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Estimation of repair parameters in mouse lip mucosa during continuous and fractionated low dose-rate irradiation

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Key words: Lip mucosa; Early reactions; Dose rate; Fractionation; Repair kinetics; LQ model

Summary

The present study investigated the effect of fractionated low dose-rate (FLDR) treatments in mouse lip mucosa, a typically early reacting tissue. The relation between dose-rate and fractionation effect has been assessed with various interfraction intervals and dose-rates. A fixed overall treatment time of 10 h has been used for the present continuous and fractionated irradiation experiments with corresponding dose-rates of 3.1–84 Gy/h. Sophisticated mathematical models are now available to estimate repair parameters from data derived with different fraction numbers, fraction sizes and dose-rates. These formulas, allowing the calculations of isoeffect relationships are based on the incomplete repair model and assume that repair can operationally be described by a monoexponential function. A further assumption of these models is that repair of sublethal damage follows the same kinetics during irradiation and between fractions. The present FLDR experiments with small interfraction spacing were performed to investigate the validity of these assumptions and consequently the applicability of the models. In addition, it has been assessed whether the experimental approach of investigating repair kinetics as such [high dose-rate (HDR) split-course vs. continuous low dose-rate (CLDR) or FLDR] influences the estimation of these parameters, as has been suggested from the analysis of in vitro studies. Using the mucosal desquamation endpoint, virtually identical repair parameters have however been estimated with different approaches (α/β = 14.1–18.2 Gy, $T_{1/2}$ = 28–37 min). The available isoeffect models seem to be applicable to the present experimental data and might after further experimental tests also involving late reacting tissues, be a useful tool for clinical isoeffect calculations.

Introduction

The sparing effect of fractionated or protracted irradiations is assumed to result from the same biological mechanism: cellular repair of sublethal radiation damage. In fractionated treatments repair occurs in between acutely delivered fractions, whereas repair takes place during the irradiation in protracted regimes. Fractionated low dose-rate (FLDR) irradiation represents an intermediate regime between a continuous low dose-rate (CLDR) and a fractionated high dose-rate (FHDR) treatment. If fractionated schedules are delivered at sufficiently low dose-rates, repair takes place both during the irradiation and during the interfraction intervals. When the dose-rate is further lowered, a limit will be reached where virtually all repairable damage is repaired during the irradiation and fractionation leads to no additional sparing. In all applied regimes however, the radiation response is mainly determined by repair of sublethal radiation damage.

The accurate knowledge of repair parameters for different tissues and for various treatment modalities is essential for an optimal design of new fractionation schedules in clinical practice. For instance, accurate estimates are necessary when switching from low to

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high dose-rate treatments or when fractionated regimes are to be used in brachytherapy. Despite the increasing importance of such treatments, little is known about the radiobiological behaviour of normal tissues to FLDR irradiations and specifically little quantitative relation between effects of protraction and fractionation in specific tissues.

The present study therefore investigated the effect of FLDR treatments in mouse lip mucosa, a typically early reacting tissue. The relation between dose-rate and fractionation effect has been assessed with various interfraction intervals and dose-rates, within a fixed overall treatment time. The rationale of keeping the available repair time constant, was to investigate the conditions in which repair was most effective when total potential repair time was constant. In addition, this approach keeps possible influences of regenerative processes as comparable as possible and thus might increase the resolution of the biological model.

In most available in vivo studies, repair parameters in terms of α/β ratios and halftimes of repair ($T_{1/2}$) have been determined from the results of FHDR studies. Several review papers however, have suggested that CLDR or FDHR studies in conditions of incomplete repair (IR) are more advantageous to study repair parameters of sublethal radiation damage [18–20]. Compared to conventional split-dose experiments in conditions of complete repair, from which only repair capacity can be determined, estimation of both repair capacity and kinetics can be obtained with CLDR or with FHDR treatments in conditions of IR. In principle, the same advantages are valid for FLDR treatments, in which IR plays a major role in determining radiation response.

Sophisticated mathematical models are now available to estimate repair parameters from data derived with different fraction numbers, fraction sizes and dose-rates. We have tested whether the present results on repair of sublethal radiation damage in mouse lip mucosa during FLDR can satisfactorily be described with the available mathematical models [16,22]. These formulas, allowing the calculations of isoeffect relationships, are based on the IR model [21] and assume that repair can operationally be described by a mono-exponential function. A further assumption of these models is that repair of sublethal damage has the same kinetics during irradiation and between fractions. The present FLDR experiments with small interfraction intervals were performed to investigate the validity of this assumption. In clinical practice it is necessary to know whether the available mathematical approaches can be applied safely or whether a more complex repair function has to be incorporated.

Finally, it has been assessed whether the experimental approach of investigating repair kinetics as such (HDR split-course vs. CLDR or FLDR) influences the estimation of these parameters, as has been suggested from the analysis of in vitro studies [19].

Materials and methods

Adult, female outbred NMRI mice with a body weight of 23–28 g were used for this study. Animals had unlimited access to water and food. Mice were immobilized without anaesthesia by the use of specially designed Perspex cylinders for the animal body and tape fixation of the extremities. Special care was taken to avoid any obstruction of blood vessels in the head and neck area of the animals, which could lead to artificial hypoxia and therefore altered radiation response. A short anaesthesia with Ethrane was used to position the mice accurately prior to treatment. A continuous flow of air (2 l/min) during irradiations was used to avoid an accumulation of carbon dioxide in the dead-air space of the treatment set. Prior to irradiation the mice were left for a period of 15 min to get accustomed to their position. The latter might minimize possible influences of stress induced during the handling of the animals [13].

Because of the lack of a reliable method to measure experimental stress for the animals it was assumed, based on several parameters (body weight loss, urination frequency, general impression) that mice tolerated the treatment adequately.

Irradiation was performed on a ^{60}Co unit at a focus to skin distance of 60 cm for the low dose-rate (LDR) experiments and of 45 cm for the high dose-rate treatments. The dose-rate of the various LDR experiments was adjusted by the use of lead filters. The snouts of mice placed in a supine position were exposed to a single field irradiation, with the remaining part of the body shielded with 8 cm thick MCP alloy (Mining and Chemical Product, melting point 70 °C, Metallurgie Hoboken, Belgium). The homogeneity and accuracy of the dose distribution was checked repeatedly with TLD and film dosimetry. Sixteen mice were used for each radiation dose point. In order to construct dose/-response relationships 5 dose levels were selected per experiment. Each experiment was repeated at least once.

Details of the scoring system for the acute lip mucosal reactions can be found in previous publications of our department [15,26]. The reactions of the lip mucosa were scored daily for a period of 3 weeks. During the observation period, all animals were also weighed every 1–2 days.

As the most relevant and reproducible endpoint the incidence of lip mucosal desquamation in a group of mice was recorded. Thus a quantal analysis of the data could be performed. Dose-response curves were constructed by plotting the incidence of desquamation against the respective total dose. ED_{50} values (radiation doses leading to lip mucosal desquamation in 50% of the animals) were determined by probit analysis [9]. Estimation of α/β values and $T_{1/2}$ was performed by use of a modified direct analysis computer program [4,22]. The values given in brackets represent 95% confidence limits.

The overall treatment time of the FLDR and FHDR experiments in conditions of IR was kept constant at 10 h. These treatments can be performed without major distress of the animals and can be compared to LDR treatments within the same overall treatment time. It should be noted that an important feature of the present investigations was the lack of any significant repopulation during the time involved in the experiments [2].

Since the mice remained without drinking and eating during protracted irradiations, the tolerance of the animals to fasting was also measured by performing sham irradiations. Mice were installed in the set-up used and left for 10 h in the same conditions as those encountered during the experiments. A rectal temperature measurement during the sham irradiation showed no difference in body temperature of animals during the 10 h treatment time compared to controls. To investigate the possible influence of the 10 h jigging procedure on the radiation response a single dose HDR irradiation was performed either 15 min after beginning the immobilization of mice or after 10 h. No difference in isoeffective dose (20.0 vs. 19.8 Gy) nor larger scatter of the individual radiation responses was detected.

Different sets of experiments have been carried out (see Table I and Fig. 1 for details). The first group of experiments was performed with 2 LDR fractions being divided by an increasing duration of irradiation-free interval (Fig. 1a). The gap was 1, 2, 4 or 8 h, respectively. The corresponding dose-rates (given at isoeffective dose) were in the range of 3 to 14 Gy/h (5–23 cGy/min).

A second set of experiments was done to assess the

TABLE 1

Details of the experiments, isoeffect doses and theoretical calculations. ΔED represents the difference of experimental and theoretical data. Calculations are based on an α/β ratio of 17 Gy and a $T_{1/2}$ of 0.6 h. The single dose HDR data have been used for reference.

Low dose-rate experiments with constant overall treatment time (10 h)

No. of fractions	Interval (h)	Dose-rate (Gy/h)	ED_{50} (Gy)	$ED_{50\ calc.}$	ΔED
1	–	3.12	31.2 (30.5–31.8)	33.2	+2.0
2	1	3.45	31.1 (30.5–31.8)	32.8	+1.7
2	2	4.03	32.2 (31.9–32.5)	32.7	+0.5
2	4	5.35	32.1 (31.5–32.8)	31.4	−0.7
2	6	7.57	30.3 (29.5–30.9)	29.7	−0.6
2	8	13.85	27.7 (27.2–28.1)	27.7	–
7	0.33	4.0	32.0 (31.4–32.6)	31.1	−0.9
13	0.33	5.53	33.2 (32.6–34.1)	30.2	−3.0

High dose-rate experiments

No. of fractions	Dose-rate (Gy/h)	ED_{50} (Gy)	$ED_{50\ calc.}$	ΔED
1	84	20.0 (19.5–20.6)	–	
2/24 h	84	24.9 (24.5–25.5)	25.0	+0.1
10/3.5 days	84	35.8 (34.4–36.8)	35.9	+0.1

Continuous low dose-rate experiments

No. of fractions	Duration (h)	Dose-rate (Gy/h)	ED_{50} (Gy)	$ED_{50\ calc.}$	ΔED
1	10	3.12	31.2 (30.5–31.8)	33.2	+2.0
1	6	5.1	30.6 (30.2–31.2)	30.2	−0.4
1	2	11.8	23.5 (22.9–24.3)	24.6	+1.1

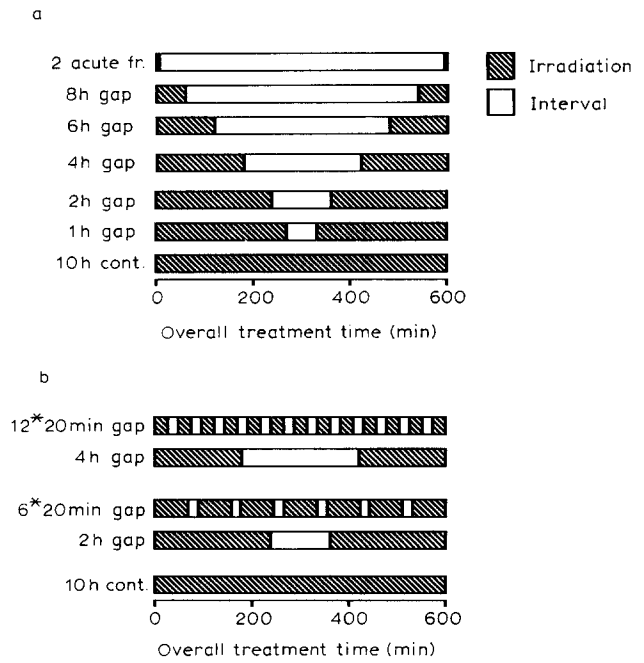

Fig. 1. Schedules of the FLDR experiments.

relative importance of further fractionation of a LDR irradiation, keeping dose-rate and overall treatment time constant (Fig. 1b). In this way the effect of fractionating a LDR irradiation in 2 to 13 fractions within the same overall treatment time and dose-rate could be assessed. Fractionated HDR treatments in conditions of IR have been performed, again within a constant overall treatment time of 10 h allowing comparisons of HDR and LDR data, within identical overall treatment time and thus potential repair time.

Both, continuous 2, 6 and 10 h LDR treatments and different conventional HDR irradiations in conditions of complete repair have also been performed to allow the use of isoeffect formulas and comparisons of repair parameters obtained with the different above mentioned experimental approaches.

Results

The present study investigated the effect of FLDR treatments and assessed the influence of various interfraction intervals in those treatments, specifically within a fixed overall treatment time. ED_{50} values for the different LDR treatments given within a constant overall treatment time of 10 h are displayed in Table I. Results from two LDR fraction experiments with 1, 2 or 4 h interval are practically not different from one another (31.1–32.2 Gy), although the dose-rate increased slightly (3.45–5.35 Gy/h). With increasing interfraction interval of 6 or 8 h duration and corresponding higher dose-rates (4 or 2 h radiation time, respectively) a decrease of isoeffective dose compared to the 10 h continuous treatment (30.3 and 27.7 vs. 31.2 Gy) was observed. Two HDR fractions within 10 h resulted in an isoeffective dose of 24.9 Gy, about 6 Gy less compared to the two fraction LDR treatments.

When comparing CLDR treatments of 6 or 2 h to a split course LDR regime with the same dose-rate, a significant increase in tolerance could be observed. Two fractions of 3 h LDR irradiation with an interval of 4 h resulted in an increase of isoeffective dose of 1.5 Gy compared to a continuous treatment of 6 h duration (32.1 vs. 30.6 Gy). A two fraction LDR regime of 1 h irradiation each and an interfraction interval of 8 h showed an increase of the isoeffective dose to 27.7 Gy compared to a 2 h continuous irradiation (23.5 Gy).

Two hyperfractionated LDR treatments have been carried out within the same total treatment time of 10 h. Seven fractions of 68 min (8 h total irradiation time, 6 × 20 min gap) and 13 fractions of 27 min (6 h total irradiation time, 12 × 20 min gap) can be compared to a 2 fraction regime with identical total treatment time and identical dose-rate. No significant effect of such a hyperfractionation could be demonstrated at these dose-rates (32.0 vs. 32.2 Gy, 33.2 vs. 32.1 Gy). The 13 fraction LDR led to an ED_{50} value being 2 Gy higher when compared with the 10 h lasting CLDR, a difference being statistically different.

The graphical Fe-analysis [8] (Fig. 2) of the FHDR data in conditions of complete repair (CR) resulted in an α/β ratio of 17.2 Gy, a value at the upper end of the range reported for rapidly proliferating tissues (for review see: [23,24]).

Using the approach published by Dale [5,6] for the present CLDR treatments, an α/β of 15.8 was estimated with a corresponding halftime of repair of 42 min (for

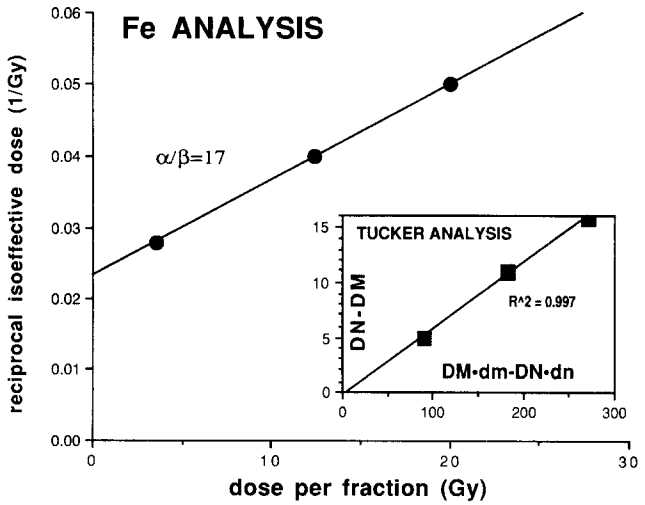

Fig. 2. Fe and Tucker analysis of the HDR data in condition of complete repair.

TABLE II

Estimation of repair parameters from different experimental schedules.

Experiments	α/B (Gy)	$T_{1/2}$ (min)
FLDR + CLDR	17.1 (15.1–19.1)	35 (31–41)
HDR	14.1 (12.1–16.4)	38 (32–47)
CLDR	18.2 (15.6–21.2)	28 (23–35)
All data	16.4 (14.6–18.2)	37 (36–43)

further methodological examples of this analysis see [17]).

Assuming monoexponential repair and the general applicability of the linear quadratic model, sophisticated mathematical approaches allow the estimation of both repair kinetics and capacity from data obtained with different treatment regimes. By use of a recently proposed isoeffect formula the analysis can also be conducted with data obtained with FLDR treatments [16]. This somewhat complicated approach enables the analysis of $T_{1/2}$ and α/β ratio from experimental quantal data gained with varying interfraction intervals, fraction numbers and dose-rates. This maximum likelihood method allows at the moment the most reliable estimation of repair parameters as the informations derived from all animals are used directly. In addition, it allows the calculation of relatively tight confidence limits, which is not adequately possible with two-step methods such as logit or probit methods, followed by fitting reciprocal isoeffect doses with regression methods [22]. The modified direct analysis led to an α/β ratio of 16.4 (14.6–18.2) Gy and a $T_{1/2}$ of 37 (34–43) min for the entire data set presented in this paper.

Table II shows the results of the direct analysis for different subsets of experiments. Virtually identical α/β ratios have been calculated, and no significant effect in estimating halftimes with different experimental approaches like CLDR, FLDR and HDR investigations could be detected.

Discussion

The biological response of FLDR treatments is largely determined by the speed with which repair of radiation-induced sublethal damage takes place. If repair is slow, an interfraction interval should result in an additional increase in tolerance as the induction of sublethal damage is not in balance with the repair process during irradiation. If repair is fast, the effect of fractionation should be rather small as most of the repairable damage is virtually repaired during the irradiation time. It therefore seems favourable to study repair kinetics with FLDR experiments and compare this approach with other experimental methods. In addition, the applicability of an extended mathematical formalism [16], allowing calculations of isoeffect relationships for continuous and fractionated treatments with varying dose-rates can be tested.

Over a broad range of dose-rates between 3.1 and 5.35 Gy/h the effect of diminishing tolerance due to increasing dose-rates could be counterbalanced by longer interfraction intervals. Results of the treatment with 1, 2 and 4 h intervals are practically not different from the 10 h continuous treatment. However, a gap of 6, 8 and 9.5 h duration steadily resulted in a decrease of isoeffective dose compared to a continuous treatment. With longer gaps but higher dose rates, interfraction repair reaches a plateau and cannot compensate for the increasing induction of lethal damage during irradiation at higher dose-rates. Loss of tolerance becomes most obvious in the 2 fraction HDR experiment within 10 h where an isoeffective dose of 24.9 Gy was seen, being approximately 6 Gy lower than the continuous 2 fraction LDR treatments. Within the limits of the applied biological model and dose-rates investigated, it was thus shown that the effect of a continuous treatment lasting 10 h could be mimicked by various 2 fraction treatments, resulting in the same isoeffective dose. The same biological effect in the tissue investigated could thus be achieved in a shorter effective irradiation time.

The effect of further fractionating a 2 fraction LDR treatment, keeping dose-rate and overall treatment time constant showed no significant effect. In other words, the result of repair of sublethal damage seems to be identical, when comparing LDR treatments with one long interfraction interval time with a treatment involving several subdivisions of this interval. However, the 13 fraction LDR experiment led to the highest isoeffective doses within the constant overall treatment time of 10 h, being statistically significant different from the 10 h CLDR experiment. Reasons for this finding, remain speculative. However, seemingly, repair was more effective in the interfraction intervals compared to the continuous irradiation time with lower dose-rates. Consequently, compared to CLDR irradiation the same or even higher isoeffective doses could be obtained with FLDR, which can be given in a relative short effective overall irradiation time.

The estimation of repair kinetics is very critical in order to calculate equivalence of HDR and LDR treatments. Based on in vitro experiments it was postulated that the experimental approach of investigating repair kinetics as such may influence the estimation of this parameter. In conventional split-course experiments

possible "multiexponential" repair kinetics might be averaged in favour of the slower component. In contrast, low dose-rate experiments may be dominated by an on average faster component [19]. Due to their clinical relevance possible influences or artefacts for determining repair parameters have to be well defined. In spite of the large fundamental scientific importance of a "polyexponential" repair of sublethal damage, it remains questionable whether this possible mechanism has relevance for clinical tolerance calculations, when changes in dose-rate or fractionation are performed.

Deriving repair parameters with different methods (see Table II) resulted in virtually identical α/β ratios for different experimental approaches. A range of values of 14 to 18 Gy was determined by direct analysis. α/β ratios derived with the Fe-method (for HDR data in conditions of CR of Dale's approach [5] for CLDR data agreed with these values.

These values are somewhat in contradiction to previously obtained values in this tissue in different treatment conditions [1,2]. Reasons for this deviation, namely the influence of anaesthesia on large doses per fraction are discussed in detail in a separate paper (Stüben et al., in preparation). In order to check the quality of the fit of the data and the applicability of the linear-quadratic (LQ) concept, an analysis described by Tucker [25] was performed (Fig. 2). The intercept of a straight line, fitting the isoeffect data nicely, is very close to zero. Thus by this analysis the applicability of the LQ formalism could not be rejected.

Evaluating this "range" of α/β ratios one has to take into account that a difference between 14 and 18 Gy in describing the fractionation sensitivity in this "high" α/β region has a very small significance for the calculations of isoeffect relationships. However, in the "low" α/β region, typically for late reacting tissues, a shift of 4 Gy would have far more weight for isoeffect relations. Taking into account the resolution and shortcomings of most radiobiological models and endpoints, repair halftimes and α/β values should be regarded to have a certain range of "adequate" values. The use of absolute values for sophisticated calculations should therefore be questioned critically.

Calculations of halftimes of repair resulted in a range of values of about 0.4 to 0.7 h (24–42 min). This is in good agreement with values previously reported in mouse lip mucosa [2] and data from the literature for rapidly proliferating tissues [23]. While examining the analysis of the CLDR experiments a slight but not significant trend toward a faster component of repair was seen.

The sparing effect due to fractionation or lowering the dose-rate is assumed to result from the same process, namely repair of sublethal damage. To our knowledge only few experimental studies investigated the effect of FLDR on normal tissues and determined repair kinetics with this approach [10,11].

The possible dose-rate dependence of repair kinetics, which has been suggested by a reanalysis of FLDR experiments in mouse jejunum [7,12], could not be confirmed. It is worth noting that the present analysis was performed assuming that the experimental data are adequately described by the LQ-concept. Inherently assumed is therefore that the α/β values determined in HDR experiments are also valid for protracted treatments, that every fraction has equal radiobiological effectiveness (for Review see [14]) and that no difference in repair rate during irradiation compared to interfraction intervals exists.

TABLE III

Isoeffect models based on the TE-concept.

(1) *Infinite dose-rate, complete repair*

$$TE = (nd)(\alpha/\beta + d)$$

TE = total effect; n = number of fractions; d = dose per fraction; α/β = constant; dosage factor = nd = total dose; fractionation factor = $\alpha/\beta + d$

(2) *Infinite dose-rate, incomplete repair*

$$TE = n\{\alpha d + \beta d^2[1 + h_n(\vartheta)]\}$$

$$\vartheta = e^{-\mu t} \qquad T_{1/2} = -\frac{\ln 0.5}{\mu} = \frac{\ln 2}{\mu}$$

($\vartheta = 0.5$ represents so-called halftime of repair, $T_{1/2}$)
μ = repair rate parameter; t = time allowed for repair

$$h_n(\vartheta) = \left(\frac{2}{n}\right)\left(\frac{\vartheta}{1-\vartheta}\right)\left(n - \frac{1-\vartheta^n}{1-\vartheta}\right)$$

fractionation factor = $\alpha/\beta + d[1 + h_n(\vartheta)]$

(3) *Low dose-rate continuous irradiation*

$$TE = \alpha(vt) + \beta(vt)^2[g_1(\mu t)]$$

v = dose rate; t = exposure time

$$g_1(\mu t) = \frac{2(\mu t - 1 + e^{-\mu t})}{(\mu t)^2}$$

(4) Fractionated low dose-rate irradiation

$$TE = n\{\alpha(vt_n) + \beta(vt_n)^2[g_1(\mu t_n) + g_2(\mu t_n)\, h_n(\vartheta)]\}$$

$$g_2(\mu t) = \frac{(e^{\mu t_n} - 2 + e^{-\mu t_n})}{(\mu t_n)^2}$$

t_n = exposure time per fraction; $\vartheta = \exp[-\mu(t_n + \Delta t_n)]$
fractionation factor = $\alpha/\beta + (vt_n)[g_1(\mu t_n) + g_2(\mu t_n)\, h_n(\vartheta)]$

One has to be aware that repair is an operational term describing very complex enzymatic processes, with probably a large number of individual kinetics. Our data however, being of relevance for TBI and brachytherapy give no evidence for a multiexponential repair process in the dose ranges investigated. It seems likely that the slowest of complex enzymatic processes determines an average speed, which may well be described by first-order enzyme kinetics. An operational mathematical description (with a simple monoexponential function) seems therefore acceptable to calculate isoeffect relationships based on the above mentioned assumptions within the limits of experimental accuracy.

In the experiments described in this paper, the applicability of a new isoeffect formula for fractionated low dose-rate data was tested. It is important to note that very similar repair parameters have been obtained with different approaches. Within the limits of our model and dose ranges investigated, no clear cut differences could be detected in estimating repair parameters with different approaches, namely HDR in conditions of CR or IR, CLDR or FLDR regimes. Based on these parameters, "predictions" for the experiments can be calculated with the available isoeffect formulas (summarized in Table III) and compared to the experimental findings, in order to demonstrate the fit of the *individual* experiment (Table I).

The HDR investigations in conditions of IR could perfectly be described by the chosen parameters (α/β = 17 Gy, $T_{1/2}$ = 0.6 h). The FLDR data are in parts adequately described, however, with some discrepancy in studies involving dose-rates of about 3 Gy/h, for which higher isoeffective doses were predicted than experimentally observed. In addition, the isoeffect dose for the 13 fraction LDR experiment, based on the defined parameters was predicted to be lower. Interestingly this experiment led to the highest isoeffective doses within the constant overall treatment time of 10 h.

Reasons for the inadequate applicability of the defined repair parameters to all individual experiments are unknown for the moment. However, in general, this formula seems to be applicable to most experimental data and might after further experimental tests, also involving late reacting tissues, be a useful tool for clinical isoeffect calculations.

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