Journal of Drug Metabolism and Toxicology

Johnson, J Drug Metab Toxicol 2012, 3:6
DOI: 10.4172/2157-7609.1000e114

Editorial Open Access

Estimating Human Cancer Risk from Rodent Carcinogenicity Studies: The Changing Paradigm for Pharmaceuticals

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The current design of animal carcinogenicity studies required for new drug approval can be traced back to John and Elizabeth Weisburger's early work at NIH fifty years ago [1]. Specific evaluation procedures have changed over the years, but the concepts behind the studies still rely on high-dose exposure to the animals and default linear extrapolation procedures, with little consideration given to newer advances in the toxicological sciences [2]. Technological and scientific advances have been incorporated into shorter term and ancillary studies – most notably in the mouse – but drug approval criteria still rely on the decades old 2-year rat study design and evaluation procedures. For years, multiple proposals for changing the requirements have appeared in the literature and the topic has been debated extensively in scientific meetings and within FDA advisory committee and ICH Expert Working Group sessions. During the last decade the emphasis has been on incorporating a "weight-of-evidence" approach into the evaluation procedures and particularly the regulatory review process [3,4]. Key questions in this approach used to estimate human risk assessment from data in rat studies are highlighted below.

1. Is there weight of evidence sufficient to establish a mode of action (MOA) in animals?
2. Are key events in the animal MOA plausible in humans?
3. Is there previous demonstration of carcinogenic potential in the product class that is considered relevant to humans?
4. Is there a structure activity relationship that suggests a carcinogenic risk?
5. Is there evidence of pre-neoplastic lesions in repeated-dose toxicity studies?
6. Is there long-term retention of the parent compound or metabolite(s) resulting in local tissue reactions or other pathophysiological responses?
7. Does the benefit of the therapy justify the risk of cancer?
8. Is there a reasonable safety margin in drug exposure at dose levels in animals where no tumors were produced vs. the drug exposure expected in patients given therapeutic dosing regimens?

A close examination of these questions points out the difficulties with the existing system and justifies current discussions on bringing a 21st century mindset to resolving the issues [5]. Of importance in these discussions are two key questions (a) what evidence would eliminate the need for conducting a rat carcinogenicity study, and (b) if a study was considered to be necessary, what data would be developed before or during the study to augment the interpretation of the results? Considering the seven questions above, 3, 4, and 7 would be known or could be ascertained prior to conducting the rat study. Questions 5 and 6 could be answered in studies typically done before the initiation of the 2-year study. Questions 1, 2, and 8 would not answered until the study had finished and specific tumors were identified, which suggests that additional mechanistic studies would need to be carried out prior to an informed regulatory decision. This adds additional time to approval and ultimately adds uncertainty to future revenue estimates for the company developing the drug. Relying on methods that require more studies to be conducted after the rat carcinogenicity study finishes, regardless of the human relevance of the findings, would be counterproductive to the call for better predictive models to identify concerns much earlier in the research and development process. New approaches must not only reduce time and costs, but accelerate the relevant evaluation of potential human drug toxicities during the drug development process [5].

Background on 2-year Rat Studies

Companies developing drugs for longer term indications (6 months or more) have been required for decades to use rodent carcinogenicity studies to assess potential human cancer risk prior to drug approval. These studies are 2-year carcinogenicity bioassays in two species, typically the rat and mouse. Unless there are specific drug or product class reasons for earlier study conduct, these studies are usually the last piece of information to be included in a registration package. Carcinogenicity standards now in use were made official through the International Conference of Harmonization (ICH); the specific guideline, ICH S1A, was published in 1996. The Guideline states that causes of concern of specific compounds included previous demonstration of carcinogenic potential in the product class that is considered to be relevant to humans, structure-activity relationships suggesting carcinogenic risk, evidence of pre-neoplastic lesions in repeated dose toxicity studies, and long-term tissue retention of parent compound or metabolite(s) resulting in local tissue reactions and other pathophysiological responses. Note that all of these concerns from the 1996 ICH document are mentioned above in the weight-of-evidence evaluation approach currently in practice. Animal carcinogenicity studies are conducted with three drug dosage groups, low, mid, and high dose and one or two concurrent control groups. Setting the dose levels in the studies has had a long history with a comprehensive evaluation published by an ILSI Working Group in 2007 [6]. The concept of a Maximally Tolerated Dose (MTD) is still considered to be the appropriate method to set the high dose in these studies. Each group has 60-70 animals per sex and the statistical analysis of tumors now follows the FDA Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent

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Received November 14, 2012; **Accepted** November 16, 2012; **Published** November 19, 2012

Citation: Johnson DE (2012) Estimating Human Cancer Risk from Rodent Carcinogenicity Studies: The Changing Paradigm for Pharmaceuticals. J Drug Metab Toxicol 3:e114. doi:10.4172/2157-7609.1000e114

Carcinogenicity Studies of Pharmaceuticals published in May 2001. Each tumor in each tissue or organ system is analyzed individually by gender, with a standardized procedure used to combine certain tumors for statistical analysis. Common tumors are considered those with a background incidence of 1% or over based on the historical database of the laboratory conducting the study, whereas rare tumors are those with a background incidence below 1%. The historical database must represent studies conducted in the same facility within a reasonably recent timeframe to avoid genetic drift of background tumor incidence. The background incidence of various tumors can vary from each breeding colony and supplier and over time even with the same strain of animals and facility, which makes the data from the actual laboratory conducting the study important. In the analytical procedures, p values for rare and common tumors are evaluated for pair-wise significance at 0.05 (for rare) and 0.01 (for common). The use of more than one control group will sometimes alter the actual classification of rare *vs.* common for a specific tumor because there is general agreement in the scientific community that the concurrent control is the most relevant comparator for determining treatment-related effects in a study [7]. The rare *vs.* common classification is essentially an arbitrary tumor threshold (not a dose threshold) and an adjustment by specific tumor to the classification of rare to common can alter the outcome of tumor statistical evaluations. This not only has consequences in the evaluation of statistical significance, but also the establishment of the No Observed Effect Level (NOEL) for particular tumors and ultimately the extrapolation to human risk.

In 1997, an additional ICH Guideline was enacted (ICH S1B) that presented the option of substituting a shorter term (6-month) mouse study using certain transgenic models which have now been widely assessed with the most appropriate model widely accepted by regulatory bodies. These models, $p53^{+/-}$, Tg.AC, and TgHras2, have been evaluated by several international scientific working groups and consortia [5,6]. Currently, the TgHras2 model with 25 animals per group has become the study of choice for both genotoxic and non-genotoxic compounds and is generally considered acceptable for all systemically administered drugs [1]. Whereas the mouse alternative models have reduced the time, cost, and potentially the false negative and positive rates of the second species, the rat studies remain the current topic of concern. The studies typically take at least 3 years to conduct from start to finish, and this is after the initial studies that establish dose levels. Depending the specific design and route of administration the studies cost between $1 and $2 million US dollars. Of major concern with 2-year rat studies is the high rate of false positives as discussed later and the high background tumor incidences in control animals which are used to set the statistical criteria for evaluation. In addition as mentioned above, mechanistic data rarely emerges from the study endpoints. An additional concern is the estimate of safety margin extrapolated to potential human use, which is discussed below.

Issues with Safety Margins Estimated from Pharmacokinetics Data

When safety margins for animals *vs.* humans are estimated, the acceptable methods are the determination of multiples of the area under the dose-response curve (AUC) from toxicokinetic studies at doses where there is a NOEL for tumors (each tumor analyzed on its own) *vs.* efficacious AUCs in clinical trials. The animal pharmacokinetic parameters are developed from exposure levels in animals typically from satellite groups in studies where dosing is maximally 1-year in duration. This is because survival in 2-year studies starts to decline after 1 year and can reach close to 20% at 2 years. Therefore AUC values used for safety margin calculations typically are not representative of values potentially seen in the second half of a 2-year study. This is particularly problematic if a capacity-limited clearance mechanism is involved and accumulation of the "active" chemical species occurs but cannot be detected in the second year of a 2-year study. Also, non-genotoxic mechanisms in animals are considered to result in later developing tumors, such as in the second year of a study. There is little disagreement that safety margins based solely on a comparison of a single rodent animal species and human PK parameters will almost always be misleading because of the potential variation in dosing regimens and dosing vehicles in humans and potentially different metabolic processes and clearance mechanisms in rats. These are precisely the reasons why allometric scaling procedures were initially developed to create a more appropriate means of comparisons of PK data across species. The uncertainty factors mentioned above, which are always present, are the primary reasons safety margins for uncertainty (the 10 to 100-fold factors) are incorporated into the rat *vs.* human comparisons. As mentioned earlier, the dose selection and subsequent safety margin calculations rely on linear extrapolation models. Most drugs showing positive carcinogenicity findings in rodents appear to be associated with hormonal or immunosuppressive mechanisms or are initiated via exaggerated pharmacologic (or biologic) activity thought to be due to high doses used in the rodent bioassays. In these cases, neoplastic induction is thought to involve a threshold and long-term exposures below that threshold may be insufficient to increase carcinogenic risk. Even in cases with genotoxic carcinogens there is a growing body of opinion that thresholds unequivocally exist. Linear extrapolation models are conventionally the default based on the one hit, no threshold concept. The re-plotting of dose and response from a linear to a more rational dose on a logarithmic scale suggests a threshold for genotoxic carcinogens [8]. The much studied genotoxic carcinogens like 2-acetyaminofluorene and diethylnitrosamine have been re-examined critically and clear thresholds shown [9]. Moreover the dose response curves are non-linear with a lower threshold for DNA adducts than hepatocyte proliferation, pre-neoplastic foci and tumor development, the latter three having supra-linear dose response curves. The shape of dose response curves may be monotonic if the DNA adducts formed by the drug induce a repair capacity or delay the cell cycle [10]. The induced repair acts on both the exogenous and the background adducts, whereas, the delayed cell cycle lowers the chance of adducts being fixed as mutations in daughter cells. Calabrese [11] has suggested that in many cases dose response is a J or U-shaped curve and that hormesis should be the default dose-response model in risk assessment, which would be consistent with the majority of immunological mechanisms. In view of the challenge in establishing the most appropriate dose-response curve for the tumor in question, which is the relevant endpoint to be assessing, the default approach has been to only use the AUC or internal exposure for safety margin calculations. In addition, when a specific tumor NOEL is established, the dose level associated with no statistically significant tumors must be one that was actually used in the carcinogenicity study, not an extrapolated value from a curve. This requirement accentuates the issue of relevance since several clinical trial associated PK values are derived initially from population PK models. If the goal of safety margins is to actually extrapolate to human clinical trial AUCs, then the argument of using multiples of AUCs in clinical trials to set dose levels of 2-year rat studies would seem appropriate. However, the opposing argument has always been that rats and humans are different, so the rat must be viewed on it's own from a study design standpoint. This then characteristically assigns the rat study margin of safety to a fall-back position during the case-by-case regulatory review process.

Questions on Relevance of Rat Tumors *vs.* Human Cancer

The relevance of 2-year rat studies has been the subject of several differing opinions, particularly over the last 10 years, where several researchers have published papers on the lack of human relevance of specific cancers in rats [12-16]. The reality in drug development is that the majority of compounds that reach the late-stage of development where carcinogenicity studies would be started have been shown to be non-genotoxic in several genetic toxicity assays because genotoxic compounds will typically be dropped much earlier from development. The exceptions are those drugs being developed for cancer or other life-threatening conditions where benefit is thought to outweigh potential risk.

In reference to the case-by-case regulatory approach, approval of pharmaceuticals with positive rat carcinogenicity studies is not unheard of Sistare et al. [17] have reviewed records of several compounds in PhRMA files that are non-genotoxic concluding these compounds should not be subject to rat carcinogenicity studies if key information is known prior to the start of the rat carcinogenicity study because of the potential of generating misleading false positive findings. These key points are: (a) negative for genotoxicity, (b) lacks histopathologic risk factors for rat neoplasia in chronic rat toxicology studies, and (c) is negative in an alternative 6-month transgenic mouse carcinogenicity study. In addition, several other authors have written opinions on the human relevance of several tumors appearing in different rat carcinogenicity studies, all questioning the relevance in reference to risk-benefit considerations for specific medical indications [4,13,14,16,18-21]. Davies and Monro [19], looked at all approved drugs in the PDR in 1994 and found 101 positive in carcinogenicity studies. Of these, 22 were positive in rats only. Two of the most common sites were thyroid and adrenal gland. Contrera et al. [22] reviewed the FDA database of 282 (229 marketed) human pharmaceuticals where a majority of studies were conducted in Sprague-Dawley rats. Positive carcinogenicity findings occurred in 44.3% of the compounds. Compounds that produce trans-species tumors, positive in both mice and rats, are considered to pose a greater risk to humans as are compounds that result in cancers in multiple tissue sites. These have been classified as one-cell positive up to four-cell positive using the trans-gender and trans-species classifications. One of the conclusions of this paper was the acceptance that negative results in an alternative, transgenic mouse model supported the conclusion that positive results only in a rat study was enough evidence to claim a single-species phenomenon, or a lower regulatory hurdle. In the FDA database, 45 drugs (33 marketed) were found with tumor findings only in rats; 15 were two-cell positive, and several approved drugs were three and four-cell positive.

Examples of Rat-Specific Modes of Action

As mentioned earlier, the human relevance of findings in rats has been extensively debated and has resulted in several publications suggesting alternative approaches to current guidelines and requirements. Below are three of the most commonly debated tumors appearing in 2-year rat studies where there are known rat MOAs considered to be different from known MOAs in humans.

In the adrenal cortex, the known MOA in rats is stimulation of adrenocorticotropic hormone (ACTH) from the stimulated pituitary [23]. Adrenal cortical tumors is rare in rodents and their presence from a non-genotoxic compound suggests potentially multiple secondary effects. The adrenal gland is composed of three zones. The zona glomerulosa which produces aldosterone is responsible for electrolyte balance. This includes sodium reabsorption and potassium excretion in the kidney. Reducing aldosterone levels can cause an increase in sodium and water excretion. The zona fasciculate produces glucocorticoids, and the zona reticularis produces glucocorticoids and sex hormones in some species. Several spontaneous lesions occur in the cortex; however, tumor formation is rare in control animals. Glucocorticoids are produced from cholesterol via the Endoplasmic Reticulum (ER) eventually forming corticosterone in rats. In humans the ER contain additional hydroxylases responsible for the synthesis of cortisol. Rats have little sex hormone production in the adrenal glands when compared to humans, although rats will respond to increased levels of androgens and estrogens with proliferative lesions. Adrenal glands have large stores of lipid and lipophillic compounds can easily accumulate in adrenals, which accounts for some of the adrenal toxicity caused by certain compounds. Altered function can elicit an inflammatory reaction or cause a disruption of normal ACTH production from the pituitary. In addition, an increased metabolic load in the liver and potential ER effects could potentially cause proliferative changes in the adrenal gland.

In the thyroid gland the known MOA in rats is increased clearance of thyroid hormones resulting in increased pituitary secretion of TSH [13,16,24-27]. The rat thyroid is more susceptible to secondary (non-genotoxic) carcinogenesis than the human thyroid because rats lack high-affinity thyroxine-binding globulin such as do humans, utilizing instead a low-affinity albumin (10^3 lower affinities). Therefore the thyroid hormone half-life in rats is 10 times shorter than in humans and turnover is more rapid requiring higher "work" to maintain homeostasis. Accordingly, rats are more susceptible to hyperplasia and neoplasia with the disruption of the synthesis, secretion, or metabolism of thyroid hormones. In rats proliferative changes are primarily due to a prolonged stimulation by thyroid stimulating hormone (TSH) released by the pituitary in response to decreases in circulating T3 and T4 levels. Alterations in the normal feedback mechanisms usually occur from: interference with iodine uptake and thyroid hormone synthesis or secretion, interference with peripheral metabolism of T3 or T4, and increased metabolism and excretion of thyroid hormone.

In the pancreas, the MOA in rats involves a gastrointestinal hormone cholecystokinin (CCK), a trophic factor for the normal pancreas. CCK is thought to act as a promoter for pancreatic tumors in rats and there is evidence that stimulation of endogenous CCK levels by different xenobiotics will lead to pancreatic hypertrophy and hyperplasia in rats. In addition, reactive oxygen species resulting in cell proliferation can be a significant factor [28-33]. Of interest, pancreatic cancers of the acinar cell type can be experimentally induced in rats. Several studies in rats have demonstrated that duct-like and undifferentiated carcinomas, as well as acinar cell carcinomas, can arise from acinar cells. In contrast to rats, humans characteristically develop duct-like carcinomas for which the hamster more closely resembles the majority of tumors seen in the human pancreas. High unsaturated fat diets, corn or safflower oil by gavage, trysin inhibitors (eg. raw soy flour), and gastrointestinal surgical procedures have been shown to induce pancreatic cancer in rats.

Examples of Case-By-Case Approvals with Positive Rat Studies

In a regulatory review when the MOA for the rat tumor becomes the issue, the specific rat tumor MOA must be proven with the compound being reviewed, rather than relying on literature or previous non-

compound specific evidence such as the summaries above. That can be a very time consuming and costly endeavor with an uncertain outcome. In many cases because of the difficulty, the evaluation usually defaults to an easier to assess endpoint such as safety margin. In another example, when a drug is being developed for a life-threatening condition the benefit to patients will typically outweigh any risk associated with positive 2-year rat carcinogenicity findings. The following examples of these two points were taken from drug approval documents from the FDA website (FDA.gov).

An example of the difficulty of using a MOA argument for approval is the review of gabapentin, where pancreatic tumors in rats were the question. The sponsor conducted several mechanistic studies involving the CCK phenomenon [28], several of which have become the standard for this phenomenon, but ultimately the CCK argument was not accepted and an alternative method to access safety margins based on pharmacokinetic data became the mechanism for drug approval.

An example where the patient population is taken into consideration from a risk/benefit standpoint is the approval of Entecavir (Baraclude) for Hepatitis B in 2005. The mechanism of action of Entecavir involves a competition with the natural substrate deoxyguanosine triphosphate, which then functionally inhibits all three activities of the HBV polymerase (reverse transcriptase) including base priming, reverse transcription of the negative strand from the pregenomic messenger RNA, and synthesis of the positive strand of HBV DNA. Upon activation by kinases, the drug has been shown to be incorporated into the DNA which has the ultimate effect of inhibiting the HBV polymerase activity. In a battery of genetic toxicology studies, Entecavir was positive in mouse lymphocytes and was clastogenic *in vitro* in human lymphocytes (without metabolic activation). However, it was negative in the Ames assay, the mammalian-cell gene mutation assay, and a cell transformation assay. It was also negative in two *in vivo* assays, one for the induction of micronuclei and one for the induction of unscheduled DNA synthesis in primary liver cells. In both mouse and rat carcinogenicity studies, several tumors were statistically significant and considered by FDA to be relevant to human safety evaluation. These included hepatocellular adenomas and carcinomas in female rats, skin fibromas in female rats and brain gliomas in both male and female rats. In addition, in the mouse carcinogenicity study, liver tumors in males and vascular tumors in females as well as lung tumors in both sexes were relevant. The opinion was that these findings in the mouse and rat studies suggested a potential cancer hazard to patients as the drug was considered a four cell positive compound. Subsequently the FDA Antiviral Drug Advisory Committee voted unanimously, 17-0, for approval and the FDA and the committee determined that entecavir should be approved for first line and second line HBV therapy based on the potential importance of the drug in this specific disease. Due to the findings of tumors in rodents given entecavir and the potential for humans to develop malignancies, the sponsoring company proposed a post approval safety study that would evaluate the risk for cancers developing in patients 5 to 8 years after starting entecavir and this was the agreement that allowed approval despite positive carcinogenicity findings in two species, both genders, and at multiple tissue/organ system sites.

The Path Forward

The current ICH Guidelines, S1A, S1B, and S1C (R2), recommend which pharmaceuticals warrant carcinogenicity testing, appropriate approaches for evaluating carcinogenicity potential, and appropriate methods for dose selection, respectively. While S1A outlines differences based on clinical indication, duration of intended exposure in humans, and a priori concern about carcinogenic potential, it does not address alternative strategies for carcinogenic assessments. There is also no guidance on important pharmacological or toxicological modes of action for cancer risk or any modification in approaches other than the 6-month transgenic mouse study in S1B. Based on the wealth of relevant information on rat studies, the ICH has appointed an Expert Working Group to recommend changes to ICH S1A. Initial discussions and communications from members were published online in October 2012 [34]. The ICH S1A modification is expected to reach the Step 2 document status in mid 2014 and to reach Step 4 finalization in mid 2017. One of the key sources of information triggering these discussions was a PhRMA group representing 13 pharmaceutical companies that proposed a new testing scheme stating that a rat bioassay need not be conducted if the compound in question has no evidence of hormonal disruption or genotoxicity and there is an absence of histopathologic risk factors in chronic toxicology studies. This has become known as the NEGCARC proposal. Under this proposal, if any one of the three criteria ends in a positive signal in previous non-clinical studies, then a 2-year rat study and a transgenic mouse study would be warranted. If all three criteria are negative (no positive signal) then a transgenic mouse study would be conducted and the 2-year rat study would be waived. The proposal gives the option to conduct either the 6-month transgenic or 2-year mouse study [34]. The sticking points in the proposal are the specific definitions of each positive signal of the three points and how each would be adjudicated prior to conducting studies or after the fact during the regulatory review process. Would a different evaluation be made on hypertrophy in the liver or in other tissues based on metabolic overload? Would a different view of positive rat findings occur if all three points were negative but the sponsoring company did the rat study anyway based on timeline demands?

This brings us to the point of translating the vision of FDA and the scientific community to develop a new approach to evaluate the potential of new drugs to increase the risk of human cancer in patients taking the drug. It is convenient to leave the 2-year rat study on the table as a default option – because we've always done it that way. This is essentially the NEGCARC proposal. The more innovative way would be to take the 2-year rat study off the table while a new approach is being formulated. It may be that it claims a place back on the table later, but it should not be the distracting element that is easy to cling to. Wiping the slate clean on the front end is the way innovative solutions to problems are discovered. These are lessons previously learned in business, the arts, and innovative drug discovery. In this extremely important discussion and decision making process, we should be attempting to formulate solutions that reflect advances in science and technology, not just the protection of 50 years of legacy data.

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