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A N I N T E R N A T I O N A L J O U R N A L

Trends in Biological Dosimetry: an Overview

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ABSTRACT

Biological dosimetry (biodosimetry) is based on investigations of induced biological effects (biomarkers) in order to correlate them with radiation dose. Among the indicators employed in biodosimetry, scoring of chromosome aberrations is the most reliable method to quantify individual exposure to ionizing radiation. The technique, applied to circulating lymphocytes, has been developed into a routine procedure to evaluate the dose in the case of real or suspected accidental exposure. Considering the radiosensitivity of lymphocytes in vitro and in vivo as being the same, the dose effect relationship obtained after in vitro irradiation of blood has been widely used, with medico-legal value, for evaluating individual radiation exposure. This report presents an overview of strengths, limitations and perspectives on biodosimetry.

Key words: Biodosimetry, physical dosimetry, biomarkers, chromosome aberrations

INTRODUCTION

Defined as the amount of energy imparted to matter by ionizing radiation (IR) per unit of mass, a*bsorbed dose* is the most important physical quantity to evaluate potential biological response as a result of exposure to radiation. In the Système International (SI) the unit of dose is the gray (1 Gy = 1 $J.kg^{-1}$) (ICRU, 1993). Although 1 Gy raises the temperature of water by only 2×10^{-4} °C, it represents a very large dose to man due to the ability of IR to deliver its energy directly to individual atoms and molecules.

The concept of absorbed dose has some limitations for evaluating biological effectiveness of radiation exposure. For example, the pattern of energy deposition in living tissues, at the cellular level, varies according to the type of IR. The concept of *linear energy transfer* (LET), introduced by Zirckle (Zirkle, 1940; ICRP, 1991), as energy deposited per unit of path length of radiation, means that the same absorbed dose can be delivered by differing track ionization densities of different radiations. Hence, equal absorbed doses from different forms of radiations (such as X and γ rays; electrons, protons neutrons and α particles), do not imply the same level of biological response. In order to contrast biological effectiveness among the different forms of IR, relative biological effectiveness (RBE) was defined as the ratio of cumulative dose of a reference radiation (generally, 250 kVp X-rays) with respect to cumulative absorbed dose of a test radiation to produce a specific biological effect.

For radiation protection purposes, the International Commission on Radiological

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Protection (ICRP) (ICRP, 1991) currently suggests the use of the quantity *equivalent dose* ($\mathbf{H_T}$) to a particular tissue, defined as:

$$\mathbf{H_T} = \sum_R \mathbf{w_R}\, \mathbf{D_{T,R}} \qquad (1)$$

with $\mathbf{w_R}$ = radiation weighting factor for a type of radiation **R** and $\mathbf{D_{T,R}}$ = absorbed dose in a tissue **T** as a result of exposure to a radiation **R**. Equivalent dose has the special unit sievert (Sv) to distinguish it from absorbed dose in gray (Gy).

As tissues and organs in the body have differing sensitivities for particular radiation-induced effects (e.g cancer), the concept of *effective dose* (**E**) was designed to take into account the contribution of all irradiated tissues and organs to the health detriment (ICRP 1991; ICRP, 1992). The effective dose is defined as:

$$\mathbf{E} = \sum_T \mathbf{w_T}\, \mathbf{H_T} \qquad (2)$$

with $\mathbf{w_T}$ = tissue weighting factor, which represents the proportionate contribution of tissue **T** to the whole body risk.

Knowledge of dose levels in radiation protection is an important step for risk assessment. Thus, to restrict the exposure to ionizing radiation, international authorities on radiation safety recommend dose limits to workers and members of the public (CNEN, 1988; IAEA, 1990; ICRP, 1991; CEU, 1996). However, in most cases of real or suspected accidental exposures to IR, physical dosimetry cannot be performed for retrospective estimates. In such situations, biological indicators (so-called biomarkers) have been proposed as an alternative (Downing, 2000; Bonassi & Au, 2002). In particular, the scoring of induced chromosomal aberration from peripheral lymphocytes has been developed into a valuable dosimetric tool in radiological protection (IAEA, 2001).

BIOMARKERS AND CHROMOSOME ABERRATIONS

Biomarkers can be defined as biological endpoints (such as cellular and molecular changes) used to indicate an exposure to IR, representing an early event that occurs as a result of IR interaction with living tissues (Horneck, 1998; Amundson et al., 2001; Bonassi & Au, 2002; Dainiak, 2002). Chromosome aberrations (CA) in circulating lymphocytes of human blood is the most extensively studied system (Bender, 1964; 1969; Lloyd et al., 1986; Bender et al., 1988; Albertini et al., 2000; Lloyd et al., 2000; Yamada et al., 2000). Having a half-life of about 3 years, blood lymphocytes are normally found at the quiescent G_0 phase of the cell cycle, which makes analysis possible a long time after a real or suspected exposure (Ramalho et al., 1995; Voisin, 1997; Testard & Sabatier, 1999). Some chromosome-type aberrations (such as dicentrics and rings) are generally considered to be specific to radiation exposure, although in certain circumstances a few chemical agents can also induce them. Considering different populations, the spontaneous frequency of dicentrics does not vary significantly, being of the order of 1 per 2000 lymphocytes (Voisin, 1997; Bonassi & Au, 2002). Several studies have shown no significantly difference between *in vivo* with *in vitro* CA in blood lymphocytes yields as a result of exposure to IR (Buckton et al., 1971; Dossou et al., 2000). Hence, the dose-effect relationship obtained after *in vitro* irradiation of blood can be used as a calibration to estimate effects from an irradiation *in vivo* (Doloy et al., 1991).

Two methods are commonly used: scoring unstable CA (such a dicentrics, rings and fragments) and the FISH (fluorescence *in situ* hybridization) method that is based on the use of fluorescence probes to visualize translocations without the need for time-consuming karyotyping (Savage, 1975; Pinkel et al., 1986).

Dicentrics, rings and fragments are referred to as unstable CA because their persistence in the body declines with cell divisions cycles. Lymphocyte cells sustaining unstable chromosomes lesions have a probability of about 50% of surviving in each mitosis. On the other hand, stable translocations are preserved for longer because they pass through cell divisions. Thus, translocations is a better biomarker for retrospective dose evaluation when there has been a long delay between exposure and blood sampling (Savage, 1975; Ramalho et al., 1995; Mclean et al., 1995; Pala et al., 2001).

DOSE-EFFECT CURVE (CALIBRATION CURVE)

Obviously, in order to interpret the scored CA in terms of radiation dose a calibration curve is necessary. This calibration curve should be constructed with respect to basic physical parameters, such as type of radiation (low or high-LET), and dose rate.

The dose/response for dicentrics as a function of low-LET radiation, is generally well fitted by a second-degree polynomial curve:

$$\mathbf{Y} = \mathbf{C} + \alpha D + \beta D^2 \qquad (3)$$

where: Y is the dicentric yield, D is dose, **C** is the control frequency of dicentrics and α and β are fitted parameters. This kind of function is widely known as the "linear-quadratic" model.

On the other hand, with most high-LET radiations, CA fit better to a linear-dose response (Testard et al., 1997; Venkatachalam et al., 1999).

Figure 1 presents the general curves' features of frequency of CA versus absorbed doses, as a result of an exposure to low or high-LET radiations.

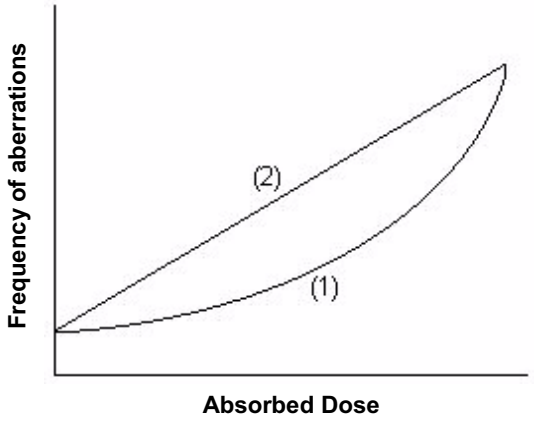
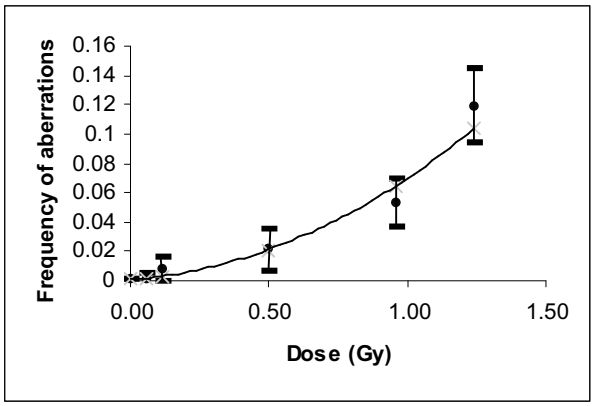


Figure 1 - Models of frequency of aberrations vs dose. Curve 1: linear quadratic model; Curve 2: linear model.

Comparisons between dose-response curves from different laboratories can be made assuming that all physical and biological parameters are the same. For this, it is important to establish reproducible sample irradiation conditions. Therefore, IAEA, in its Technical Report Series No 405 recommends that each laboratory should generate its own dose-response curve (IAEA, 2001). This is particularly difficult in the case of internal contamination.

In this context, Monte Carlo calculations represent an important tool for more accurate evaluation of the relationship between dose and chromosome aberrations in irradiated lymphocytes, particularly in the case of studies concerning internal exposure to radiation (Briesmeister, 1997; Amaral et al., 2001; Ottolenghi et al., 2001; Thierens et al., 2001). The interest in the use of Monte Carlo calculations to estimate dose stems mainly from the possibility of utilizing theoretical experiments in Nuclear Science, in order to infer the average behavior of particles/photons in a biological system by simulating their interactions with absorbing medium. Based on the physical principles and transport data, it is possible to "follow" each of particles/photons from its source throughout the medium, recording information such as the energy deposited in a finite volume. As an example of Monte Carlo simulations, an application is presented in the Figure 2, which shows doses correlated with chromosomal aberrations induced by *in vitro* ^{99m}Tc.



$\mathbf{Y} = (8.99 \pm 2.06).10^{-4} + (1.24 \pm 0.62).10^{-2}\mathbf{D} + (5.67 \pm 0.64).10^{-2}\mathbf{D}^2$

Figure 2 - Frequency of unstable chromosome aberrations versus calculated absorbed dose - observed results (showing 95% uncertainty intervals) and fitted function (Amaral et al., 2001).

The Monte Carlo approach reproduces *in vitro* ^{99m}Tc irradiation blood conditions, and takes into account the relationship between activities introduced into blood samples and the observed

frequencies of CA. Although these calculations were performed for nuclear medicine purposes, this example points out that by employing Monte Carlo simulations one may bypass some problems concerning the precise reproduction of radiation conditions.

LIMITATIONS AND PERSPECTIVES ON BIODOSIMETRY

Limitations and strengths on biodosimetry have been fully discussed in the IAEA Report 405 (IAEA, 2001). Some examples are the response to high radiation doses (> 4 Gy) where cell death in interphase and delays in progression through the cycle as cells are cultured to metaphase represents a pitfall for estimations of acute irradiation particularly when non-uniform or partial body irradiations have occurred. The method is time consuming, which has led to considerable efforts towards automation of scoring CA (Lloyd, 1984; Prassana et al., 1997; Huber et al., 1998). Apart from precise dose reconstructions biodosimetry can also be used in the immediate response to accidents. Lloyd and coworkers suggest an approach in which only 20 and 50 cells need to be scored initially, for medical triage of whole body and partial-body irradiation, respectively (Lloyd et al., 2000). This would play an important role in national emergency responses to a large-scale accident where many persons may have been exposed.

Scoring of micronuclei has been proposed as an alternative to conventional CA, being more sensitive and faster. A kind of unstable CA's byproduct, micronuclei are cytoplasm chromatin masses that arise from chromosome fragments. They have the appearance of small nuclei, besides the cell's nucleus. Although micronuclei method has been improved, its use as biomarker of human exposure to IR demands further studies (Voisin, 1997).

In many countries, biodosimetry has medical-legal recognition. However, discrepancies in recent inter-comparisons involving cytogenetic dosimetry have emphasized the need for better standardization (Turai, 2000; Voisin et al, 2002).

CONCLUSION

In the response to radiation accidents, rapid and reliable dose estimates are crucial for risk assessments, and also for clinical planning of the treatment of highly exposed victims. However, in many accident situations initial information is often scant and confused and only becomes available later after detailed complex dose reconstruction procedures. The development of cytogenetic dosimetry has made possible the use of chromosome aberrations in lymphocytes as biomarker of exposure to ionizing radiation. This is complementary to physical dosimetry, when it is available, and thus cytogenetics has made a significant contribution to the radiation protection programs of many countries.

RESUMO

A dosimetria biológica (biodosimetria) é baseada na investigação de efeitos biológicos induzidos (bioindicadores) objetivando relacioná-los com dose absorvida pela exposição à radiação ionizante. Entre os indicadores empregados na biodosimetria, a quantificação de aberrações cromossômicas é o método mais confiável na avaliação de uma exposição à radiação ionizante. A técnica, aplicada para análises de linfócitos do sangue periférico, tem sido empregada em procedimentos de rotina para avaliação de dose em caso de acidentes ou de suspeita de exposição. Considerando a radiosensibilidade de linfócitos como sendo a mesma tanto *in vivo* quanto *in vitro*, a relação dose-efeito obtida após uma irradiação *in vitro* do sangue tem sido amplamente utilizada, com valor médico-legal, para avaliação de exposições de indivíduos à radiação. Neste contexto, este trabalho apresenta uma breve revisão das potencialidades, limitações e perspectivas da biodosimetria.

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