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THE POTENTIAL OF IN VIVOEPR IN EVALUATING FREE RADICAL REACTIONS IN IRRADIATED RATS AND MECHANISMS OF RADIOPROTECTION

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ABSTRACT

We investigated the potential of *in vivo* electron paramagnetic resonance (EPR) in assessingchemical reactions that occur during ionizing irradiation and capabilities of two radioprotectors (an antibiotic, anisomycin and a naturally occurring tiol, Gl2011) to enhance survival during irradiation. Radioprotectors selected are supposed to have different modes of action and *in vivo* EPR was used to assess whether free radical scavenging has a role in their action as radioprotectors. Both compounds exhibited similar and highly efficient radioprotective capabilities according to the 30-days survival test (96% and 87%, respectively after the dose of 6.7 Gy). *In vivo* EPR showed that free radical scavenging has an important role in the action of GL2011, but not in the case of anisomycin. In conclusion, EPR have unique capabilities in studying free radicals *in vivo* and can be useful addition to battery of methods needed to study radioprotectors *in vivo*.

INTRODUCTION

Investigation of mechanisms of radioprotection *in vivo* is a challenging task due to the complexity of biochemical and physiological events that occur during and after irradiation of the body. Radiation-induced cell damage is mostly done via free radicals produced by radiation, hence, molecules with scavenging properties for radicals, such as thiols, attracted attention as potential radioprotectors and a number of synthetic or naturally occurring thiols have been investigated as potential radioprotectors. These and other protectors have been investigated through survival studies or experiments using *in vitro* samples (cell cultures or isolated cells and organs). However, it is difficult to translate results of later studies to the *in vivo* conditions; hence there is a need to develop methods suitable for direct *in vivo* investigations of free radicals involved into radiation damage and radiation

protection. The EPR can, in principle, detect free radicals but their direct observation in vivo is almost impossible. One of the potential solutions is to use *in vivo* EPR technique to study interactions between the endogenous free radicals, produced by the irradiation, with the exogenous radicals (nitroxides, stable free radicals),which were added after irradiation[1]. Here, we used thistechnique to study action of natural tiol (GL2011), a substance we previously investigated as a potential radioprotector [2]. We also included in the study the anisomycin, an antibiotic, well-known as the inhibitor of protein synthesis. It has been suggested that it can have radioprotecting capabilitiesso it is included here for comparison with GL2011, as an agent acting through different mechanisms than GL2011.

EXPERIMENTAL

Healthy 2 month's old male albino Wistar rats grown under standard conditions were used. Total body irradiation was performed on non-anaesthetized rats using 60-cobalt gamma ray source (dose = 6.7 Gy). For EPR measurements, anesthetized rats were placed in supine position into the rat-bed, loaded into the Bruker Elexsys II EPR spectrometer and L-band surface coil was placed above the rat liver area. Body temperature was maintained at around 33ºC.Administration of radio protectors: GL2011, intraperitoneally 30 min prior to radiation, dose = 100 mg/kg body weight; anisomycin, subcutaneously 1 hour prior to irradiation, dose = 150 mg/kgb.w. One milliliter of the 3CP spin probe (3-carbamoyl proxyl, Sigma), dose = 2μmol/g b.w., was injected via the tail vein and spectra were recorded for the period of 30 min using 20s scan time. For survival studies, animals were kept in cages (two per cage), regularly inspected twice a day and moribund animals were killed according to the IACUC Guidelines, Policy#5.

RESULTS AND DISCUSSION

Figure 1. demonstrates radio protecting capabilities of both compounds used, GL2011 leading to a survival of 87%, while protection with anisomycin was 96%. This is a rather high radio protective efficiency achieved without apparent toxic effects, except for occasional local skin irritation at the injection site of anisomycin.

The EPR signal decay in control, unirradiated rats (Fig. 2) shows pronounced non-exponential curve reflecting participation of several processes beside reduction of injected 3CP to EPR silent hydroxylamine in the overall decay. These can include the rate of distribution of the spin probe from the blood to the tissue and within different tissue compartments, urinary excretion through kidneys, fecal excretion through liver and bile, etc and such curves should, in principle, be analyzed using multicompartment pharmcaceutical models [3].

Here, we will only use half-elimination times ($t_{1/2}$) to analyze the effect of irradiation and radioprotectors on *in vivo* reduction of nitroxide.

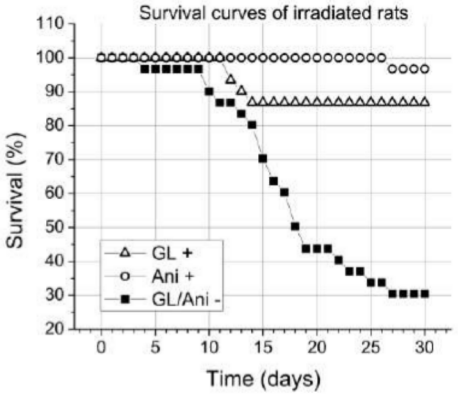

Figure 1. Survival of rats after radiation (6.7 Gy) and different treatments. *Squares* – no treatment (protection); *Triangles* – protection with GL2011; *Circles* – protection with ansomycin. The survival curves were significantly different as predicted by log rank test. (+++p value <0.001). Thirty rats pergroup.

Data in Fig.2 illustrate the basic concept of the experimental approach. The curve for irradiated rats with no protection showed faster signal decay, most certainly due to the reduction of injected 3CP to hydroxylamine with additional amount of reactive free radicals produced during and after irradiation. Measured $t_{1/2}$'s are 14.5 min and 11 min, respectively. In rats, protected by the GL2011, measured $t_{1/2}$ is the same as in control, indicating that GL2011 scavenged extra free radicals produced by irradiation thus restoring the original redox capacity of tissues. Noapparent changes in the redox status were observed in rats receiving anisomycine since $t_{1/2}$ in these ratshad the same value as in non-protected rats.

In biological systems, ionizing radiation induces formation of reactive oxygen radicals ($OH^{\bullet}$ and $O_2^{\bullet ?}$) through radiolysis of water.

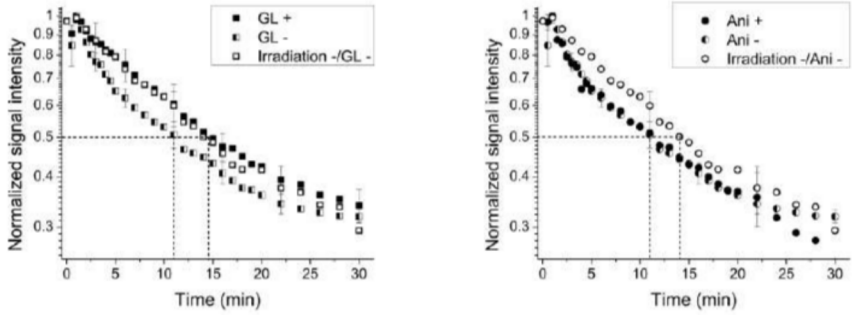

Figure 2. Semilogaritmic plots (pharmacokinetic curves) of *in vivo* reduction kinetic of 3CP nitroxide from the liver region of rats. *Open symbols* – control rats, no irradiation and no protection; *closed symbols* – irradiated rats protected with either GL2011 (left) or anisomycin (right); *semifilled symbols*- irradiated animals without protection. Dotted lines denote decay half-times. Error bars are standard deviations, n – 3 animals per group.

Nitroxides can readily react with these species, but it is virtually impossible that nitroxides,injected 1 h after irradiation to study pharmacokinetic, interact with these primarily formed species,because of their extremely short life time. It is more likelythat injected nitroxides react with secondary radicals formed in radicals chain reactions initiated by the primary ROSinduced by irradiation. In any case, these data show that at least part of action of the GL2011 can be attributable to the free radical scavenging.

This research demonstrate usefulness of EPR in investigating of radioprotection mechanisms *in vivo*. It also can be useful in connecting mechanisms of protection and efficiency of certain radioprotector,but one has to be cautious while doing this. There are substances that suppress various oxidative stresses *in vivo* but exhibit no radioprotective action and others, such as anisomycin,studied here, exhibiting good protection actions but through different mechanisms.*In vivo* EPR can't replace the 30-day survival test in studying the efficiency of certain substance as a potentialradioprotector since the survival presents is a systemic effect while reduction of nitroxides *in vivo* EPR is observing short term effect immediately following irradiation.Nevertheless, besides being useful in revealing underlying mechanisms of radioprotection *in vivo*, EPR can be used, to a certain extent, as ascreening techniquefor assessing potential radioprotectors instead of the animal consuming 30-day survival which requires the large number of animals (typically 30 per group).

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