Is Saccharin Safe? Animal Testing Revisited

Joanne Zurlo, Robert A. Squire*

The ability to predict harmful effects of chemicals, drugs, and food additives has historically depended on the process of extrapolating from animal studies. The current guidelines of the U.S. Food and Drug Administration recommend nine animal-based studies to assess the safety of additives used directly in food handling or preparation or at the table in amounts in excess of 1 part per million in the diet. With respect to the controversial food additive saccharin, there remains an unresolved debate about its future as a commercial product, despite a seemingly exhaustive list of studies that have been completed to date. The article by Takayama et al. (1) in this issue of the Journal gives us further pause and forces us to think about our present use of animal tests for risk assessment.

The issue of the carcinogenicity of saccharin has been the subject of numerous animal and epidemiologic studies. Results from standard long-term rodent carcinogenicity tests indicate that sodium saccharin is a weak carcinogen and tumor promoter in the male rat bladder, particularly when it is administered in a two-stage, initiation–promotion protocol with specific bladder genotoxic carcinogens such as N-(4-hydroxybutyl)-N-nitrosamine and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (2). Results in female rats and in mice have been equivocal (3), and several hypotheses have been presented for the specificity of saccharin induction of bladder, causing irritation, hyperplasia, and ultimately tumors.

In the absence of any conclusive epidemiologic data that saccharin is associated with increased tumors in humans, it has been argued that the mechanism for saccharin induction of bladder tumors in rats is specific to the physiology of the male rat bladder and that saccharin should be taken off the National Toxicology Program’s list of agents that are “reasonably anticipated to be carcinogen[s].” Thus, given these unique circumstances for this particular carcinogen, it might also be argued that the rodent bioassays of saccharin were irrelevant for human studies. Indeed, the practice of giving animals the highest possible amount of the substance under study—the maximum tolerated dose—long a requirement for such bioassays, has raised questions about the relevance of rodent bioassays in general (4).

The study presented in this issue of the Journal by Takayama et al. (1) was undertaken “to determine the effects of long-term feeding of sodium saccharin to three species of nonhuman primates.” No effects were found. Sodium saccharin did not cause increased urothelial cell proliferation, bladder tumors, or the appearance of large crystals in the urine of the monkeys. How should/can these data be interpreted? Is the evidence persuasive that sodium saccharin is not carcinogenic for humans because it was without effect in nonhuman primates?

One must look at this study carefully before judging whether it warrants primacy in the assessment of human risk from sodium saccharin. This study was begun in 1970, before any information had been elucidated about the mechanism of saccharin-induced bladder carcinogenesis in the rodent. We now recognize that, under the conditions of the experiment, it is unlikely this crystallization phenomenon would have been observed; i.e., the dose of sodium saccharin was insufficient, and there are important differences between the composition of primate urine and rodent urine. Moreover, the small number of animals, the multiplicity of species, and the low doses of saccharin greatly reduced the sensitivity of the study to subtle effects. With the benefit of hindsight, one may question whether there was a high probability that compelling information could have been gleaned from this study. The level of precision in the data, given the multiplicity of species and the small number of animals, was less than optimal, limiting the ability to extrapolate the results to humans.

This latter issue also brings to mind how approaches to animal tests have changed over the last few decades since the beginning of this study. For example, the “three Rs” have become more of a driving force. The three Rs were first described in a book by Russell and Burch published in 1959 (5) and are defined as methods that refine existing animal protocols by minimizing pain and distress, reduce animal usage to the minimum necessary to produce statistically significant data, or replace whole animal methods. In a recent article by Balls et al. (6), the concepts originally presented by Russell and Burch were reaffirmed, and numerous recommendations were put forth. The concept of reduction includes increasing the level of precision, given the number of animals, as well as optimizing experimental design and statistical considerations. Adherence to these prin-

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Policies would likely lead to a different study design if the study by Takayama et al. were initiated today.

In light of the apparent lack of human relevance of the rodent data on saccharin carcinogenicity and the impracticality of long-term, large-scale studies involving nonhuman primates, in what direction should we be moving to revise our current methods for risk assessment? Two-year carcinogenicity bioassays in rodents may be relevant as long as positive findings do not result in knee-jerk extrapolations to humans before evidence is available that similar mechanisms are likely to be operative at human exposure levels. Bioassays in presumably more relevant species, i.e., nonhuman primates, are more expensive and impractical because, once again, one cannot use large enough numbers and because of public sensitivity to the use of animals, particularly primates. Neither of these choices is ideal; yet the need to adequately test for safety and efficacy remains compelling.

More effort must be devoted to developing better methods of risk assessment, in essence a new paradigm possibly relying on extrapolation from mechanistic data from in vitro studies on human tissues as well as from tiered, mechanistic strategies in animals. Noninvasive in vivo human studies, e.g., those designed to look at biomarkers, can also shed light on exposure and mechanisms of toxicity in humans. Better mechanistically based in vitro tests using cells and tissues from animals must also be designed and conducted side by side with in vivo tests on human tissues to ascertain interspecies variabilities early, before long-term expensive bioassays are conducted.

The U.S. Food and Drug Administration has been very forward in its thinking and has established a Subcommittee on Toxicology as part of its Science Board to make recommendations on revising current animal testing strategies that address some of these concepts. Similarly, the National Institute of Environmental Health Sciences has taken the lead in the establishment of the Interagency Coordinating Committee on the Validation of Alternative Methods. One would hope that the time has come to identify our basic science needs and to apply these data to risk assessment.

References


Note

R. A. Squire was the Head of the Tumor Pathology Section and Director of the Carcinogenesis Testing Program at the National Cancer Institute, Bethesda, MD, 1973–1977.

Psychologic Stress, Immunity, and Cancer

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Why publish an article in a major cancer journal that demonstrates an association between psychologic stress and cellular immune function in cancer patients? The potential interest in articles such as Andersen et al. (1), published in this issue of the Journal, is based on the premise that stress may alter the function of the immune system in a manner that influences the development or growth of malignant tissue. This premise is quite controversial and we use this editorial to discuss its underlying assumptions.

1) Psychologic stress can alter immune function.

There is evidence for a number of mechanisms through which psychologic stress might alter immune function (2). These include direct innervation of lymphatic tissue by the central nervous system and stress-elicted release of hormones from the brain that bind to and alter the functions of immunologically active cells. The mechanisms also include behavioral changes that often occur in response to stress: an increase in smoking, an increase in drinking alcohol, a loss of sleep, a reduction in exercise, a degradation of the diet, and a decrease in adherence to medical regimens.

In fact, healthy humans exposed to stressful tasks that last only a few minutes, including difficult cognitive tasks and tasks that induce social anxiety, show suppression of T-cell mitogenesis, and increased numbers of circulating CD8 and natural killer cells (3). Studies of real-life stressors show similar alterations. Living near the Three Mile Island nuclear power plant at the time of the accident, caretaking for a relative with Alzheimer’s disease, taking medical school examinations, and clinical depression have all been associated with alterations in both the numbers and functions of various subpopulations of lymphocytes (4). These alterations include a reduced proliferative response to mitogen stimulation, reduced natural killer cell cytotoxicity, as well as changes in the production of cytokines. Although the range of stress’ effects on immune function is

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