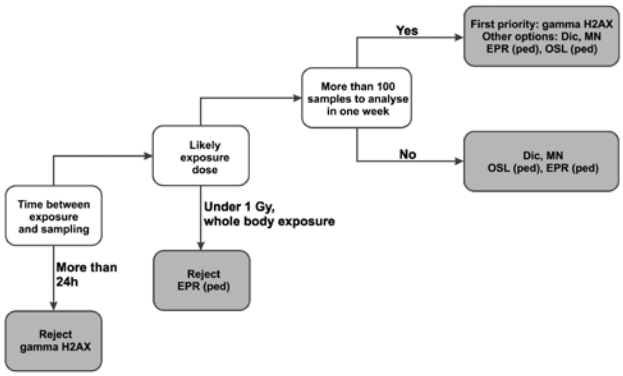

Fig. 1. Decision tree on the use of the biodosimetric tools when the time of exposure is known.

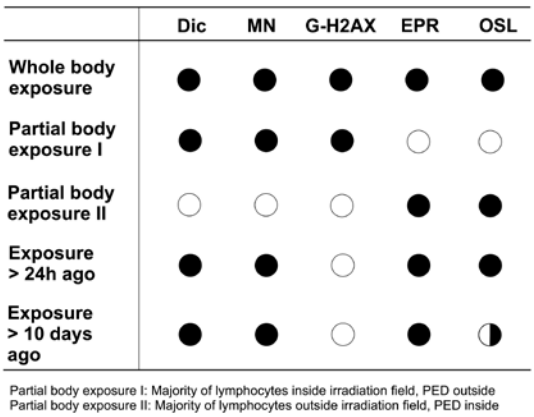

Fig. 2. An example of how combined application of the biodosimetric tools enables scenario identification. Dots indicate whether an assay demonstrates a significant radiation exposure. Black dot = yes, white dot = no. The black/white dot indicates a lower dose detected as compared to the other assays. ped: portable electronic devices.

longer due to the necessity to culture lymphocytes for several days.

Based on the values shown in Table 2 it was interesting to calculate the time required to obtain dosimetric results for different numbers of samples and with different numbers of participating laboratories. The results are shown in Table 3. When the number of samples is low EPR and OSL are the fastest methods. The situation changes, however, when the number of samples increases to reach a value of 1000 or more. Here, EPR and OSL perform less well in terms of speed. The reason for this is that both methods include manual steps (sample preparation for EPR and OSL and measurement for EPR) and none of the steps can be performed in parallel using several samples, as is the case for harvesting lymphocytes. Consequently, both assays perform less well than Dic, MN and gamma-H2AX, where not only fixation steps are done in parallel, but where also the analysis can be carried out automatically 24 hours per day. It should be stressed that the calculated times can be reduced by involving more people in the analyses and by deploying additional EPR and OSL readers.

Where information is available about the scenario of the radiation emergency, a decision tree can be applied to choose the optimal biodosimetric tool. An example of such a decision tree is shown in Figure 1. Due to the relative short signal stability, the gamma-H2AX assays should not be used if the accident took place more than 24 h before blood samples are available. Also, EPR is not permitted if absorbed doses in excess of 1 Gy can be excluded, even for heterogeneous irradiation.

A radiation emergency can easily be envisaged where no reliable information exists about the exposure scenario. In such a case the different characteristics and performance profiles of the assays can contribute valuable information that may enable an estimation of the time point of the exposure and whether it affected the whole or only part of the body. This is illustrated in Figure 2. The time of exposure can be assessed based on the short signal stability of the gamma-H2AX assay and

the fast decay of the OSL signal. Partial body exposure can be detected either if a personal electronic device (ped) was outside the radiation field while the majority of lymphocytes were exposed (partial body exposure scenario I) or a ped was inside the radiation field while the majority of lymphocytes received a dose below the triage threshold (partial body exposure scenario II). In view of this it is recommended to use the full battery of assays whenever possible.

4. Conclusions

Within the Multibiodose project five biodosimetric assays were tested, adapted, and validated for their use in triage biodosimetry in a mass casualty situation. Each assay has specific characteristics and the combined use of all assays allows important conclusions to be drawn about the individual exposure scenario of each victim. In a large accident, exceeding 1000 cases, it may take several days before the dosimetric information is available, so parallel application of the assays in as many laboratories as possible is recommended. In Europe, the assays are now being implemented in a large number of laboratories in the framework of the European network RENEB[22].

Acknowledgment

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 241536.

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