TUMOR PROMOTION, GROWTH FACTORS AND DIOXIN RISK ASSESSMENTS

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ABSTRACT: In many cases hepatic tumor promotion by exogenously administered chemicals appears to be related to an anti-proliferative selection environment associated with prolonged mitogenic stimulation. In this paper, a stochastic simulation-based clone growth model has been used to analyze the U-shaped promotion curves for dioxin and other hepatic promoters based on the assumption of an anti-proliferative mechanism of action. These U-shaped curves were reproduced with a two-cell model of promotion: the growth of one initiated cell type is repressed by the anti-proliferative environment at low dioxin doses; the growth of the second cell type, refractory to mito-suppression, is stimulated at higher doses of dioxin. The biological basis of the U-shaped curve may be related to stimulation of negative growth factors, such as transforming growth factor beta1 (TGF-β1), in liver by these promoters.

INTRODUCTION: Dioxin is a tumor promoter in rat liver and mouse skin model systems. The current cancer risk assessment process for dioxin and for other liver tumor promoters could be made more biologically-realistic by taking into consideration emerging knowledge of the molecular and cellular events associated with treatment with these promoters. In this paper we note some of the attributes of liver promotion with phenobarbital - PB [1] - and the dose-response curves for promotion with both PB and dioxin [2]. We then examine these curves with a preliminary form of a stochastic, clone growth simulation model [3,4] and make use of our modeling results to propose incorporating mechanistic insights about tumor promotion into the dioxin risk assessment both for liver cancer and for certain non-cancer endpoints.

Growth Factors and Tumor Promotion: Both stimulatory and inhibitory growth factors influence the cell cycle. Transforming growth factor alpha (TGF-α) and hepatocyte growth factor cause liver cells to progress from a resting state (G0) to the G1 portion of the cell cycle. Other factors, such as TGF-β1, inhibit the passage from G1 to S phase - the DNA synthesis phase of the cell cycle. With PB, Jirtle and co-workers [1] developed a hypothesis for hepatic

promotion that focused on the role of inhibitory growth factors. They found that PB initially increases DNA synthesis, primarily in pericentral portions of the liver lobule. On continuous PB exposure, the liver response to mitogens was markedly suppressed. There were decreased levels of EGFR (epidermal growth factor receptor) which binds TGF-α and increased periportal TGF-β1. In addition, preneoplastic lesions had considerably less TGF-β1 than the rest of the liver. TGF-β1, a potent negative growth factor in the liver, induces programmed cell death (apoptosis) in both cultured hepatocytes and in regressing livers [5].

These observations led to the following general hypothesis: promoters induce cell proliferation; in tissues in a mature animal, proliferation cannot be sustained and the organism expresses increased levels of inhibitory growth factors to restrain cell proliferation; the anti-proliferative environment provides selection pressure for the growth of initiated cells that are relatively resistant to the effects of TGF-β1; these specific cells then grow out into clones from which fully malignant tumors can arise. There is limited evidence that a similar mechanism of promotion may be operative with dioxin, which causes decreased hepatic EGFR[6] and appears to reduce cell proliferation throughout the liver at low doses while enhancing proliferation within the pericentral lobule [7].

Dose-Response Behaviors for Promoters: With both dioxin and PB, dose response curves for promotion, measured as foci number per volume or volume of foci per liver, were U-shaped [2]. Low doses decrease promotion compared to initiated animals receiving no promoter treatment or to rats receiving higher doses of the promoter. Other hepatic promoters, including ethinyl estradiol [8], are reported to decrease cell proliferation in the liver on chronic treatment, consistent with a role of growth suppression in this organ during promotion.

In developing a model for promotion, we assumed that dioxin, and perhaps most hepatic promoters, act in a biphasic manner. Initial proliferative stimuli associated with chemical exposure induce a secondary response (i.e., a homeostatic response) to suppress further proliferation and restrain growth of the liver. The anti-proliferative environment selects initiated cells resistant to inhibitory growth factors. Growth of these altered cells are then 'stimulated' at higher concentrations of the promoters. This general hypothesis is not new. A mathematical model of promotion as an escape from mitotic inhibition was previously discussed [9].

STOCHASTIC SIMULATION MODELING OF PROMOTION: We analyzed these U-shaped dose-response curves for dioxin promotion with a preliminary version of a stochastic clone growth simulation model [3,4]. In these simulations, initiated cells are formed only by the treatment with diethylnitrosamine (DEN) followed by the 70% partial hepatectomy (PH). (No clones are found in the animals treated with dioxin alone.) In the initiated cells, birth (α1) and death rates (β1) are constant over time but are affected by dioxin concentrations. Clones are observable when they contain 24 cells. Estimating the volume of liver occupied by foci and the

number of foci per volume constrained the possible values of $\alpha 1$ and $\beta 1$, and of N_0, the number of initiated cells formed by the DEN/PH.

Model 1: In the simplest simulation model for these U-shaped curves, only one type of initiated cell is assumed to be produced by the DEN/PH-treatment and $\alpha 1$ and $\beta 1$ for that single cell are differentially altered by dioxin. Cell death rates are affected at lower concentrations than cell birth rates, but the overall increase in birth rate is larger than the increase in death rate. To model the dioxin data there have to be enough initiated cells formed to give the maximum number of foci observed in the highest dioxin dose group at six months (i.e., 9200/liver). Most of these clones either become extinct or never reach observable size at the lower doses; only 2800 foci/liver are found at 0.001 ug/kg/day. This model was parameterized by fixing the mutation rate to give the number of foci observed at high doses (i.e., N_0). To simulate the lower doses, ($\alpha 1 - \beta 1$) was adjusted to give the correct volume fraction, and $\alpha 1$ and $\beta 1$ were altered together to predict the number of clones observed. This model works well only with unrealistically high values of birth and death rates for the initiated cells (i.e., about 0.5 divisions/cell/day).

Model 2: In the next level of model complexity there are two different initiated cell types. The first cell type responds to inhibitory growth factors with an increased death rate as dioxin concentration is increased. It is the precursor cell for most clones observed in control rats (+DEN; 0 ug/kg/day dioxin). These cells respond to the anti-proliferative environment with increased death rate and contribute fewer and fewer clones as dioxin dose is increased. The second cell type is not responsive to the anti-proliferative environment, is stimulated to divide at higher doses of dioxin, and grows out to observable clones as dioxin dose is increased. This model accounts for U-shaped dose response curves with more reasonable growth parameters for these initiated cells. The process of stochastic model building for dioxin hepatic promotion will be improved as direct measurements become available for the birth rate of cells in foci and on the H&E staining characteristics of the foci at various doses.

The two cell type model suggest different cells of origin for the foci observed at low versus high doses. Altered hepatic foci produced by PB have different H&E staining characteristics compared to AHF in control rats [10]. With PB there is a large increase in pale pink eosinophilic foci in treated rats. It remains to be seen whether predictions of the 2 cell type stochastic clone growth models will find a direct experimental analog in different types of H&E staining for clones at different dioxin doses.

CONCLUSIONS: Dioxin risk assessments must eventually focus on the conditions under which dioxin, via effects on growth factors, alters cells growth and differentiation, leading to frank toxic effects. To date, homeostatic mechanisms in affected tissues have been largely ignored in risk assessment strategies. These secondary, anti-proliferative responses of normal cells to continuous mitotic stimulation may be crucial in determining the shape of the dose-response curves for endpoints important for risk assessments. We argue here that this may be

the case with hepatic promotion where an anti-proliferative environment may restrict growth of some initiated cells but select for growth of specific mutant cells, unresponsive to the anti-proliferative environment. These U-shaped curves for hepatic promotion may well have a basis in cell biology and create highly non-linear regions in dose-response curves.

Homeostatic processes suggested to play a role in tumor promotion might also be involved in other endpoints. Chronic mitogenic stimuli could desensitize other cell types to proliferative stimuli. Other adverse effects could ensue if desensitization occurred in cells that respond to mitogenic stimuli as part of their normal function. This desensitization might play a role in immunotoxicity (decreased responsiveness to cytokines) or impaired sexual development in utero (decreased responsiveness of the developing fetus to sex hormones). In light of the increasing emphasis on these other endpoints in the current dioxin risk assessment initiatives, it appears timely to focus attention on the possible commonality of mechanism that might underlie a variety of endpoints, a common basis captured in this working hypothesis for hepatic promotion by these various chemicals.

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