The Two-Year Rodent Carcinogenesis Bioassay — Will It Survive?

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Abstract: For over 35 years, many synthetic and natural chemicals have been tested by government agencies, private companies and research institutes for carcinogenic activity in rats and mice in classical 2 year studies as part of a toxicity profile ultimately used for human toxicity and carcinogenicity risk assessment. With an increasing number of pharmaceutical and agricultural chemicals shown to be carcinogenic in these bioassays, research into the mechanisms of toxicity and carcinogenesis has intensified. The relevance of the induced tumors in rodents has been questioned after much research. Research has provided evidence to some scientists that doses used in the bioassays may represent situations where toxicity pathways do not develop in humans exposed to levels of these chemicals, toxicity itself may create situations where tumors develop only under those situations, species specific responses may exist, and tumors induced may not be relevant to human risk. Regulatory agencies have considered these and other factors when preparing regulatory decisions on regulation of these chemicals. Thus, the USA FDA often has approved drugs despite their carcinogenicity in rodents and the USA EPA has explored many situations where considerations of the mechanisms of carcinogenesis in rodents and humans play a role in their regulatory decisions. Unfortunately, much of the decisions are based on unproven and hypothetical mechanisms of carcinogenesis in rodents and humans. Despite this situation, the impact of these decisions on future considerations and decisions for regulation of chemicals suggests that the US regulatory agencies consider that the occurrence of increased incidences of tumors in standard 2 year rodent carcinogenesis bioassay is often not relevant to human carcinogenesis risk assessment.

Key words: bioassay, carcinogenesis, rodents, toxicology

Introduction

Natural and manmade chemicals have been evaluated for their toxicity in rats and mice for over 40 years in order to provide information to be used for estimating risks to humans. Of particular popularity has been the 2 year carcinogenesis bioassay. It has always been assumed that the acute and chronic toxicity of a specific chemical in rodents has relevance to the risk of acute and chronic toxicity and carcinogenesis in humans. With the discovery of the toxicity of chemicals found naturally in the environment and the growth of the pharmaceutical industry during the past 20 years, it has become evident that there are numerous toxins and rodent carcinogens that humans are exposed to on a daily basis. The relevance of the carcinogenicity findings in rats and mice to human risk has been investigated, reviewed and challenged in many publications by individuals, institutions and scientific committees during the past ten years. The federal regulatory agencies in the United States and other countries have responded. Numerous drugs that have caused tumors in rodents in 2 year carcinogenesis bioassays are on the market, approved for use by the regulatory agency responsible for assessing efficacy and safety. The EPA evaluates mechanism of carcinogenesis of environmental agents prior to approving their use. This review will present the major issues involved.

History of the Bioassay

Chemicals in our environment include pharmaceutical agents, food additives, industrial intermediates, agricultural chemicals, natural endogenous and exogenous chemicals and air pollutants. Most are potentially toxic to living creatures including wild animals, insects, laboratory rodents, domesticated animals and humans. Since Percivall Pott showed that chimney soot was associated with scrotal cancer and Yamigiwa & Ichikawa first showed that coal tar could cause skin cancer in rabbits, it was assumed that chemicals that humans are exposed to may lead to toxic and carcinogenic hazards. Thus, the various federal
government regulatory agencies developed rules and regulations for chemicals that humans may be exposed to at work, in the air, at home, in food and medications. Much of these regulations accelerated in the 1970s when agricultural chemicals were found to be carcinogenic in rodents. At first simple bioassays were developed which exposed rats or mice to a test chemical in the diet, by gavage or by inhalation for up to 2 years. Chemicals were given usually at 2 doses and controls were used. The highest doses were at a maximally tolerated level, which was subsequently defined as a dose which would produce not more than 10–15% depression in body weight gain, early illness or death due to the chemical. The largest efforts in using, developing and improving these bioassays were first done by The US National Cancer Institute in the 1970s until the formation of The National Toxicology Program under the leadership of the National Institute of Environmental Health Sciences in the late 1970s. Since that time, various guidelines have been developed by regulatory agencies and have evolved for use in requirements for carcinogenesis testing in the USA and other countries (The US FDA Redbook; the US EPA guidelines for carcinogenicity risk assessment, http://epa.gov/iris/cancer032505.pdf; European and international guidelines, http://www.emea.europa.eu/htms/human/ich/ichsafety.htm). Some old concepts still exist, however, such as The Delaney Clause of the Federal Food Drug and Cosmetic Act of 1958 which states that no additive will “be deemed safe if it is found to induce cancer when ingested by man or animal.” Advances in using rodents for human carcinogenesis risk assessment have been suggested.

Since that time, over 500 chemicals were tested for toxicity and carcinogenicity by the US government and probably over 1500 chemicals by various sponsors throughout the world. The International Agency for Research in Cancer (IARC) and its expert committees have reviewed data on over 900 agents (http://monographs.iarc.fr/). This large amount of accumulated data has led to questions concerning the validity of the standard 2 year bioassay for uses in human risk assessment. Most recently, modifications in standard procedures have been suggested and used by many interested parties.

The Rodent Carcinogens and Pathology of Rodent Tumors

Over the past 50 years, many chemicals (probably thousands) have been shown to cause tumors in rodents with laboratory experiments (by oral administration, injection of newborns or adults, inhalation, or gavage) of short or long term even up to the lifetime of the animals. These studies have induced tumors in various tissues most commonly in liver, lung, skin and mammary gland. Naturally occurring tumors of rats and mice occur in a sex, age and strain or stock dependent manner. Those of the most common strains and stocks of mice (CD-1, B6C3F1) and rats (F344, Sprague-Dawley) used in toxicity have been characterized as to incidence and pathology including natural history. Rodents do not commonly develop spontaneous tumors most prevalent in humans including those of the colon and prostate, in part, due to differences in genetics, diet, specific natural chemical exposures and infectious agents.

Naturally occurring and induced tumors of rats and mice progress through a sequence of histomorphologic and when known, molecular events. In epithelial tissues the first visible evidence of neoplastic progression is a focal proliferative lesion followed by focal nodular lesions and benign neoplasia. Benign neoplasia can progress to malignant neoplasia, especially in skin, liver, and lung. Epithelial lesions lining tubular organs often develop dysplastic lesions which can progress to malignancy. The rodents lesions are not unlike those observed in humans except for the more common benign tumors progressing to malignancy in rodents. Also, some normal rodent tissues (especially mammary gland and prostate) and rodent tumors often have less stroma than do their human counterparts. Some scientists have suggested over the years that rodent tumors were, in fact, not tumors but lesions of hyperplasia and reactivity to toxic damage of an organ.

The Human Carcinogens

Many chemicals or processes, viruses, bacteria, parasites, and irradiation have been associated with increased cancer risk in humans, as studied by epidemiological investigations (http://monographs.iarc.fr/ENG/Classification/index.php, http://ntp-server.niehs.nih.gov/ntpweb). Most all of these chemicals or processes cause tumors in rats and mice. IARC reports that 400 agents have been identified as carcinogenic or potentially carcinogenic to humans (www.iarc.fr). Many of these chemicals, but not all of them, are multiple organ rodent carcinogens and most are genotoxic. Human cancer drugs carry increased risk for secondary cancers 10–30 years later (www.iarc.fr, IARC monographs, volumes 50 and 66).

Human Pharmaceutical Agents are Often Rodent Carcinogens

In light of the great expansion of the pharmaceutical industry over the past 30 years, many drugs have been developed for promoting human health. During this development, many prospective drugs have been found to be carcinogenic in 2 year rodent studies. Despite these findings, the drugs are often approved based on important efficacy in humans and risk/benefit analysis by FDA for use in humans, either as prescription drugs or over the counter. Many advisory committees in the USA provide guidance to the US regulatory agencies. For reasons that are not published, the regulatory agencies have approved for human use many drugs which cause tumors in rodent studies. Fortunately, most of these drugs are not genotoxic. Regulatory agencies use rules, regulations and guidelines for...
approval of new drugs for doses given to rodents in 2 year studies which have been many times above the normal human therapeutic doses. Rarely, the rodent carcinogenic dose is only 1–3 times the human therapeutic dose. Many rodent carcinogens are FDA-approved drugs, primarily prescription drugs. Some examples of commonly approved drugs which have been shown to cause rodent tumors in bioassays are shown in Tables 1 and 2. Some of the drugs are multiple organ carcinogens in rodents and a few studies included doses barely above the human therapeutics dose. The information on toxicity or carcinogenicity in rodents is often obtained only from product inserts rather than from published reports.

Mechanisms of Carcinogenesis in Rodents and Humans

Abundant research has provided evidence that chemicals may cause (induce) tumors in animals and humans by one of several general mechanisms, most commonly referred to as genotoxic and non-genotoxic. Also, more than one mechanism may be found for any target site. Genotoxic chemicals may cause tumors by directly or indirectly damaging DNA, and a genetic change that may eventually end in neoplasia. Genotoxic chemicals can also be toxic at high doses causing tissue damage that may also contribute to promotion of carcinogenesis, perhaps initiated by DNA damage. Nongenotoxic carcinogens are often given at high toxic doses to rats and mice causing chronic target organ toxic lesions which are suggested to be the cause of tumors found in these tissues after chemical exposure. Associated with chronic toxicity is often chronic regenerative lesions with enhanced cell proliferation and turnover. This finding has lead to hypotheses that the increased rate of cell proliferation and other mechanisms involved in chronic disease promote carcinogenesis in rodents and humans. Other suggested mechanisms in rodent liver include induction of receptors and enzymes, metabolic overload, hormone perturbation, and cytotoxicity. Variability and high incidences of tumors in controls have also been indicated as rodent bioassays problems. These nongenotoxic agents have been suggested to promote naturally occurring tumors, as well, perhaps in tissues with high spontaneous rates of tumors.
Yet, others have shown that many toxins cause chronic tissue damage sometimes with proven chronic cell proliferation that is not associated with increased carcinogenesis in humans and animals. The alpha 2u globulin hypothesis for male rats seems to be related to the low incidences of renal tumors induced; yet some scientists have shown that renal toxicity induced by these chemicals is sometimes not associated with renal carcinogenesis. Others have found that renal toxins often are not renal carcinogens in 2 year bioassays (J Ward, unpublished observations).

Other research has reported that the potential mechanism of target organ toxicity and/or carcinogenesis in rodents is not relevant to humans. These include tumors in many tissues including bone, liver, lung, forestomach, Harderian gland, hematopoietic tissues, kidney, liver, lung, oral cavity, nasal cavity, preputial and Zymbal’s glands, skin, testis, thyroid, urinary bladder, and reproductive systems (Table 3). Others have shown, however, that toxicity of drugs in humans and animals are concordant and that misconceptions have been reported concerning these postulated mechanisms.

The USA EPA proposed to classify thyroid carcinogens by possible mechanism (Hill). More recently, EPA cancer guidelines (http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=116283), shows guidelines that allows EPA to make regulatory decisions concerning potential modes of action of a chemical to cause tumors and its relevance to humans, even if all aspects of the evaluation are based on hypothetical and unproven modes of action. Yet, no one has proven that any chemical causes tumors, benign or malignant, specifically by any one or more of the mechanisms hypothesized. This phenomenon may be especially relevant when the postulated mode of action also occurs in studies where no target organ carcinogenesis is seen despite target organ toxicity.

The International Agency for Research in Cancer (IARC) has suggested that chemicals that cause tumors in rodents may do so by mechanisms that do not operate in humans (http://monographs.iarc.fr/ENG/Preamble/index.php).

An expert committee and other groups and individuals have recently suggested a human relevance framework for analyzing rodent carcinogenesis data. The analysis attempts to postulate a mode of action (MOA) for the rodent carcinogenesis of a specific chemical. After it is defined, an analysis of the MOA in human is assessed based on the weight of the evidence. For genotoxic agents, a comparative mechanism of carcinogenesis in rodents may be more plausible than for non-genotoxic agents at doses for which humans are exposed and for which no comparative toxic histopathologic lesions are induced in humans.

### Table 3. Example of Tumor Induction in Rodent Carcinogenesis Bioassays for Which the Postulated Mechanism of Carcinogenesis in Rodents is Suggested to be not Relevant to Humans

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Postulated Confounding Factor</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessels</td>
<td>Toxicity</td>
<td>2-butoxyethanol</td>
<td>77</td>
</tr>
<tr>
<td>Bone</td>
<td>Retrovirus</td>
<td>Chronic toxicity</td>
<td>63</td>
</tr>
<tr>
<td>Forestomach</td>
<td>Irritation</td>
<td>BHA</td>
<td>69, 70, 81</td>
</tr>
<tr>
<td>Harderian gland</td>
<td>Tissue not found in humans</td>
<td>Lyphomas</td>
<td>68</td>
</tr>
<tr>
<td>Hematopoietic system</td>
<td>High incidence in controls</td>
<td>Lymphomas</td>
<td>74, 75</td>
</tr>
<tr>
<td>Kidney</td>
<td>Alpha 2u globulin</td>
<td>Male specific toxicity, cell proliferation</td>
<td>49, 71, 72</td>
</tr>
<tr>
<td>Liver</td>
<td>Toxicity</td>
<td>Enzyme inducers, toxicity</td>
<td>39, 52, 65</td>
</tr>
<tr>
<td>Lung</td>
<td>Different tumor type than in humans</td>
<td>Chronic toxicity</td>
<td>68</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>Toxicity</td>
<td>Chronic toxicity</td>
<td>76</td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>Toxicity</td>
<td>Chronic toxicity</td>
<td>58</td>
</tr>
<tr>
<td>Preputial/Clitoral gland</td>
<td>Tissue not found in humans</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>Skin</td>
<td>Sensitive skin in inbred mice</td>
<td>Shaving skin</td>
<td>1</td>
</tr>
<tr>
<td>Testis</td>
<td>High incidence in controls</td>
<td>Endocrine</td>
<td>94</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Pituitary-thyroid axis</td>
<td>Rodent-specific endocrine effects</td>
<td>23, 71, 78</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Stones, inflammation</td>
<td>Chronic damage, cell proliferation</td>
<td>24, 71</td>
</tr>
<tr>
<td>Zymbal’s gland</td>
<td>Tissue not found in humans</td>
<td></td>
<td>68</td>
</tr>
</tbody>
</table>

### Alternatives to the 2 Year Rodent Carcinogenesis Bioassay

Scientists and others have offered many alternatives to rat and mouse toxicity tests including 2 year carcinogenesis bioassays (83, http://iccvam.niehs.nih.gov). These have included the use of no rodents (http://www.stopanimaltests.com/u-ntp.asp), in vitro cell transformation and other assays, mutagenesis tests, computerized prediction of carcinogenicity of a chemical based on structure and chemical class, use of the toxicity level (LD50) in rodents for estimating human carcinogenic risk, the neonatal mouse assay, 6 month genetically engineered mouse assays, medium term liver rat bioassays, and medium term multi-organ rat bioassays. Each assay has its advantages and disadvantages, perhaps as many as the 2 year assay itself. It is up to the federal regulatory agencies to determine if any of
these assays will replace the 2 year carcinogenesis bioassay, supplement the 2 year bioassays or not be considered for human risk assessment.

**Future Considerations**

Human epidemiological studies often require 20–30 years to establish whether a chemical can cause cancer in humans. Few such studies have been accomplished for present day pharmaceuticals\(^{82,93}\). Also, the popularity of specific drugs changes over the years and many are discontinued and replaced by more effective agents. For pesticides, low doses in food, water and the environment, make it difficult to establish any carcinogenic activity of these compounds in humans by epidemiological studies. Cigarette smoking and high industrial exposures provide much more convincing evidence of human carcinogenicity\(^{40}\). Thus, we may never know if drugs at therapeutic doses or pesticides at low environmental doses or other chemicals actually cause cancer in humans. The pool of potential carcinogenic agents to which we are exposed grows every year. Will it take an outbreak of a specific cancer 30 years after a drug was introduced to provide evidence of human carcinogenicity\(^{40}\)? Thus far, no such evidence has been found.

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