Stochastic Models for Papilloma Formation Following Exposure to TCDD

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Introduction

Biologically-based mathematical models (BBM) provide a means for both design and analysis of toxicology studies. In the design phase, these models can be used to estimate equivalent doses across different routes of exposure (e.g. physiologically-based pharmacokinetic models), determine sample size (e.g. power for testing a given mechanistic assumption; maximum standard error for a given parameter to be estimated), and for spacing of dosing (e.g. dose-response shape predicted by a model can be used to place doses to get optimal clarification of a given pattern). In the analysis phase, BBM's can be used to estimate mechanistic quantities (e.g. mutation rates, birth rates, number of stages in the carcinogenic process), test hypotheses (e.g. the importance of exposure effects on the mutation rate and birth rate; significance of adding more stages to a cancer model), and to include additional data into the analysis (e.g. cell labeling data for estimating birth rates).

This abstract describes the analysis of skin papillomas from an experiment originally designed using a BBM for tissue concentration. The analysis is focused on three issues: determining what multistage model of carcinogenesis fits these data; ascertaining if equivalent skin concentrations of TCDD from different routes of exposure are predictive of papilloma yield; and determining if TCDD affects all rates in the process of formation of papillomas or if it only affects the growth rate of papillomas.

Materials and Methods

The data

Briefly, the data[1] used here arise from a dermal study using nine experimental groups with 20 female hemizygous Tg.AC mice in each group. The groups of mice received 0, 5, 17, 36, 76, 121, 166, 355, or 760 ng/kg of TCDD in acetone three times a week for 26 weeks. Also included was an oral study in which groups of 20 mice received 0, 105, 405, or 1250 ng/kg of TCDD in corn oil containing 1% acetone five times a week for 26 weeks. Papilloma counts were recorded every week for each animal. Papillomas were counted if their size exceeded a diameter of 1 mm. There was a maximal count of 20 papillomas per animal.

The model

A standard one-stage model for papilloma development[2] was used initially and discarded for lack of fit. Considered here is a two-stage model for the description of the papilloma data. It is assumed for the purposes of papilloma formation that a starting cell must undergo two mutations before becoming the initial cell developing eventually into a papilloma. We also assume that a papilloma becomes detectable if it contains M or more actively dividing cells where M is estimated to be 364,000 cells (assuming one cell has a diameter of 14 μm and that a sphere of cells 1 mm in diameter contains approximately 364,000 cells).

Let X_0 be the number of cells present at the start of the experiment, $t = 0$. It is assumed that these cells may undergo an instantaneous mutation with some small probability γ. Note that γX_0 actually follows a gamma distribution; however, since the number of starting cells at risk is high, the probability of mutation is small, and we are assuming instantaneous mutation, we may treat γX_0 as a Poisson random variable. In essence, γX_0 represents the initial number of cells with the active transgene and at risk of continuing on to papilloma development. We then assume that at any time t these active cells have a small probability μ of becoming the initial papilloma cell. Hence, the number of initial papilloma cells at time t is Poisson with parameter $\mu\gamma X_0 t$. Initial papilloma cells are then assumed to be subject to a linear birth-death process. Note that this model can be treated as a birth-death process with immigration where the immigration rate is $\mu\gamma X_0$. It also may be noted that this two-stage process is not sequential since the initial mutation γ is inherited.

Initial papilloma cells may divide into two cells with stochastic rate $\beta(t)$ and may die or differentiate with stochastic rate $\delta(t)$. In this model we assume that the birth rate is of the form $\beta(t) = \beta_0 + \beta_1 \cdot c(t)$ and the mutation rate μ is of the form $\mu(t) = \mu_0 + \mu_1 \cdot c(t)$, where $c(t)$ is the predicted concentration of TCDD in the skin at time t.[3] The death rate $\delta(t) = \delta$ is assumed to be constant. The number of detectable papillomas at each time point follows a Poisson distribution with parameters specified previously.[4]

Statistical Methods

To simplify the analysis, all observations (papilloma counts) are treated as independent of each other. Parameter estimates for β_0, β_1, μ_0, μ_1, and δ were obtained using maximum likelihood techniques. Likelihood ratio tests were used to evaluate the importance of concentration effects on the birth rate and the mutation rate.

Results and Discussion

Dermal Study

The lack of fit to the experimental data of the standard one-stage birth-death model for papilloma formation[2] led to our investigation of another birth-death process which allowed for mutation as a second stage to papilloma formation. Initial comparisons of predictions of a time-homogeneous two-stage model (assuming constant birth, death, and mutation rates associated with dose) with the papilloma data indicated both that the second mutation was necessary to papilloma formation and that the birth and mutation rates were approximately linear in dose.

The non-homogeneous (rates that vary with time) two-stage model presented here, in which we allow the birth and mutation rates within each dose group to depend on predicted TCDD concentrations in the skin over time, provided the best fits to the skin painting data. To determine if there was evidence of both a mutation and a birth effect of TCDD, we investigated several variations of the model. Skin Model 1 is the full model in which we use maximum likelihood techniques to obtain estimates of all model parameters. In Skin Model 2 we force no birth effect by setting $\beta_1 = 0$ (the parameter linking birth rates to tissue concentration) and estimating only the remaining parameter values. Similarly, in Skin Model 3 we force no mutation effect by setting $\mu_1 = 0$ (the parameter linking mutation rates to tissue concentration). Table 1 lists the values of the parameters for each model type which provided the best fits to the data as well as the resulting loglikelihood. Likelihood ratio tests provide evidence for a TCDD-induced mutation effect ($p<0.01$ for no mutation effect), but not of a birth effect ($p>0.2$). Note that in all cases, the death rate was restricted to be less than 0.3 deaths per cell per day and the resulting estimate fell on the boundary.

Table 1: Two-Stage Model Parameter Fits to Papilloma Data from Dermal Study

Skin Model	β_0	β_1	μ_0	μ_1	δ	*Loglike*
1	.4573	.0007	0	.1175	.3	1622.83
2	.4577	0	0	.1175	.3	1622.82
3	.3671	.3197	.0516	0	.3	1072.66

Oral Study

The non-homogeneous two-stage model also was applied to the papilloma data obtained from the oral study. Again, we investigated several variations of the model. Oral Model 1, the direct application of the parameters obtained from Skin Model 1 to the oral data, resulted in a very poor visual fit to the data. In Oral Model 2 we allowed all model parameters to vary in order to obtain the best fit to the oral data. Finally, in Oral Model 3 we used the birth and death rate parameters obtained from the skin painting study and just fit the mutation parameters to the oral data. The resulting parameter values and loglikelihoods are given in Table 2. It is interesting to note that the best fit to the oral data was achieved with a death rate of zero. Furthermore, the birth rate which best fit the oral data, while certainly much smaller than that obtained from the skin painting study, shows a greater dependence on the predicted concentrations of TCDD in the skin over time. Here, the likelihood ratio test argues that, as a whole, the skin painting parameters statistically fail to fit the oral data ($p<0.01$); however, when using only birth rates from the skin painting study, the resulting fits do not differ significantly ($p>0.3$) from those achieved by fitting the parameters directly to the oral data.

Table 2: Two-Stage Model Parameter Fits to Papilloma Data from Oral Study

Oral Model	β_0	β_1	μ_0	μ_1	δ	*Loglike*
1	.4573	.0007	0	.1175	.3	-442.6
2	.0909	.1106	.0022	.015	0	-122.497
3	.4573	.0007	.0002	.0085	.3	-133.19

It is not clear why the birth rate of papillomas in the skin painting study appeared to be unaffected by TCDD exposure. Current understandings of the effects of TCDD on tissues would argue for a clear effect on the birth rate. A previous analysis of TCDD-induced lesions in the liver[5] described a similar finding. Several possibilities exist including improper model specification and activation of processes in the skin which could result in an increase in mutations independent of TCDD. For example, this could be due to enzyme induction as has been hypothesized for the role of p-450's in liver cancer in female rats.

While the two-stage model applied to these data does a fairly good job of fitting, there are mechanistic aspects of this model which fail to describe properly the biology of the skin. The simple, linear birth-death process used in this analysis does not consider the controlled growth which occurs in the skin. A more appropriate model will need to consider a more carefully defined process which properly accounts for the multi-layered structure of the skin and the programmed cell death which occurs as skin cells move from the basal layer to the dermis. In addition, nonlinear relationships between tissue concentration and the parameters of the two-stage model were not considered in this analysis and should be in later evaluations.

References

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