SYMPOSIUM PRESENTATION

An Enhanced Thirteen-Week Bioassay as an Alternative for Screening for Carcinogenesis Factors

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Abstract

Utilizing basic concepts of chemical carcinogenesis and the human relevance framework based on mode of action analysis of animal carcinogens, an alternative is proposed for the two-year bioassay for screening chemicals for potential carcinogenic risk in humans. This model includes short-term screening of chemicals for DNA reactivity, immunosuppressive and, estrogenic activity, and potential increased cell proliferation. Follow-up studies can provide detailed information with regard to dose response and mode of action, with a detailed evaluation of potential relevance to humans. It is no longer appropriate to continue performing two year rodent bioassays.

Key Words: Two-year bioassay - carcinogenesis screening - immunosuppression - estrogen - cell proliferation

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Introduction

Society has considerable interest in screening chemicals for possible carcinogenic activity. The emphasis is whether a chemical has carcinogenic activity in humans, but testing for this has been relegated to a screening process involving two-year bioassays in rodents, usually rats and mice (Cohen, 2004). Others have delineated the numerous difficulties with this bioassay, including excessive cost, time, and high doses, but most importantly is the increasing awareness that many of the results in the two year bioassay do not have relevance to human cancer risk. This lack of relevance to human risk can be due to the excessive doses used in the rodent bioassay, or, increasingly common is the observation that the mode of action involved in producing tumors in rodents is not qualitatively or quantitatively relevant to humans.

Carcinogenesis and Human Relevance

Any animal model of human disease makes two basic assumptions (Greenfield et al., 1984; Cohen and Ellwein, 1991; Cohen, 1998a): 1) the doses used in the animal studies are relevant to the exposures to which humans are exposed (dose extrapolation); and 2) the results in animal studies are relevant to humans (interspecies extrapolation). It has become apparent that the induction of tumors involves precursor lesions or processes which can be identified in a shorter time than is required for the malignant lesion to develop. This has been captured in the formulation of the human relevance framework based on the animal mode of action analysis that was developed initially by ILSI/RSI, sponsored by the US EPA and Health Canada (Cohen et al., 2003; Meek et al., 2003). This framework has more recently been extended on a global basis by the WHO International Programme on Chemical Safety (IPCS) (Boobis et al., 2006). Although this framework was originally developed to address mode of action for non-DNA reactive carcinogens, it has been extended to include DNA reactive carcinogens (Cohen et al., 2006) as well as other toxicologic endpoints in addition to cancer (Seed et al., 2005). The cancer framework is based on the identification of these precursor changes, with a more rational, scientific, mechanistically based assay possible for screening chemicals for potential carcinogenic activity in humans. The emphasis must be on identifying possible risk in humans; the results in the two-year rodent bioassay are not an acceptable standard by which to evaluate such a proposal.

Fundamentally, an increased cancer risk can be produced by a chemical by directly damaging DNA (DNA reactivity) or by increasing the number of DNA replications in the pluriopotent (stem) cells of the target tissue (Greenfield et al., 1984). DNA reactivity can be evaluated on a weight of evidence basis utilizing structure activity relationships, in vitro assays such as the Ames test, or in vivo analysis such as DNA binding. Evidence of increased cell proliferation can be demonstrated frequently on the basis of histopathological evaluation, but occasionally requires screening for increased DNA synthesis utilizing
a label such as bromodeoxyuridine (BrdU), PCNA, or Ki-67. For agents which are DNA reactive the potential target tissues can be identified utilizing an evaluation for increased cell proliferation and/or cytotoxicity, since DNA reactive carcinogens are usually cytotoxic in the target tissue at high concentrations (Cohen and Ellwein, 1990). For non-DNA reactive carcinogens, increased cell proliferation is the underlying and ultimate mode of action which leads to an increase in DNA errors resulting from spontaneous mistakes occurring during DNA replication (Cohen, 1998a). An increase in cell proliferation can occur by either an increase in cell births (mitogenesis or cytotoxicity with consequent regeneration, or by inhibiting cell deaths (apoptosis or cell differentiation) leading to an accumulation of the proposed number of target cells (Cohen, 1998a).

**Thirteen-Week Bioassay**

The proposed screening process is envisioned as a two step process (Cohen, 2004). The first involves a general screening for any potential activity in any target tissue, whereas the second is a more detailed evaluation of the specific tissues identified by this screening procedure to determine detailed dose response and mode of action. The aim of this evaluation is to examine possible relevance to humans as well as establish margins of exposure. Obviously, if the mode of action is relevant to humans, some data regarding possible human exposure levels need to be known to establish safety margins.

In addition to DNA reactivity, two other properties of chemicals are significant indicators of potential carcinogenic activity in humans: 1) immunosuppression (Cohen et al., 1991); and 2) estrogenic activity (Cohen, 2004). These can be readily screened utilizing in vitro and/or in vivo methods. For other targets, specific indicators need to be established to evaluate possible activity in the tissue, such as cytotoxicity, hyperplasia, increased organ weight, histopathologic indicators of toxicity and regeneration or other features.

**Bladder Carcinogens**

For example, there are numerous chemicals known to increase the risk of bladder cancer in humans (Cohen et al., 2000). In addition, numerous other chemicals have been identified as urinary bladder carcinogens in rodent bioassays, including some of which have DNA reactivity as well as others that are not DNA reactive (Cohen, 1998b). All known human bladder carcinogens produce increased urothelial proliferation when administered at high doses to rats and/or mice.

In screening for potential urinary bladder carcinogens in a thirteen-week screening assay, routine histology is not always adequate for detecting chemicals which produce urothelial cancer in rodents in a full two-year bioassay. However, if a labeling index assay (BrdU, PCNA, or Ki-67) is added to the screening battery after 13 weeks (frequently less time), all rodent bladder carcinogens can be detected.

Once the urinary bladder has been identified as a potential target, a second, short term assay, usually less than thirteen-weeks, can be performed utilizing multiple doses at and below the maximum tolerated dose (MTD), including detailed examination of the urine and urothelium. The urine needs to be examined with a focus on the urothelium rather than the kidney, including NOT fasting the animals and collecting fresh void urine specimens at a specific time of day, depending on circumstances. The details of urine and urinary bladder collection and processing have been presented elsewhere (Cohen et al., 2007). Based on these observations concerning non-DNA reactive bladder carcinogens, there are only a limited number of modes of action that lead to bladder cancer (Cohen, 1998b). These include mitogenesis, of which there is only one example (Propoxur), or cytotoxicity and regenerative proliferation. Cytotoxicity can be produced by either formation of urinary solids (precipitate, crystals, and/or calculi) or by generation of a cytotoxic metabolite in the urine (sometimes this is the parent, administered chemical). There is also some evidence that striking alterations in urine composition, such as extremes of pH, can also be cytotoxic to the rodent urine. Once the mode of action and dose response is identified, further detailed mechanistic information can also be evaluated. However, with the mode of action and detailed dose response information available, an evaluation utilizing the human relevance framework can be performed. Potential risk of carcinogenesis to humans can be assessed with this information without having to resort to a complete two-year bioassay.

**Summary and Conclusion**

During the past fifty years or more we have learned much about the carcinogenic process in animal models and in humans (Cohen, 2004). Increasingly, it has become evident that there are certain lesions that are unique to rodents that do not have relevance to humans, such as the splenic leukemia that occurs in F344 rats or the urinary bladder submucosal mesenchymal lesion in mice. Furthermore, there are tissues in rodents that do not have a counterpart in humans, such as Zymbal’s gland, the Harderian gland, and forestomach. In addition, except for estrogenic activity, the relationship of endocrine changes in rodents to carcinogenesis appears to have little relevance to human cancer risk. This has been studied extensively in the thyroid (IARC Working Group, 1999). Short term studies demonstrating toxicity in endocrine tissues continue to be important, but these are not predictors of carcinogenic activity in humans in these tissues.

An additional difficulty with rodent models has been the paucity of chemicals that produce cancer in those tissues for which cancer occurs commonly in humans, such as the glandular stomach, large intestine, pancreas, or prostate. Given these circumstances, the specific tissues that need to be examined for potential carcinogenic activity in the rodent models are actually quite limited. Furthermore, there is a growing body of evidence that screening for carcinogenic activity in rats is sufficient; evaluation in mice does not add significantly to the overall
assessment of risk.

Based on evolving knowledge of carcinogenesis, it is no longer appropriate or necessary to continue performing two-year bioassays to screen chemicals for carcinogenic risk in humans. Extrapolating from two or three doses in a two-year rodent bioassay giving a positive result to humans is no longer an appropriate approach to cancer risk assessment. Short term screens involving detailed dose response and mechanistic examination provide much more useful information that is scientifically based.

References