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Pharmacodynamic modeling of the effect of changes in the environment on cellular lifespan and cellular response

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Abstract

Lifespan-based pharmacodynamic (PD) models of cellular response assume that the lifespan of cells is predetermined at the time of cellular production, despite recognized changes in the cellular environment following production that may alter the survival of the cells. This work extends previously proposed cellular lifespan PD models to incorporate environmental effects on the cell lifespan by considering two basic classes of models from survival analysis: accelerated life and relative risk models. Cellular responses using both model classes were simulated using a steady-state cellular production rate with changes in the environmental effects resulting from three different basic profiles. The environmental effect models were also fitted to the red blood cell (RBC) and hemoglobin concentration data from six sheep following hematopoietic ablation by busulfan administration. The simulations indicated that the basic shapes of the cellular responses were different between the accelerated life and relative risk models. Due to the more direct physical interpretation, relatively simple steady-state relationship between the cellular response and environmental effects, and the ability to reduce the model to a “point” baseline lifespan distribution, the accelerated life model appears to be a more realistic and flexible model. The analysis of the sheep RBC and hemoglobin data indicated that the environmental effect began to decrease the survival of cells 1–2 weeks following initiation of ablation and that the average “severity” of the environmental effect increased 3.49 (29.5%) (mean (C.V.)) fold under the accelerated life model. Alternative models without an environmental effect did not describe the observed data as well. The proposed environmental effect cellular lifespan PD models allow for the incorporation of arbitrary changes in the conditions of the cellular environment and modeling of environmentally dependent cellular survival. These PD models have potential applications in hematological management of end-stage renal disease, transfusion medicine, and patients undergoing chemotherapy, among other diseases and therapies.

Keywords

Non-stationary; Time variant; Cellular kinetics; Mean potential lifespan; Survival function; Proportional hazards; Accelerated failure; Oxidative stress; Hematology; Systems analysis; Transfusion

Introduction

Many drugs impart their primary pharmacodynamic (PD) effect on the number of cells of a particular cell type. Cell populations commonly targeted include erythrocytes, leukocytes, platelets, cancerous cells, and bacteria. Additionally, some drugs may have side effects on non-targeted cellular populations, e.g. chemotherapeutic agents on non-cancerous cells. In order to optimally dose these drugs to properly balance efficacy and cellular toxicity an understanding of the PD effect on the production and destruction of these cells is needed. Thus, lifespan distributions and age structure of the cellular population are important to accurately quantify pharmacodynamically.

The lifespan based models of cellular response fall into the broad class of indirect response models [1–8]. Early lifespan based PD models assumed a single “point distribution” of cellular lifespans that does not vary over time (i.e. time invariant). More recently, models have been introduced that account for a time invariant [2,6] and time variant distributions of cell lifespans [4]. The previous PD models of time variant cellular lifespans [3,4,7], as well as the time invariant lifespan models, assumed that the lifespan distribution and thus probability of cellular survival was defined for an individual cell at the time of cellular production. Hence the time variant disposition of the cellular lifespans was assumed to be only due to changes in the conditions cells were produced or formed under; and subsequent changes in the environmental conditions after their production were assumed to have no effect on the disposition of the cells. This assumption may not be reasonable if the environmental conditions that a cell is exposed to vary substantially over time. The term “environmental effect” in this manuscript will refer specifically to effect of the environmental conditions on cellular lifespan that are induced by changes in the environment occurring *after* the cell is produced (e.g. while present in the sampling space), and is to be distinguished from changes in the conditions under which the cell is formed.

The neglect of and need to account for environmental effects on cellular behavior using mathematical models has been recently recognized in areas such as red blood cell (RBC) survival in patients with uremia that are treated with erythropoietin and RBC transfusions [9]. Other examples of “environmental effects” on cellular lifespan include: increases in the concentration of reactive oxygen species that RBCs are exposed to due to chemotherapy administration [10,11], increases in RBC oxidative stress due to hemodialysis [12], decreased platelet activation due to administration of antiplatelet drugs [13], and changes in the availability of nutrients of cancerous cells due to the administration of anti-angiogenic drugs [14]. The study of the effect of different storage conditions and time periods on RBC aging and the survival of the transfused RBC cells is also directly related to the environmental effects on cell lifespan, where storage induced acceleration of the aging process is thought to occur [15].

The objectives of the current work are: (1) to extend the previously formulated time variant cellular lifespan PD models to allow for the incorporation of environmental effects on the cellular lifespan, (2) to examine by simulation the effect of varying the level of the environmental effects on the observed cellular response curve, and (3) to present a data analysis

example of the proposed environmental effect models considering the cellular response following hematopoietic busulfan ablation in sheep.

Theoretical

Time variant cellular disposition and the effect of changes in the cellular environment

Let $\ell_b(\tau, z)$ denote the “baseline” probability density function (p.d.f.) of cellular lifespans where τ is the cellular lifespan in the sampling space and z is an arbitrary time of production. Cellular production is here defined as the physical input of cells into the sampling space. The p.d.f. $\ell_b(\tau, z)$ is the baseline lifespan distribution that is defined at the time of production for cells produced at time z , which would be observed if the sampling space environment had no effect on the cellular lifespan distribution. Thus, in the context of a constant cellular environment that has no effect on the cell lifespans, a corresponding baseline time variant survival function, S_b , can be defined:

$$S_b(t, z) = P(T > t - z, z) = 1 - \int_0^{t-z} \ell_b(\tau, z) d\tau, t \geq z \quad (1)$$

where t is the current time, T denotes a random cellular lifespan variable, and $P(T > t - z, z)$ denotes the probability that $T > t - z$ for cells produced at time z . Accordingly, $S_b(t, z)$ defines the cellular disposition or probability of a cell being present in the sampling space if the environmental conditions cells are exposed to after production does not affect the baseline lifespan p.d.f.. The baseline survival function is analogous to the previously defined unit response function [3,4]. However, a unit response does not apply when dealing with environmental effects because the contribution of prior production is no longer an exclusive function of elapsed time since production (i.e. $t - z$).

The effect of the environment on the cellular disposition can be considered, as previously proposed in survival analysis [16,17], by utilization of a positive function, $e(t, z)$, that is the effect of the sampling space environment on the cellular disposition:

$$S_e(t, z) = P(T > e(t, z) \cdot [t - z], z) = 1 - \int_0^{e(t, z) \cdot [t - z]} \ell_b(\tau, z) d\tau, t \geq z \quad (2)$$

where S_e is the observed survival function due to the environmental effects. The function $e(t, z)$ is a *cumulative measure* of the effect of the environment over the time period from time z of production to the current time t (i.e. time period that the cells have been in the environment since production) and acts by accelerating and decelerating the age related death/removal process or life of the cells. Accordingly, $e(t, z) > 1$ and $e(t, z) < 1$ correspond to a reduction and a prolongation of the cellular survival, respectively. The constraint that $e(t, z) > 0$ ensures that all cells have a finite lifespan. Furthermore, to ensure that the probability a cell is present cannot increase with time, i.e. ensure that the survival function is not increasing, the following additional constraint on $e(t, z)$ must be considered:

$$\left[\frac{d}{dt} e(t, z) \right] \cdot [t - z] + e(t, z) > 0 \quad (3)$$

Taking the negative derivative of Eq. 2 gives:

$$-\frac{d}{dt}S_e(t, z) = \left[\frac{d}{dt}e(t, z) \cdot [t - z] + e(t, z) \right] \cdot \ell_b(e(t, z) \cdot [t - z], z), t \geq z \quad (4)$$

Followed by substitution of $z + \tau$ for t in Eq. 4 gives the observed cellular lifespan distribution due to the environmental effects of cells produced at time z , denoted $\ell_e(\tau, z)$, since the lifespan (i.e. τ) being evaluated in Eq. 4 is given by $t - z$:

$$\ell_e(\tau, z) = \left[\frac{d}{d\tau}e(z + \tau, z) \cdot \tau + e(z + \tau, z) \right] \cdot \ell_b(e(z + \tau, z) \cdot \tau, z), \tau \geq 0 \quad (5)$$

The necessity of the constraint imposed by Eq. 3 is also evident in Eq. 5, as it ensures that the observed lifespan p.d.f. can never take on a negative value.

Let $f_{\text{prod}}(t)$ denote the production (i.e. input) rate of cells into the sampling space, which is typically a function of time through endogenous growth factors and/or exogenous drug. Then as previously presented [4], the number of cells present in the sampling space, denoted $N(t)$, is given by the integral of the product of the number of cells produced at a previous times and the probability that the cells produced at the previous times are present in the sampling space at the current time (i.e. $S_e(t, z)$):

$$\begin{aligned} N(t) &= \int_{-\infty}^t f_{\text{prod}}(u) \cdot S_e(t, u) du \\ &= \int_{-\infty}^t f_{\text{prod}}(u) \cdot \left[1 - \int_0^{e(t, u) \cdot [t - u]} \ell_b(\tau, u) d\tau \right] du \end{aligned} \quad (6)$$

Equation 6 gives the *key general equation* for modeling time variant cellular lifespans using an accelerated life model that allows for the incorporation of changes in the cellular lifespans due to both time variant effects of the environment following production (i.e. through $e(t, z)$) and due to time variance in the conditions cells are formed under at the time of production (i.e. through $\ell_b(\tau, z)$). The lower integration limit of $-\infty$ in Eq. 6 is to be interpreted to consider “all prior history” of the system that affects $N(t)$. Due to the finite lifespan of cells, the lower limit can alternatively be explicitly stated as t minus the maximal cellular lifespan, if known.

Functional forms to incorporate the effect of the cellular environment

The constraints imposed on $e(t, z)$, particularly by Eq. 3, limits the functional forms that may be considered to incorporate the environmental effect. One particularly attractive formulation of $e(t, z)$ proposed here is given by:

$$e(t, z) = \begin{cases} \frac{\int_z^t g(u) du}{g(t)} & \text{if } t > z, g(t) > 0 \\ g(t) & \text{if } t = z \end{cases} \quad (7)$$

where $g(t)$ is the environmental effect function, which is a measure of the effect of the current environmental conditions at time t . Thus, if $g(t) = 1$ then environmental conditions at time t would have no effect on the baseline lifespan distribution. Calculation of Eq. 7 when $g(t) = 1$ for all t results in a value of $e(t, z) = 1$ for all t and z such that $t \geq z$, agreeing with the interpretation of this function. Further inspection of Eq. 7 reveals that under this formulation $e(t, z)$ is the

average value of the environmental effect function over the time period that a cell has been in the sampling space environment following production. Thus this mathematical formulation of $e(t, z)$ agrees with the definition given above as $e(t, z)$ being a *cumulative measure* of the effect of the environment on the cellular disposition. Furthermore, $e(t, z)$ given by Eq. 7 satisfies the constraint that $e(t, z) > 0$ and the constraint given by Eq. 3 (Appendix A). However, any number formulations of $e(t, z)$ that satisfy the above constraints are possible (i.e. formulations where $e(t, z)$ is *not* the average value).

Alternative formulations of the environmental effect on cellular lifespan

The above formulated accelerated life time variant lifespan model given by Eq. 2 and Eq. 6 represents just one formulation for incorporating the environmental effects on cellular disposition. Alternatively, the post-production time variant environmental effects could be incorporated using a time dependent relative risk model for the survival function [17], which represents the second class of the most commonly used models in survival analysis. Accordingly, the environmental effect function, now denoted by $h(t)$ for the relative risk model, directly affects the baseline hazard function, denoted by $\lambda_b(t, z)$, by altering the instantaneous risk of death/removal relative to the risk of death/removal given by the baseline hazard function. By definition [16–18], the hazard function is the rate of cell removal from the sampling compartment at time t conditional on survival of the cells to time t . Thus:

$$\lambda_b(t, z) = \frac{\ell_b(t - z, z)}{S_b(t, z)}, t \geq z \quad (8)$$

If in the model formulation $h(t)$ directly affects $\lambda_b(t, z)$, as proposed above, then the observed hazard function, $\lambda_e(t, z)$, is given by:

$$\lambda_e(t, z) = \lambda_b(t, z) \cdot h(t) = \frac{\ell_b(t - z, z)}{S_b(t, z)} \cdot h(t), h(t) > 0, t \geq z \quad (9)$$

Resulting in the following observed survival function [17]:

$$\begin{aligned} S_e(t, z) &= \exp \left[- \int_z^t \lambda_e(v, z) dv \right] \\ &= \exp \left[- \int_z^t \lambda_b(v, z) \cdot h(v) dv \right], h(t) > 0, t \geq z \\ &= \exp \left[- \int_z^t \frac{\ell_b(v - z, z)}{S_b(v, z)} \cdot h(v) dv \right] \end{aligned} \quad (10)$$

where $S_b(t, z)$ is given by Eq. 1. Similar to the accelerated life model, when $h(t) > 1$ the survival of the cells is reduced and conversely when $h(t) < 1$ the survival of the cells is prolonged. However, it is observed from the model formulation and the different entry of $g(t)$ and $h(t)$ into Eq. 2 (through Eq. 7) and Eq. 10, respectively, that the exact interpretation and the effect of the environmental effect function is not the same in the accelerated life and relative risk model formulations. Paralleling the derivation of Eq. 5, taking the negative derivative of Eq. 10 followed by substitution of $z + \tau$ for t gives the observed cellular lifespan distribution due to the environmental effects of cells produced at time z for the relative risk model:

$$\ell_e(\tau, z) = h(z + \tau) \cdot \frac{\ell_b(\tau, z)}{S_b(z + \tau, z)} \cdot \exp \left[- \int_z^{z+\tau} \frac{\ell_b(v - z, z)}{S_b(v, z)} \cdot h(v) dv \right], h(t) > 0 \quad (11)$$

Furthermore, substitution of Eq. 10 into Eq. 6 as the observed survival function due to the environmental effects gives an alternative *general equation* for modeling time variant cellular lifespans that incorporates the effect of the environment using a relative risk model instead of an accelerated life model:

$$\begin{aligned} N(t) &= \int_{-\infty}^t f_{\text{prod}}(u) \cdot S_e(t, u) du \\ &= \int_{-\infty}^t f_{\text{prod}}(u) \cdot \left[\exp \left[- \int_u^t \frac{\ell_b(v - u, u)}{S_b(v, u)} \cdot h(v) dv \right] \right] du \end{aligned} \quad (12)$$

However, in contrast to the accelerated lifespan model above there is no direct physical interpretation of the effect of the environmental conditions (i.e. $h(t)$) on the probability of survival of the cells in the sampling compartment [17]. The absence of a physical interpretation of $h(t)$ may limit the applicability of the relative risk model formulation from a physiological perspective.

Materials and methods

Simulations

A series of simulations were conducted to compare the two environmental effect models by varying the magnitude and shape of the environmental effect functions on the observed cellular response curve ($N(t)$). For all simulations the production rate, denoted by $f_{\text{prod}}^{\text{SS}}$, remained constant with a value of 3×10^9 cells/kg/day, the normal steady-state RBC production rate in humans [19]. The constant baseline cellular lifespan p.d.f. followed a two parameter Weibull distribution with a mean of 120 days and a standard deviation of 15 days. The Weibull distribution was chosen due to the analytical solution to the integral given in Eq. 2 and in Eq. 10 if $g(t)$ and $h(t)$, respectively, are represented as polynomials. The mean and standard deviation were chosen to match typical RBC lifespan values in humans [5, 19–21]. The environmental effect functions, $g(t)$ and $h(t)$ for the accelerated life and relative risk models, respectively, were arbitrarily chosen to result in a 16-fold range in mean steady-state cellular lifespans (μ_{SS}): 30, 60, 120, 240, and 480 days, which would be observed if the extremum from baseline (i.e. $g(t), h(t) = 1$) of the simulated environmental effect function profiles were maintained indefinitely. Three different environmental effect function profiles were considered: (1) a gradual change in the environmental conditions from baseline followed by an immediate return to baseline, (2) an immediate change from baseline followed by gradual return to baseline, and (3) moderate to rapid change from baseline followed by the temporary establishment of a new steady-state environmental conditions before a final moderate to rapid return to baseline environmental conditions (Fig. 1).

Specifically, profile 1 (Fig. 1, Panels A and B) was simulated as follows:

$$g(t), h(t) = \begin{cases} 1 & \text{if } t \leq 0 \\ 1 + \left[\frac{M-1}{3 \cdot \mu_b} \right] \cdot t & \text{if } 0 < t \leq 3 \cdot \mu_b \\ 1 & \text{if } t > 3 \cdot \mu_b \end{cases} \quad (13)$$

where μ_b is the mean baseline cellular lifespan (i.e. 120 days) and M is a positive multiple of the baseline environmental effect function value that was standardized for each model to give steady-state, i.e. $g(t), h(t) = M$, mean cellular lifespans of 30, 60, 120, 240, and 480 days (i.e. 0.25X, 0.5X, 1X, 2X, and 4X of μ_b , respectively). Profile 2 (Fig. 1, Panels C and D) was simulated as:

$$g(t), h(t) = \begin{cases} 1 & \text{if } t \leq 0 \\ M - \left[\frac{M-1}{3\mu_b} \right] \cdot t & \text{if } 0 < t \leq 3 \cdot \mu_b \\ 1 & \text{if } t > 3 \cdot \mu_b \end{cases} \quad (14)$$

Finally, profile 3 (Fig. 1, Panels E and F) was simulated as:

$$g(t), h(t) = \begin{cases} 1 & \text{if } t \leq 0 \\ 1 + \left[\frac{M-1}{\frac{1}{4}\mu_b} \right] \cdot t & \text{if } 0 < t \leq \frac{1}{4} \cdot \mu_b \\ M & \text{if } \frac{1}{4} \cdot \mu_b < t \leq \frac{11}{4} \cdot \mu_b \\ M - \left[\frac{M-1}{\frac{1}{4}\mu_b} \right] \cdot \left[t - \frac{11}{4} \cdot \mu_b \right] & \text{if } \frac{11}{4} \cdot \mu_b < t \leq 3 \cdot \mu_b \\ 1 & \text{if } t > 3 \cdot \mu_b \end{cases} \quad (15)$$

The observed number of cells, $N(t)$, were simulated using the accelerated life model given by Eq. 6 and Eq. 7 and the relative risk model given by Eq. 12. For the accelerated life model the mean cellular lifespan under steady-state environmental conditions (i.e. $g(t) = M$) is given by (Appendix B):

$$E_{\ell_e}\{T|z, g(t)=M\} = \frac{E_{\ell_e}\{T|z, g(t)=1\}}{M} = \frac{E_{\ell_b}\{T|z\}}{M} \quad (16)$$

where T is a random cellular lifespan variable, $E_{\ell_e}\{\cdot\}$ is the mathematical expectation taken with respect to $\ell_e(\tau, z)$ (i.e. Eq. 5), and $E_{\ell_b}\{\cdot\}$ is the mathematical expectation taken with respect to $\ell_b(\tau, z)$. Thus from Eq. 16 the standard M value equaled 4, 2, 1, 0.5, and 0.25 for the accelerated lifespan model to achieve the selected steady-state mean cellular lifespans of 30, 60, 120, 240, and 480 days, respectively. For the relative risk model, the standardized value of M for $h(t)$ giving the above selected mean cellular lifespans under steady-state conditions was determined numerically from the mathematical expectation of the negative first derivative of $S_e(t, z)$ (e.g. Eq. 11) for different steady-state values of $h(t)$. The resulting values of M for the relative risk model were 6.05×10^5 , 7.78×10^2 , 1, 1.29×10^{-3} , and 1.65×10^{-6} corresponding to mean steady-state cellular lifespan of 30, 60, 120, 240, and 480 days, respectively. Additionally, to directly observe the effect of $g(t)$ and $h(t)$ on the baseline cellular lifespan distribution, the observed cellular lifespan distributions due to the environmental effect (i.e. $\ell_e(\tau, z)$) were plotted under steady-state environmental conditions for both models with $g(t), h(t) = M$ from above.

Environmental effect of hematopoietic ablation by busulfan in sheep

The accelerated life and relative risk environmental effect cellular lifespan PD models given by Eq. 6 and Eq. 12, respectively, were fit to the data following hematopoietic ablation by busulfan administration in sheep to present an example of how the environmental effects may be estimated by the two models.

Animals—All animal care and experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee. Six healthy young adult sheep approximately 4 months old and weighing 29.9 (8.67%) kg (mean (C.V.)) at the beginning of the experiment were utilized. Animals were housed in an indoor, light and temperature-controlled environment, with ad lib access to feed and water. Prior to study initiation, jugular venous catheters were aseptically placed under pentobarbital anesthesia. Intravenous ampicillin (1 g) was administered daily for 3 days following catheter placement.

Study protocol—The busulfan chemotherapy was chosen to ensure complete ablation of the bone marrow and erythropoietic cells without jeopardizing the animal health [22]. Following catheter placement, 5.5 mg/kg busulfan was administered orally twice a day for three consecutive days. Intravenous ampicillin (1 g) was administered daily following chemotherapy initiation to prevent infection due to the ablation of the immune system. Animals were clinically monitored for adverse events of chemotherapy such as weight loss, wool loss, blood in urine or stools, fever, etc. Blood samples (~1 ml/sample) were collected approximately once per day and measured for hemoglobin concentrations using an IL482 CO-oximeter (Instrumentation Laboratories, Watham, MA) and RBC concentrations using a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). To prevent undue animal distress, studies were terminated and sheep were euthanized using an intravenous over-dosage of pentobarbital when the hemoglobin concentrations reached approximately 3 g/dl.

Specific model formulation—The production rate was assumed to be constant for $t \leq t_0$ (denoted by $f_{\text{prod}}^{\text{SS}}$) where t_0 is the time of initiation of the busulfan administration, and then to linearly drop to a production rate of 0 over 8 days (T_{decline}). The production rate was assumed to drop to 0 by 8 days post-initiation of the busulfan therapy because previous experiments indicated that by 8 days no colony-forming units erythroids (CFU-E) are present [22], which are the bone marrow erythroid progenitor cells that directly develop into hemoglobinized cells [19]. The RBC lifespan distribution in the absence of environmental effects (i.e. $\ell_b(\tau, z)$) was assumed to be time invariant (i.e. $\ell_b(\tau, z) = \ell_b(\tau)$) and follow a Weibull distribution with a fixed mean (μ) of 114 days as previously determined in sheep [23]. The standard deviation (σ) of the baseline lifespan distribution was assumed to be similar to that typically cited for humans and fixed to 15 days [5,21]. A time invariant baseline lifespan distribution was assumed since the focus of this work was the time variance of cellular lifespan due to *environmental effects* and to minimize the complexity of the analysis. Additionally, a time invariant baseline normal distribution of the RBC lifespan with mean μ of 114 days and standard deviation σ of 15 days was fitted to the data; however, the results were not presented in detail due to numerical problems encountered in the evaluation of relative risk environmental effect model in this case. For the accelerated life model $e(t, z)$ was given by Eq. 7. A “bottom-up” approach was used to estimate the value of the environmental effect functions without creating a functional relationship to any measured variables [24], since the exact mechanism that causes the reduced lifespan of the RBCs is unknown. The environmental effect functions, $g(t)$ and $h(t)$, were semi-parametrically represented and assumed to be equal to 1 for $t \leq t_0$, and then follow a positive and continuous linear spline with constant forward extrapolation beyond the last node. The number of nodes utilized, which was four, was empirically chosen to provide sufficient flexibility to adequately fit the data from all subjects. To convert from number of cells per kg to concentration of cells, the estimated number of RBCs from Eq. 6 and Eq. 12 for the accelerated life and relative risk models, respectively, were divided by a fixed, previously determined, blood volume (BV) of 74.4 ml/kg [25]. The hemoglobin concentrations were estimated by multiplying the estimated concentration of RBCs by the model estimated mean corpuscular hemoglobin (MCH) parameter in picograms per cell.

Computational details

All simulations and modeling were conducted using WINFUNFIT, a Windows (Microsoft) version evolved from the general nonlinear regression program FUNFIT [26], using ordinary least squares. The simulated and fitted models required a solution to multiple integrals. The integral in the observed survival functions (i.e. $S_e(t, z)$) given by Eq. 2 and Eq. 10 were analytically determined, as was the integral in Eq. 7 ($e(t, z)$) for the accelerated life model. Finally, the numerical solution to the outer integral from $-\infty$ to t in Eq. 6 and Eq. 12 was determined using the FORTRAN 90 subroutine QDAGS from the IMSL® Math Library (Version 3.0, Visual Numerics Inc., Houston, TX). QDAGS is a univariate quadrature adaptive general-purpose integrator that is an implementation of the routine QAGS [27]. The relative error for the QDAGS routine was set at 0.1% for all numerical integrations. The model given by Eq. 6 for the accelerated life model and by Eq. 12 for the relative risk model was simultaneously fit to both the sheep RBC and hemoglobin data by multiplying the estimated RBC concentrations by the estimated *MCH* parameter. The model fits to the sheep RBC and hemoglobin data were compared using the Akaike's Information Criterion (AIC) value, with the number of estimated parameters and spline coefficients included in the calculation [28].

Results

Simulations

Figure 2 displays the effect on the cellular response of a gradual change from baseline environmental effect (i.e. baseline effect given by $g(t)$ or $h(t) = 1$) followed by an immediate return to baseline after three mean (μ_b) baseline cellular lifespans (Profile 1, Eq. 13), as displayed in Fig. 1, Panels A and B for the accelerated life and relative risk models, respectively. An increase (decrease) in the value of $g(t)$ or $h(t)$ in the accelerated life and relative risk models caused a decrease (increase) in the cell number. For the accelerated life model, all five magnitudes of simulated environmental effect function values resulted in distinct cellular response curves. However, for the relative risk model the two lowest values of simulated environmental effects, corresponding to the two highest μ_{SS} (i.e. 2X and 4X of μ_b), resulted in cellular response curves that virtually overlaid each other. For the accelerated life environmental effect model, all cell number response curves deviated and returned to baseline cell numbers at the same time regardless of the magnitude of the perturbation from steady-state but for the relative risk model the time to return to steady-state appears to vary with both the magnitude and direction of the change in the environmental effect functions. For both models, the return to baseline cell numbers begins immediately upon the drop in the environmental effects, as can be observed from the “break” in the curve at 360 days (i.e. $3 \cdot \mu_b$). Additionally, as can be analytically determined from Eq. 6 or Eq. 12 when the cellular production rate and lifespan distribution are constant (i.e. at steady-state) with no environmental effects (i.e. $g(t)$ or $h(t) = 1$) (Appendix C), the baseline number of cells is given by $f_{\text{prod}}^{\text{SS}} \cdot \mu_b = 360 \times 10^9$ cells/kg.

Figure 3 displays the effect on the cellular response of an immediate change from baseline environmental effects followed by gradual return to baseline environmental effects (Profile 2, Eq. 14), as displayed in Fig. 1, Panels C and D for the accelerated life and relative risk models, respectively. Similar to before, an increase (decrease) in the environmental effects caused a decrease (increase) in the cell number. Again for the relative risk model the two lowest values of simulated environmental effect function values resulted in cellular response curves that virtually overlaid each other. Unlike the previously simulated cell number response curves (Fig. 2), the shapes of the curves are “smoother,” as expected.

Figure 4 displays the effect on the cellular response of a moderate to rapid change from baseline environmental effects followed by a new temporary steady-state environmental effect function

values and a final moderate to rapid return to baseline environmental effects (Profile 3, Eq. 15), as displayed in Fig. 1, Panels E and F for the accelerated life and relative risk models, respectively. Steady-state cell numbers were achieved within the time period of perturbation of the environmental effects for four out of the five simulated steady-state environmental effect function values. However, steady-state cell numbers were not achieved for the lowest value of the environmental effects, which corresponds to the longest μ_{SS} (i.e. 4X of μ_b). As designed, the same steady-state number of cells was achieved for both models when steady-state cell numbers were reached. The relative risk model reached the new steady-state values more rapidly than the accelerated life model for the same standardized environmental effect function profiles. Unlike the previous simulated profiles using the relative risk model (Fig. 2, Fig. 3), each level of environmental effects resulted in a distinct cellular response profile. Additionally, the time for the relative risk model to return to baseline cell number following the drop in the environmental effects back to baseline appeared to depend on the direction and the magnitude the environmental effects were changed. For the accelerated life model, the time to return to the baseline cell number was independent of the direction and magnitude the environmental effects were changed.

Figure 5 displays the effect of steady-state environmental conditions on the observed cellular lifespan distribution for both the accelerated life and relative risk models. As expected, as μ_{SS} increased the mass of the distribution shifted to longer lifespans. Additionally, the variance in the lifespan also increased with increasing μ_{SS} for both models. Furthermore, under steady-state environmental conditions the observed lifespan distributions were identical for the accelerated life and relative risk models when they were constrained to have the same mean observed cellular lifespan arising from a baseline Weibull distribution. However, this is not generally true for any baseline cellular lifespan distribution. If a normal distribution is instead used as the baseline distribution then the observed cellular lifespan densities would not have been equal even when constrained to have the same mean observed cellular lifespan.

Environmental effect of hematopoietic ablation by busulfan administration in sheep

The profiles and simultaneous fit to the RBC and hemoglobin concentrations using the accelerated life and relative risk models, as well as the estimated environmental effect function values, for a representative sheep following hematopoietic ablation by busulfan administration at time 0 is displayed in Fig. 6. In general, the sheep RBC and hemoglobin concentrations gradually declined for the first 2 weeks following busulfan administration, and then rapidly declined at an increased rate over the next week before study termination. The rapid decline in the RBC and hemoglobin concentrations was paralleled by a rapid increase in the estimated environmental effect function values. Both models fit the data similarly well and gave similar parameter estimates (Table 1), except for the parameters related to the environmental effects, consistent with the different magnitude of environmental effects necessary to achieve the same steady-state cell number. The relative risk model gave the lowest mean AIC value; however, in 2 of the 6 subjects the AIC was lowest for the accelerated life model. The steady-state cellular production rate (f_{prod}^{SS}) was estimated at 6.20 (6.50%) and 6.15 (6.53%) 10^9 cells/kg/day (mean (C.V.)) for the accelerated life and relative risk models, respectively, and the mean corpuscular hemoglobin (MCH) was estimated at 11.8 pg per cell for both models. The average environmental effect function values (e_{avg}) from the initial time of busulfan administration (t_0) to the last observation (t_{last}) were calculated at 3.49 (29.5%) and 115 (103%) for the accelerated life and relative risk models (Note: for the accelerated life model, $e_{avg} = e(t_{last}, t_0)$, where $e(t, z)$ is given by Eq. 7). Using a normal p.d.f. instead of a Weibull p.d.f. to represent the baseline cellular lifespan distribution for the accelerated life environmental effect model resulted in very similar estimates of the model parameters (6.12 (4.43%) 10^9 cells/kg/day, 11.8 (5.10%) pg, and 3.52 (29.0%) for f_{prod}^{SS} , MCH , and e_{avg} , respectively). The use of a normal

distribution for the relative risk environmental effect model could not be successfully implemented due to excessive numerical integration errors associated with the lack of an analytical solution to the Eq. 10 when the environmental effects are represented as a linear spline function.

Assuming that no environmental effect was present but allowing the cellular production rate to drop to 0 immediately following busulfan administration initiation did not result in as good of a fit to the data compared to the environmental effect model, as shown for a representative subject by the dashed and solid lines, respectively, in Fig. 7. Additionally, assuming the cellular production rate dropped linearly over 8 days, as done above, and no environmental effect but allowing the mean and standard deviation of the baseline cellular lifespan distribution to be estimated also did not result in as good of fit to the data (dotted line, Fig. 7). The AIC's were 37.0 (74.7%), 3.39 (477%), and -37.3 (81.6%) for the instantaneous stop in production (no environmental effect), mean and standard deviation of cellular lifespan estimated (no environment effect), and accelerated life environmental effect models, respectively. The AIC was -38.4 (78.0%) for the relative risk environmental effect model. In fact, both the accelerated life and relative risk environmental effects models had a lower AIC value for all subjects than either of the above sub-models that assumed no environmental effect. Furthermore, without the environmental effect being incorporated the mean baseline cellular lifespan distribution was estimated at 41.9 (32.1%) days, much lower than previous estimates in sheep of 114 (10.5%) days using a [^{14}C] cyanate label [23] and 75 to 153 days using ^{59}Fe pulse-chase methodology [29].

Discussion

A PD model for incorporating time variant environmental effects on cellular lifespans and cellular responses was formulated and presented using the two basic classes of models from survival analysis, an accelerated life and a relative risk model. The models extend previous models of time variant cellular lifespans [3,4], allowing for time variance in the cellular lifespans to be due to both changes in the baseline cellular lifespan distribution (i.e. $\ell_b(\tau, z)$) and changes in the environmental effects (i.e. $g(t)$ or $h(t)$). The model formulation allows for a flexible incorporation of the environmental effect function with minimal constraints on the form of the function representing the environmental effects other than what is dictated from obvious physiological considerations. The environmental effect of drugs on the cellular lifespan can readily be incorporated into the model through the environmental effect functions (i.e. $g(t)$ and $h(t)$), which can be modeled to depend on time through changes in drug concentrations. Additionally, multiple changes in the environment that effect the cellular lifespan can be incorporated into the model through the environmental effect functions if a specific model of these functions of the environmental changes is proposed. The model formulation also allows for the utilization of any arbitrary p.d.f. to represent the baseline cellular lifespan distribution for the accelerated life model. The formulated accelerated life and relative risk models of environmental effects were successfully fitted to the RBC and hemoglobin concentration profiles of six sheep following hematopoietic ablation by busulfan administration. Comparisons of the environmental effect models to models without the environmental effect component indicated that the environment effect needs to be considered to more fully explain the observed change in the cellular response.

Simulations demonstrated a different shape to the cellular responses following standardized changes in the environmental effects using the accelerated life model compared to the relative risk model (Fig. 2 through Fig. 4). Another prominent difference in the models demonstrated by the simulations is the time to return to steady-state cellular response following the return of the environmental conditions to baseline. The time to return to baseline depended on the direction and magnitude of the changes in the environmental effects for the relative risk model,

but appeared to be independent of the environmental effect change for the accelerated life model. The shape in the cellular responses is in some ways intrinsic to the model formulations, as the environmental effect functions, $g(t)$ and $h(t)$, enter the observed survival functions (Eq. 6 through Eq. 7 and Eq. 12, respectively) in very different mathematical forms for the accelerated life and relative risk models, respectively. This can be illustrated by examining the relationship between the steady-state cell number, denoted by N_{SS} , and the steady-state environmental effects when assuming both the cellular production rate and the baseline cellular lifespan (i.e. $\ell_b(\tau, z) = \ell_b(\tau)$) are constant. From Appendix B and Appendix C it is evident that the relationship between N_{SS} and $g(t)$ for the accelerated life model is given by:

$$N_{ss} = \frac{f_{\text{prod}}^{SS} \cdot \mu_b}{g_{ss}} \quad (17)$$

where g_{ss} is the steady-state environmental effects. Thus the steady-state cellular response is inversely proportional to the steady-state environmental effects for the accelerated life environmental effect model. In contrast, for the relative risk model the relationship between N_{SS} and $h(t)$ at steady-state, denoted by h_{ss} , is given by (using results from relative risk regression modeling [17]):

$$N_{ss} = f_{\text{prod}}^{SS} \cdot \int_0^{\infty} \left[1 - \int_0^u \ell_b(\tau) d\tau \right]^{h_{ss}} du \quad (18)$$

Obviously the relationship between the steady-state cellular response and the environmental effects for the relative risk environmental effect model is more complex than the inversely proportional relationship that exists for the accelerated life model.

Additionally, there are several limitations to the relative risk model that are not present for the accelerated life model. One limitation is the absence of a direct physical interpretation of the environmental effect function on the probability of the survival of cells in the relative risk model [17]. This limitation reduces the ability to directly interpret the meaning of the changes in the environmental effects. A second limitation of the relative risk model is not being able to define the baseline hazard function ($\lambda_b(t, z)$) in Eq. 8 when a dirac delta function is used as the baseline lifespan distribution. In that case, $\ell_b(\tau, z) = \delta(\tau - a, z)$, where $\delta(\cdot, z)$ is a dirac delta function defined at time z and a is the single “point” cellular lifespan. Defining $\lambda_b(t, z)$ as zero except for at $t = z + a$ results in a model that cannot account for an environmental effect since the observed hazard function in Eq. 9 will be equal to the baseline hazard function regardless of the value of the environmental conditions ($h(t)$). Therefore, the relative risk environmental effect model does not apply to a “point distribution” of cellular lifespans, which is often a practical and sufficient simplification of the cellular lifespan PD model [2]. The accelerated life model does not have this limitation.

A clear distinction of the presented environmental effects cellular lifespan model must be made from other cellular response PD models where environmental effect mediated cell removal is a random, i.e. age-independent, process. Examples of these types of models include simplified models that assumed a time invariant “point distribution” of cellular lifespans and a time invariant first-order removal processes in addition to the age-dependent removal processes [30,31]. The removal of cells from the sampling space in the current models is still an age-dependent process; however, the age-dependent removal is modified by the environmental effects. This removal process due to the environmental effects is different than that given by environmental effects that cause cell removal by an age-independent mechanism, as would be

the case if some cells are removed by first order processes prior to the predetermined lifespan [30,31]. A discussion of modeling environmental effects that cause an age-independent removal process is beyond the scope of this work.

Hematopoietic ablation of six sheep by busulfan administration caused very little initial increase in the estimated environmental effects in the first week, followed by a rapid increase in $g(t)$ and $h(t)$ beginning between 1 and 2 weeks following busulfan administration initiation (Fig. 6). The exact cause of the environmental effect increase is unknown, but could be due to a variety of factors. One likely candidate for accelerating the removal of the RBCs from the systemic circulation is an increase in the oxidative stress that the RBCs are exposed to in the systemic circulation. Previous research has demonstrated an increase in the oxidative stress status of individuals undergoing high-dose chemotherapy for peripheral blood stem cell transplantation through a decrease in the plasma antioxidant concentrations and a corresponding increase in oxidative stress markers [11,32]. Furthermore, the peak increase in the oxidative stress markers corresponded to 7–9 days following the initiation of high-dose chemotherapy, similar to the 1–2 week time frame for the rise in the estimated environmental effects. Other studies have also demonstrated increases in free radical generation and lipid peroxidation in vivo following chemotherapy administration [33]. Increases in oxidative stress and oxidant damage of RBCs are important determinants in the premature removal of erythrocytes from the systemic circulation [10,34]. Since oxidative damage to RBCs would accumulate over time, it is mechanistically appealing to use the accelerated life environmental effect model compared to the relative risk model, as the accelerated life model is formulated in terms of the cumulative effect of the environment over the lifespan of a cell (i.e. $e(t, z)$ in Eq. 6), even though on average AIC was lowest for the relative risk model (Table 1). Additionally, other researchers also hypothesize that RBC senescence is primarily due to the cumulative stress a cell is exposed to during its time in the systemic circulation [21], further supporting the use of the accelerated life environmental effect model in this instance. However, since no markers of oxidant stress were measured in the present study other environmental effects of hematopoietic ablation by busulfan administration certainly could have caused the observed increased removal of cells.

The importance of including an environmental effect into the cellular lifespan distribution model under certain situations is illustrated with a representative subject fits given in Fig. 7 for models that assumed no environmental effects. Neither estimation of the baseline mean and standard deviation of the baseline cellular lifespan distribution nor assuming that busulfan administration immediately dropped the production rate to zero resulted in models that fit the observed RBC data as well, based on AIC. While the assumption of the functional form of the RBC production rate (i.e. $f_{prod}(t)$) will influence the observed cellular response (Fig. 6 and Fig. 7) and the estimated environmental effects (Fig. 6), alternative assumptions on the production rate, such as it remained positive beyond 8 days, would result in a more gradual decline in the RBC and hemoglobin concentrations for the same given environmental effect.

The proposed environmental effect PD models may have applications in a variety of disciplines involving the PDs and/or kinetics of cellular populations that are exposed to changing environmental conditions over time. One area of potential application is the management of anemia in patients with limited renal function by treatment with erythropoietin and RBC transfusions [9]. These patients often have impaired RBC survival due to the uremic environment that alter the RBC membrane and cytoskeleton characteristics and are subjected to increased RBC oxidative stress through hemodialysis [9,12]. The study of the effect of different storage conditions and time periods on RBC aging and the survival of the transfused RBC cells is also closely related to the proposed models, where storage induced acceleration of the aging process is thought to occur [15]. As discussed above, the model has further potential applications in understanding the decreases in RBC cell survival due to increases in the

concentration of reactive oxygen species that RBCs are exposed to from chemotherapy administration [10,11]. Finally, other areas of application include dynamically accounting for decreased platelet activation and increased survival due to administration of antiplatelet drugs and decreased survival of cancerous cells due to changes in the availability of nutrients from the administration of anti-angiogenic drugs [13,14].

Conclusion

In summary, the previously formulated time variant cellular lifespan PD models were extended to incorporate environmental effects on the cell lifespan using the two basic classes of models from survival analysis: accelerated life and relative risk models. Due to the more direct physical interpretation of the effect of the environmental effects, a relatively simple steady-state relationship between cellular response and environmental effects, and the ability to reduce the model to a “point” baseline lifespan distribution, the accelerated life environmental effect model may offer some advantages. The analysis of a data example using RBC and hemoglobin concentration data of six sheep following hematopoietic ablation by busulfan administration indicated the environmental effects began to increase from baseline 1 to 2 weeks following initiation of ablation and that competing models that did not incorporate an environmental effect could not describe the data as well. The presented cellular lifespan PD model allows for the incorporation of arbitrary changes in the conditions of the cellular environment and the effect of these changes on the probability of age-dependent cellular survival.

Glossary

AIC, Akaike's Information Criterion
 BV, Blood volume
 $e(t, z)$, Cumulative measure of the effect of the environment
 e_{avg} , Average value of the environmental effect function
 $E\{\cdot|\cdot\}$, Conditional mathematical expectation of a random variable
 $f_{prod}(t)$, Production rate (i.e. input rate into the sampling compartment)
 f_{prod}^{SS} , Steady-state production rate
 $g(t), h(t)$, Environmental effect functions
 $\ell_b(\tau, z)$, Baseline p.d.f. of cellular lifespans
 $\ell_e(\tau, z)$, Observed p.d.f. of cellular lifespans with an environmental effect
 $\lambda_b(t, z)$, Baseline hazard function
 $\lambda_e(t, z)$, Observed hazard function with an environmental effect
 μ_b , Mean of the baseline cellular lifespan
 μ_{SS} , Steady-state mean cellular lifespan
 MCH, Mean corpuscular hemoglobin
 M, Positive multiple of the baseline environmental effect function value
 v, u , Arbitrary integration variables
 $N(t)$, Number of cells in the sampling compartment
 $P(\cdot)$, Probability
 p.d.f., Probability density function
 PD, Pharmacodynamic
 RBC, Red blood cell
 σ , Standard deviation of the baseline cellular lifespan
 SD, Standard deviation
 $S_b(t, z)$, Baseline survival function
 $S_e(t, z)$, Observed survival function with an environmental effect
 SS, Steady-state (super- and sub-scripted with other terms)

τ , T, Cellular lifespan, i.e. the time from input into the sampling space to the output from the sampling space (capital tau refers to a random cellular lifespan variable)

t , Time

t_0 , Time of initiation of busulfan administration

T_{decline} , Time period over which the production rate declines from $f_{\text{prod}}^{\text{SS}}$ to zero

Z , Time of production

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Appendix

Appendix A

Proof that Eq. 7 satisfies the condition imposed on $e(t, z)$ that $e(t, z) > 0$ and the condition imposed by Eq. 3.

Since by definition $g(t)$ is >0 for all t , then for $t > z$ both the numerator and denominator of Eq. 7 are greater than zero. Thus $e(t, z)$ is >0 when $t > z$. Furthermore, since $g(t)$ is >0 then $e(t, z)$ is >0 if $t = z$, proving that Eq. 7 satisfies the positive constraint imposed on $e(t, z)$.

Differentiation of Eq. 7 with respect to t when $t > z$ results in:

$$\frac{d}{dt}e(t, z) = \frac{g(t) \cdot [t - z] - \int_z^t g(u) du}{[t - z]^2} \quad (\text{A1})$$

Substitution of Eq. A1 into Eq. 3 results in:

$$\begin{aligned} \left[\frac{d}{dt}e(t, z) \right] \cdot [t - z] + e(t, z) &= \frac{g(t) \cdot [t - z] - \int_z^t g(u) du}{[t - z]^2} \cdot [t - z] + e(t, z) \\ &= \frac{g(t) \cdot [t - z] - \int_z^t g(u) du}{[t - z]} + e(t, z) \\ &= g(t) - \frac{\int_z^t g(u) du}{[t - z]} + e(t, z) \\ &= g(t) - e(t, z) + e(t, z) \\ &= g(t) > 0 \end{aligned} \quad (\text{A2})$$

Finally, since at time $t = z$ the difference $t - z$ in the left-hand-side of Eq. A2 equals zero, Eq. 3 is also equal to Eq. A2 when $t = z$. Proving that Eq. 7 also satisfies the conditions imposed by Eq. 3 and completing the proof.

Appendix B

Derivation of Eq. 16

From Eq. 7, if $g(t) = M$ for all t then $e(t, z) = M$ for all $t \geq z$. Thus the mathematical expectation of T taken with respect to $\ell_e(\tau, z)$ is given from Eq. 5:

$$\begin{aligned}
E_{\ell_e} \{T|z, g(t)=M\} &= \int_0^{\infty} \tau \cdot \ell_e(\tau, z) d\tau \\
&= \int_0^{\infty} \tau \cdot \left[\left[\frac{d}{d\tau} e(z+\tau, z) \right] \cdot \tau + e(z+\tau, z) \right] \\
&\quad \cdot \ell_b(\mathbf{e}(z+\tau, z) \cdot \tau, z) d\tau \\
&= \int_0^{\infty} \tau \cdot [[0] \cdot \tau + M] \cdot \ell_b(M \cdot \tau, z) d\tau \\
&= \int_0^{\infty} M \cdot \tau \cdot \ell_b(M \cdot \tau, z) d\tau
\end{aligned}$$

By change of variables:

$$\begin{aligned}
\int_0^{\infty} M \cdot \tau \cdot \ell_b(M \cdot \tau, z) d\tau &= \frac{\int_0^{\infty} u \cdot \ell_b(u, z) du}{M} \\
&= \frac{E_{\ell_b}\{T|z\}}{M}
\end{aligned}$$

Completing the derivation of Eq. 16.

Appendix C

Derivation of the baseline number of cells

From both Eq. 6 and Eq. 12:

$$N(t) = \int_{-\infty}^t f_{\text{prod}}(u) \cdot s_e(t, u) du \quad (\text{C1})$$

If the production rate remains constant, denoted by $f_{\text{prod}}^{\text{SS}}$, then:

$$\begin{aligned}
N(t) &= \int_{-\infty}^t f_{\text{prod}}^{\text{SS}} \cdot S_e(t, u) du \\
&= f_{\text{prod}}^{\text{SS}} \cdot \int_{-\infty}^t S_e(t, u) du
\end{aligned} \quad (\text{C2})$$

If additionally the baseline distribution is constant (i.e. $\ell_b(\tau, z) = \ell_b(\tau)$) and there are no environmental effects present (i.e. $g(t) = 1$ or $h(t) = 1$), then:

$$S_e(t, z) = S_b(t, z) = 1 - \int_0^{t-z} \ell_b(\tau) d\tau \quad (\text{C3})$$

for $S_e(t, z)$ given by both Eq. 2 (with Eq. 7) and Eq. 10. Thus Eq. C2 becomes

$$\begin{aligned}
 N(t) &= f_{\text{prod}}^{\text{SS}} \cdot \int_{-\infty}^t S_b(t, u) du \\
 &= f_{\text{prod}}^{\text{SS}} \cdot \int_{-\infty}^t \left[1 - \int_0^{t-u} \ell_b(\tau) d\tau \right] du \\
 &= -f_{\text{prod}}^{\text{SS}} \cdot \int_0^t \left[1 - \int_0^u \ell_b(\tau) d\tau \right] du \\
 &= f_{\text{prod}}^{\text{SS}} \cdot \int_0^t \left[1 - \int_0^u \ell_b(\tau) d\tau \right] du
 \end{aligned} \tag{C4}$$

Using a well known identity from survival analysis (17, 35), the integral in Eq. C4 is equal to the mathematical expectation of τ taken with respect to the p.d.f. of the survival function being integrated across, giving:

$$N(t) = f_{\text{prod}}^{\text{SS}} \cdot \mu_b \tag{C5}$$

Completing the derivation of the baseline number of cells.

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Fig. 1.

The three simulated environmental effect function profiles (Profile 1: Panels **A** and **B**, Profile 2: Panels **C** and **D**, Profile 3: Panels **E** and **F**) for the accelerated life (Panels **A**, **C**, and **E**) and the relative risk (Panels **B**, **D**, and **F**) models. μ_{SS} is the steady-state mean cellular lifespan, as a multiple of the baseline mean cellular lifespan (i.e. 0.25X, 0.5X, 1X, 2X, and 4X), that would be reached if the extremum from baseline of the simulated environmental effect function profile was maintained indefinitely and μ_b is the mean baseline cellular lifespan. The simulated environmental effect functions for the relative risk model (right Panels) are plotted on a log y-axis scale due to the extreme values needed to achieve the multiples of μ_b .

Fig. 2.

The effect on the cellular response of a gradual change from baseline environmental effects followed by an immediate return to baseline environmental effects (Profile 1, Eq. 13 as displayed in Fig. 1, Panel **A** and **B**) for an accelerated life and a relative risk environmental effect model. The cellular production rate and baseline lifespan distribution were constant with values of 3×10^9 cells/day and a mean and standard deviation of 120 and 15 days, respectively

Fig. 3.

The effect on the cellular response of an immediate change from baseline environmental effects followed by gradual return to baseline environmental effects (Profile 2, Eq. 14 as displayed in Fig. 1, Panel C and D) for an accelerated life and a relative risk environmental effect model. The cellular production rate and baseline lifespan distribution were constant with values of 3×10^9 cells/day and a mean and standard deviation of 120 and 15 days, respectively

Fig. 4.

The effect on the cellular response of a moderate to rapid change from baseline environmental effects followed by a new steady-state environmental effect and a final moderate to rapid return to baseline environmental effects (Profile 3, Eq. 15 as displayed in Fig. 1, Panel E and F) for an accelerated life and a relative risk environmental effect model. The cellular production rate and baseline lifespan distribution were constant with values of 3×10^9 cells/day and a mean and standard deviation of 120 and 15 days, respectively

Fig. 5.

The effect of steady-state environmental conditions ($g(t), h(t) = M$) on the observed cellular lifespan distribution ($\ell_e(\tau, z)$ given by Eq. 5 and Eq. 11 for the accelerated life and relative risk models, respectively) for the accelerated life and relative risk environmental effect model. The baseline lifespan distribution ($\ell_b(\tau, z)$ 1X) was a Weibull distribution with a mean and standard deviation of 120 and 15 days, respectively

Fig. 6.

Representative animal subject fit (*lines*) of the accelerated life and relative risk environmental effect cellular lifespan **PD** model to the observed sheep **RBC** and hemoglobin concentrations (*squares*), as well as the estimated environmental effects, following hematopoietic ablation with busulfan

Fig. 7.

Accelerated life environmental effect model fits (*lines*) to a representative animal subject RBC concentrations (*squares*) following hematopoietic ablation with busulfan assuming three different model scenarios: (1) Environmental Effect (*solid line*)—environmental effects estimated, production rate drops linearly over 8 days to 0, mean and standard deviation of the baseline cellular lifespan distribution fixed to 114 and 15 days, respectively, (2) No Environmental Effect, Instantaneous Stop of Production (*dashed line*)—no environmental effects incorporated, production rate drops instantly to 0 at time 0, mean and standard deviation of the baseline cellular lifespan distribution fixed to 114 and 15 days, respectively, and (3) No Environmental Effect, Mean and SD of Cellular Lifespan Estimated (*dotted line*)—no environmental effects estimated, production rate drops linearly over 8 days to 0, mean and standard deviation of the baseline cellular lifespan distribution estimated (37.6 and 1.47 days, respectively, for this subject)

Table 1
Parameter summary and comparison between the accelerated life (Eq. 6 and Eq. 7) and relative risk (Eq. 12) environmental effect cellular lifespan PD model assuming a Weibull distribution of cellular lifespans fitted to sheep RBC and hemoglobin concentrations following hematopoietic ablation with busulfan ($n = 6$)

Parameter	Accelerated life model		Relative risk model	
	Mean	C.V.	Mean	C.V.
AIC	-37.3	81.6%	-38.4	78.0%
f_{SS}^{prod} (10^9 cells/kg/day)	6.20	6.50%	6.15	6.53%
$T_{decline}$ (days)	8.00 ^a	-	8.00 ^a	-
MCH (pg)	11.8	5.04%	11.8	5.20%
BV (ml/kg)	74.4 ^a	-	74.4 ^a	-
μ (days)	114 ^a	-	114 ^a	-
σ (days)	15.0 ^a	-	15.0 ^a	-
e_{avg}^b	3.49	29.5%	115	103%

^aFixed parameter

^bSecondary parameter, average environmental effect function values from the time of initiation of busulfan administration (t_0) to the last observation time (t_{last})