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Evaluation of the Carcinogenic Potential of Pharmaceuticals

Opportunities Arising from the International Conference on Harmonisation

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Summary

The evaluation of the carcinogenic potential of pharmaceuticals is currently undergoing dramatic changes. For the past 25 years the regulatory expectation for agents intended for long term use has been that lifespan studies (usually lasting 2 years) in 2 rodent species be conducted. These studies take at least 3 years to plan, execute and interpret, and use over 1200 animals. It is now recognised that the quality of the information obtained from these studies is unreliable for prediction of carcinogenic risk to humans.

Over the past 4 years, the International Conference on Harmonisation (ICH) has recommended changes in approaches to assessing the carcinogenic potential of pharmaceuticals. In future, only one long term rodent study will be routinely required (usually in rats), provided this is complemented with a short or medium term test in one of the emerging new models for carcinogenicity, such as transgenic mice or newborn mice. However, the relevance of these new models to human cancer and their use in risk assessment is still largely unknown and this situation must be kept under review as knowledge accumulates. A long term study in a second rodent species is still an option.

Dose selection has also been improved inasmuch as there are now several alternatives to the use of the maximum tolerated dose (MTD). In the past, the use of the MTD, when the normal homeostasis of the test animals is disturbed, has been considered one of the major problems with the rodent carcinogenicity bioassay. However, one of the alternative end-points to the use of the MTD, i.e. the comparison of plasma concentrations in rodents and humans, must be viewed with caution. While this may contribute to limiting the high dose level for agents of very low toxicity, the concept should not be interpreted as signifying that plasma concentrations provide a sound basis for comparing the carcinogenic activity of agents in different species.

Recognition of the 4 properties (genotoxicity, immunosuppression, steroid hormonal activity and long term tissue damage), at least one of which is associated with each of the pharmaceuticals known to be carcinogenic to humans, should focus more attention on a search for these properties in patients. Absence of these properties at clinically relevant dose levels indicates that a pharmaceutical is highly unlikely to be carcinogenic to humans.

Approaches to evaluating the carcinogenic potential of pharmaceuticals have been changing significantly over the past few years. The International Conference on Harmonisation (ICH) on Technical Requirements for the Registration of Pharmaceuticals for Human Use is an organisation comprised of scientists from the pharmaceutical industry associations and the government authorities of the European Union, Japan and the US. The underlying purpose of ICH is to improve, through harmonisation, the efficiency of the process for developing and registering new medicinal products in order to make these products available to patients with a minimum of delay. Since the first meeting of ICH in 1991 enormous progress has been made in harmonising, and usually simplifying, the requirements for establishing the quality, efficacy and safety of human pharmaceuticals in the 3 geographical areas.[1] Evaluation of the carcinogenic potential of pharmaceuticals has been a major topic and the discussions are leading to important changes to practices in this activity.

To put the current changes into perspective, one needs to look back at the early 1970s. Up until that time, the relatively few agents recognised as being carcinogenic to humans were genotoxic (*vide infra*) chemicals such as polycyclic hydrocarbons, azo dyes, aromatic amines and nitrosamines and these agents were also found to display potent carcinogenic activity in rodents. A series of questionable assumptions followed. First, chemicals that are carcinogenic to rodents will also be carcinogenic to humans. Second, carcinogenicity is a property associated with relatively few substances; one way of reducing the burden of human cancer would be to identify these carcinogens, in laboratory animals, and then eliminate them from our environment. Third, these animal tests should be made as sensitive as possible: the animals should be given the maximum tolerated dose (MTD) for most of their lifetime (about 18 to 24 months in laboratory mice and rats). The US government, in its 'war on cancer' in the early 1970s, made funds available for hundreds of chemicals to be subjected to these rodent carcinogenicity 'bioassays' and, after about 20 years' testing, analysis of the results of this programme revealed that the assumptions were ill-founded.

To much surprise, a relatively high proportion of agents tested provided evidence of carcinogenic activity and, indeed, we now know that about 50% of agents tested in these bioassays gave positive results.[2-6] There was widespread consternation in pharmaceutical medicine when positive results were reported for some agents that had been in use for many years, for example, phenobarbital (phenobarbitone), clofibrate, reserpine, phenothiazines, oral contraceptives, etc. From the early 1970s, the testing pattern expected for new drugs was for companies to conduct bioassays in 50 male and 50 female animals per dose level in 2 rodent species at dose levels up to the MTD, with the exposure starting soon after weaning and continuing on a daily basis for most of the lifespan (i.e. till survival is down to about 50%) of the animals. The design, conduct and interpretation of each study requires at least 3 years and 600 animals per species. This testing schedule is now open to change since ICH no longer requires 2 rodent bioassays at the MTD to be routinely conducted.

In this overview, we summarise the main progress made by ICH in allowing more flexibility in the evaluation of the carcinogenic potential of pharmaceuticals to better reflect current understanding of the causes of cancer in humans. We also add, in several sections, our personal views on the areas that still need to be further discussed, and perhaps revised by ICH in the future.

1. Deficiencies and Redundancies in Rodent Carcinogenicity Bioassays

The deficiencies and redundancies of rodent bioassys are summarised briefly in the following sections.

1.1 Too Many Positive Results

A major problem in a simplistic sense is that bioassays, as currently conducted, produce too many positive results. Surely the finding that about 50% of agents tested positive[2-6] cannot mean that

half of the chemicals in our environment pose a carcinogenic hazard to humans. It has been estimated that environmental chemicals (including drugs) are responsible for only 1 to 3% of preventable cancer.[7] Therefore, it is a real dilemma for regulators and those developing new drugs to decide which results have human relevance. Mechanism and potency must both be evaluated. It would surely be better to re-design the test so that it produced less positives in the first place with the proviso, of course, that the positives that are produced include those agents that are most likely to pose a hazard to humans. Ways to improve the specificity of testing, i.e. to produce fewer false positives, are discussed later in section 3.

1.2 Target Organs: Rodents *vs* Humans

An underlying problem is that rodents are fundamentally flawed as surrogates for humans by virtue of the difference between the species in terms of aspects of their anatomy, physiology and metabolism that are important in chemical carcinogenesis (table I). It should be no surprise, therefore, to find that there are marked inter-species differences in organ susceptibility to cancer. Thus, some of the rodent strains commonly used in bioassays have high (30 to 100%) spontaneous incidences of tumours in liver, pituitary and testis, sites in which cancer in humans is relatively rare.[8]

In the US the descending order of the incidence of human cancer is prostate, breast, lung and colorectal until, after some sites with intermediate frequency, one arrives at low incidence sites such as liver, thyroid, testis, pituitary and adrenal. Yet, these last 5 sites (plus the lung in mice) are the commonest sites of cancer for pharmaceuticals in rodent studies (fig. 1). These differences send a strong signal that humans and rodents possess different intrinsic responses to carcinogenic stimuli.

1.3 Paradoxical Results

Confusion arises when bioassays produce conflicting patterns of results, with simultaneous increases in the incidence of one tumour type being accompanied by decreases in another.[11,12] While, understandably, regulatory attention in the past has

Table I. Some differences between rats and humans that are critical in chemical carcinogenesis

Parameter	Rat	Human	Comment
Lifespan (years)	2.5	70	Longer exposure in humans, but rates of carcinogenesis and the aging process are related
Food consumption (g/kg/day)	50	10	Intake of lipid and protein leads to cumulative oxidative damage that contributes to aging and cancer
Basal metabolic rate (kcal/kg/day)	109	26	High metabolic rate correlates with DNA oxidative damage
Forestomach, Zymbal's gland, Harderian gland, preputial gland, clitoral gland	Present	Absent	Difficult to interpret significance of tumours present in organs not present in other species
Reproductive cycle	Oestrus	Menstrual	Different patterns and roles for estrogen and progestogen might affect susceptibility to some tumour types
Parity	High	Low	Pregnancy protects against some cancers
Role of prolactin in mammary gland activity	High	Questionable	Modulation of prolactin secretion by a drug will have different consequences for mammary cancer
Stomach pH	4-5	1-2	Can affect activation/reactivation of some xenobiotics that will undergo enterohepatic cycling
Gastrointestinal bacterial flora	Numerous	Few	Can affect metabolism of bile acids that may have a role in colon cancer
α_2-Microglobulin	Present (especially in males)	Virtually absent	Protein necessary for some renal (and perhaps bladder) tumour types in rats
DNA excision repair	Low	High	Factor in determining lifespan

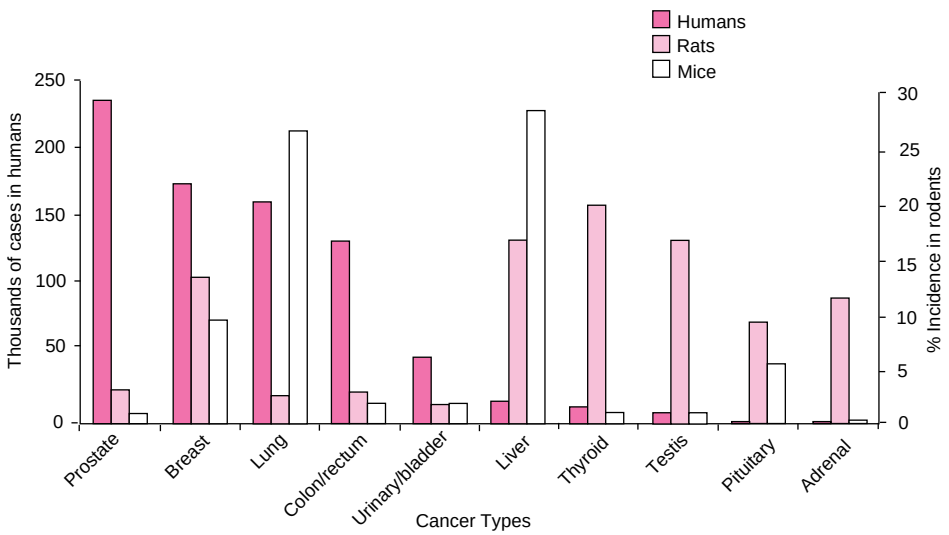

Fig.1. Incidence in the US of human cancers occurring at different sites[9] and incidence of cancers in rodents by site.[10]

focused on tumour incidence increases, should a chemical that both increases and decreases tumour incidences be stigmatised as a 'carcinogen'?

1.4 Use of the Maximum Tolerated Dose

In addition to all the above inherent weaknesses in the use of rodents as models for chemically-induced carcinogenesis in humans, the use of the MTD has been described by many as the most serious problem with the bioassay.[13-17] The use of the MTD implies, by definition, that this dose level causes some toxicity or at least a disturbance of homeostasis. It is this disturbance of homeostasis, often associated in the case of drugs with hormonal imbalance, that leads to many carcinogenic effects. The phenomenon usually has an easily definable threshold dose level and, for most drugs, it would correspond to an adverse effect in humans which would be expressly avoided for a drug that is to be used for an extended period. Increased cell proliferation caused by reparative hyperplasia after chronic organ damage or by a mitogenic stimulus, e.g. a hormonal effect, is a predisposing condition for neoplasia.

The use of dose levels that are much higher than that required for the therapeutic effect of a drug may also (but not inevitably) give rise to differences in the bioavailability, metabolism and kinetics of the drug at the various dose levels. However, even if such differences are detected it is usually not obvious how this information can be applied to interpretation of the results of the bioassay; it simply adds another confounding factor.

In summary, the rodent bioassay as conducted over the past 25 years has several important defects. The only defect readily amenable to improvement is the exposure of animals to the drug, i.e. dose selection and duration of administration. However, changes in exposure strategies would still not circumvent the main defects that are inherent in the use of rodents as surrogates for humans.

2. New Strategies based on Pharmaceuticals Known to be Carcinogenic to Humans

While rodent bioassays for assessing the carcinogenic hazard of chemicals in general are receiving widespread criticism,[13,18-20] pharmaceutical agents are a special sub-group of chemicals that lends itself readily to the development of new strategies that diminish the dependency on rodent bio-

assays. The strategies are based on our knowledge of the properties of the 20 pharmaceutical agents considered by the International Agency for Research on Cancer (IARC) to be carcinogenic to humans (table II). These agents all possess at least one of 4 properties: genotoxicity *in vivo*, immunosuppression, steroid hormonal activity, or chronic irritation/inflammation.

It has been found useful to categorise carcinogenic chemicals as genotoxic or epigenetic (non-genotoxic).[24] The former are DNA-reactive, causing gene mutations and/or chromosomal damage.

Table II. Pharmaceutical agents carcinogenic to humans[21,22]

Agent	Genotoxicity	
	Salmonella (Ames test)	BM[a]
Antineoplastics		
Busulphan	+	+
Chlorambucil	+	+
Chlornaphazine	+	+
Lomustine	+	+
Cyclophosphamide	+	+
Melphalan	+	+
MOPP & combinations	+	+
Thiotepa	+	+
Treosulfan	+	+
Immunosuppressants		
Azathioprine	+	+
Cyclosporin	–	–
Dermatologicals		
Arsenic salts	–	+
Coal tars	+	ND
Methoxsalen + UVA	+	ND
Hormonal		
Diethylstilbestrol	–	(+)
Estrogen replacement therapy	ND	ND
Sequential oral contraceptives	ND	ND
Combined oral contraceptives	ND	ND
Tamoxifen[23]	–	–
Miscellaneous		
Phenacetin (in analgesic mixtures)	+	+

a Rodent bone marrow (micronucleus or chromosomal aberrations)

Abbreviations and symbols: MOPP = chlormethine (mechlorethamine), vincristine, procarbazine and prednisone; ND = no data; UVA = ultraviolet$_A$ radiation; + = positive; – = negative; (+) = equivocal.

The universal role of DNA in the control of cell division and death suggests that genotoxic agents are more likely than epigenetic agents to be carcinogenic in all species. The first chemicals found to be carcinogenic to humans were genotoxic, and indeed they were also carcinogenic to rodents. As methods for assessing genotoxicity evolved during the 1970s there appeared to be a strong correlation between genotoxicity and carcinogenicity. However, with the accumulation of data from hundreds of chemicals, it transpires that up to a third of chemicals with evidence of genotoxicity *in vitro* are not carcinogenic to rodents even at the MTD.[2] Nevertheless, if a potential new drug is determined to be unequivocally genotoxic *in vivo*, its development will usually be terminated unless there is a strong benefit-risk argument to the contrary, such as for antineoplastic or some antiviral agents.

Chemicals that cause cancer by epigenetic mechanisms in rodents are diverse in character. Immunosuppressants certainly induce tumours in rodents and in humans and, irrespective of findings in animals, evidence of immunosuppression in humans is an indication of a carcinogenic risk, the magnitude of which will depend on the degree and duration of the immunosuppression. The routine performance of haematological examinations during clinical trials will give an indication of a potential immunosuppressant effect. Any perturbation of lymphocyte parameters can be followed by investigation of lymphocyte sub-populations, cytokine levels and so on.

Similarly, any evidence of a steroid hormonal imbalance in short term animal tests should alert the clinician to seek such effects in humans treated with the drug. The high incidences of human cancers of the breast, uterus, ovary, prostate and testis are heavily dependent on prolonged exposure to estrogens, progestogens and/or androgens and, in particular, the ratios of these hormones to each other.[25,26] Laboratory assays (often radioimmunoassays) are now widely available for estradiol, progesterone, testosterone, luteinising hormone, follicle stimulating hormone, prolactin, steroid hormone binding globulin and other hormones that

may be appropriate to the particular drug. This opens the way for a clinical chemistry screen to detect changes in these critical hormonal parameters. Lack of an effect is reassuring; finding a change would trigger more detailed investigations into prevalence, mechanism, and likely target organs. Chronic organ damage during prolonged drug use in humans, such as the nephrotoxicity incurred by chronic use of phenacetin, is also a carcinogenic risk factor. For both hormonal effects and chronic irritation, in the last analysis, it is a persistent increase in cell proliferation that leads to an increase in site-specific cancer.[13,14,27]

Extensive investigations over the past 2 decades have characterised several of the mechanisms whereby epigenetic pharmaceutical agents increase tumour incidence in rodents (table III). This knowledge and the ability to assess the relevance of the mechanism in humans under actual conditions of use has allowed the approval for use in humans of over 100 drugs that are known to cause cancer in rodents.[9]

Table III. The carcinogenicity in rodents of some epigenetic pharmaceuticals

Type of drug	Target
Liver enzyme inducers that increase liver weight	Liver
Liver peroxisome proliferators	Liver
Inhibitors of thyrotrophin synthesis	Thyroid
Inhibitors of T3-monodeiodinase (converts T4 to T3)	Thyroid
Liver enzyme inducers (increase disposition of thyroid hormones)	Thyroid
β-Adrenergic agonists	Mesovarium
Dopamine antagonists (increase prolactin)	Mammary, pituitary
Dopamine agonists (decrease prolactin)	Endometrium (rats)
Gastric acid antisecretory agents (H_2-antagonists, proton pump inhibitors)	Stomach
5α-Reductase inhibitors (and anti-androgens)	Testis
LHRH-analogs	Pituitary, testis
Estrogens	Pituitary, mammary, uterus
Immunosuppressants	Lymphatic system
Drugs that affect calcium absorption and homeostasis	Adrenal
Drugs that affect catecholamine release from the adrenal medulla	Adrenal
Drugs that affect FSH or LH secretion	Testis, ovary
Drugs that bind α_2-microglobulin	Kidney
Carbonic anhydrase inhibitors	Bladder
Drugs that affect urinary pH	Bladder

Abbreviations: FSH = follicle-stimulating hormone; LH = luteinising hormone; LHRH = luteinising hormone-releasing hormone.

In summary, we argue that if a drug does not possess any of the 4 properties discussed (genotoxicity, immunosuppression, steroid hormonal activity and long term tissue damage) it is unlikely to pose a carcinogenic hazard to humans. Information on these properties can be obtained in short term tests in the laboratory, plus a study in laboratory animals of up to 3 months' duration. If any of the properties are manifest, the focus switches to clinical investigation to determine the extent of their existence in the key species under conditions of exposure relevant to the use of the drug. It is important to note that the investigations should target biological end-points. At this point, pharmacokinetic data have little value for inter-species comparisons. However, such data may be useful within the human population to seek, *inter alia*, population subgroups, dose-dependent kinetics and potential for drug interactions.

3. Changes in Testing Brought About by the International Conference on Harmonisation (ICH)

The scheme outlined in the section 2 is based largely on current knowledge of chemical carcinogenesis and ICH has taken several significant steps in the same direction.[1] The changes in carcinogenicity testing introduced by ICH are summarised in the following sections.

3.1 Need for Studies

ICH Topic S1A, 'The Need for Studies', was adopted in November 1995.[1] This guideline clarifies the confusion that had existed previously on the duration of clinical exposure that would trigger the need for a rodent bioassay. It was decided, arbitrarily, that rodent bioassay testing should be re-

1 Guidelines agreed up to and including July 1997 are available on the Internet at http://www.ifpma.org/ich1.html

quired for drugs that are likely to be used continuously for 6 months or longer or frequently in an intermittent manner for chronic recurrent conditions (e.g. allergic rhinitis, depression). The need for testing could also be triggered by various 'causes for concern' such as findings in genotoxicity studies, evidence of carcinogenic activity (relevant to humans) in the product class, chemical structure, putatively preneoplastic lesions in short term animal studies, or tissue inflammatory reactions that are associated with prolonged tissue retention of drug or its metabolites. It was also decided that unequivocally genotoxic compounds need not be subjected to a rodent bioassay. A few other clarifications were made in order to reduce the need for duplicate bioassays for agents that have 2 or more acid/base/salt forms or that are given by 2 or more routes of administration.

3.1.1 Comment

We feel that it is disappointing that ICH did not take a bolder position. We have argued that for pharmaceutical agents which possess one or more of the 4 properties (genotoxicity, immunosuppression, steroid hormonal activity and long term tissue damage), important information on carcinogenic potential can be obtained directly from studies in humans. Conversely, agents that are devoid of these 4 properties in animal studies do not provide cause for concern. In a simplistic sense, one might agree that in neither of these extreme cases is a long term rodent bioassay useful. The predictability of the outcome of rodent bioassays supports this view. In a landmark investigation carried out between 1990 and 1994, the outcome of bioassays on 40 (nonpharmaceutical) chemicals was predicted from knowledge of chemical structure and the results of genotoxicity testing and of 90 day toxicology studies.[28] For about half the chemicals – those judged most likely to be not carcinogenic or to be definitely carcinogenic – the prediction was 86% correct. For the other chemicals for which prediction was less confident, the success was only modest. For pharmaceuticals, the more extensive information available on pharmacology, metabolism and pharmacokinetics should lead to even better predictivity. It is difficult to justify the conduct of a bioassay (or any experiment) for which the outcome is highly predictable.

3.2 Testing for Carcinogenicity

Under the heading 'Testing for Carcinogenicity' (Topic S.1B), which was adopted in July 1997,[1] the ICH has made a major advance by abandoning the requirement for the routine conduct of bioassays in 2 rodent species. Instead, a bioassay is required in 1 species and this should be accompanied by at least one additional short or medium *in vivo* test. This explicit acknowledgement of the limited value of rodent bioassays was arrived at after a thorough examination of the data available from the hundreds of pharmaceuticals that have been registered in the 3 ICH regions.[4] The overall conclusion was that bioassay data from mice rarely contributed information that had any bearing on carcinogenic risk assessment in humans.[5,6,29] The guideline suggests that the single species chosen for the bioassay should be an appropriate one based on considerations such as pharmacology, short term toxicology, metabolism, toxicokinetics and route of administration. In the absence of clear evidence favouring any one species, the rat should be selected.

3.2.1 Additional Tests

An additional short- or medium term test should ideally be able to contribute additional information specific to the agent under evaluation and which is relevant to human carcinogenesis. Tests suggested in the guideline are:

Transgenic Rodents

These are rodents that have been genetically modified to make them more susceptible than normal wild strain animals to carcinogenic stimuli. Generally, they have a very low incidence of spontaneous tumours and show clear responses to potent carcinogenic agents within 6 months. The most studied transgenic rodents are the Hras2, p53 deficient, Tg.AC and the XPA deficient models.[30-32]

Newborn Rodents

These animals are extremely sensitive to at least some carcinogenic chemicals. Two or 3 doses of the test substance are administered within the first 2 weeks of life and the animals are then maintained for a year and examined for the incidence of tumours.

Initiator-Promoter Studies

Rodents are 'initiated' with a single dose of a potent genotoxic carcinogen such as diethylnitrosamine, and this is then followed by administration of the test chemical for several months. An increased tumour count would indicate that the test substance has the ability to 'promote' i.e. facilitate the progression of an initiated cell through the several stages required to arrive at malignancy. This information may have value for understanding the mechanism of a carcinogenic effect, but since the model essentially just replicates the results of rodent bioassays and is restricted to those few target organs that may have been 'initiated' it is still deficient as a predictor of human risk.

3.2.2 Comment

At the time of writing, relatively few chemicals have been evaluated by any of these new short term tests, so their value in uncertain. An enormous multi-laboratory collaborative investigation, sponsored by the International Life Sciences Institute, Washington, DC, in the US is currently in progress to systematically evaluate the responses of these test systems to about 20 carefully chosen pharmaceuticals that either possess various types of carcinogenic activity – genotoxic or epigenetic – and have produced positive results only in rodents or in both rodents and humans, or agents that are putatively not carcinogenic to humans.[33] As the results of this collaboration emerge from 1998 onwards, it is possible that this guideline may need revision to reflect any new knowledge acquired.

3.3 Dose Selection for Carcinogenicity Studies

Finally, the citadel of the MTD has started to crumble! This is probably easier for pharmaceuticals than for other chemicals; the availability of data from humans allows dose selection to be linked to the human situation. Indeed, the guiding principle of the new guideline, 'Dose Selection for Carcinogenicity Studies' (Topic S.1C), which was adopted in October 1994,[1] is that the doses selected should be tolerated without significant physiological dysfunction and should permit data interpretation *in the context of clinical use*. The guideline emphasises flexibility and suggests that any one of several end-points may be used:

- Toxicity – the MTD is still acceptable
- Pharmacokinetics
  (a) A rodent/human plasma concentration ratio [area under the curve (AUC)] of drug and/or metabolites of at least 25
  (b) Saturation of systemic absorption
- Pharmacodynamics – avoidance of loss of a selectivity that may characterise the action of the drug at the therapeutic dose level
- Maximum feasible dose – historically at 5% in the diet, an addendum to the guideline provides for an upper limit dose of 1500 mg/kg for drugs for which the human dosage is less than 500 mg/day.

3.3.1 Comment

While alternatives to the MTD are now available, it seems likely that the MTD will continue to be used by investigators for the foreseeable future. A recent survey of the industry conducted in 1997 by the UK Centre for Medicines Research International revealed that about 80% of pharmaceutical companies were still using the MTD because a 25 × AUC ratio was unobtainable at a dose level below the MTD.[34]

However, by listing a pharmacokinetic endpoint, the guideline is well intentioned in that it recognises that the administered dose as a comparative measure of systemic exposure in 2 species has limitations and may sometimes be misleading. Comparison of systemic concentrations (in blood, serum or plasma) of drug and its metabolites is one step closer to the site of action. The ICH Guideline S.3, 'Toxicokinetics and Pharmacokinetics', emphasises the value of pharmacokinetic data in elu-

cidating how systemic exposure within a toxicology study may be affected by dose level, route and means of administration, duration of administration, age, gender, etc. However, the process is still defective in that it does not address the important question of whether a given concentration of an agent produces the same pharmacodynamic (in this case, carcinogenic) response in the 2 species.

In view of the differences in species' susceptibility to cancer cited earlier, the diversity of epigenetic mechanisms involved (table III), the cumulative nature of the carcinogenic process and the different rates of the various stages in species of vastly different lifespans, it would be surprising to find a general correlation between drug plasma concentration and carcinogenic response across species. The ICH guidance that invokes a 25 × AUC factor should be regarded, therefore, as a pragmatic alternative to reducing the need to use the MTD; it should not be interpreted as indicating that plasma concentrations of a chemical can be used to predict carcinogenic risk across species.

The invalidity of using blood or plasma concentrations of chemicals for estimation of cancer risk across species has been demonstrated.[35] Examination of the systemic concentrations for 5 chemicals with some of the strongest evidence for lack of carcinogenicity in humans [phenobarbital, DDT, dieldrin, salbutamol (albuterol) and clofibrate] revealed that the concentrations in humans were similar to or greater than those associated with carcinogenicity in rodent tests. For similar reasons, one must question the validity of the prediction of a carcinogenic risk to humans of hypolipidaemic agents, based essentially on a comparison of systemic concentrations of these agents in rodents and in humans.[36]

Some data from 35 pharmaceuticals tested in rodent bioassays that purportedly supported the use of plasma concentrations in cancer risk assessment were analysed by Contrera et al.[37] They demonstrated a loose correlation between drug plasma concentrations (AUC) and dose, but they did not discuss whether there was a correlation with carcinogenicity and nor was any apparent. However, they reported that for one-third of the 35 compounds studied, the plasma concentration at the MTD in rats was *less* than the concentration in humans receiving the recommended therapeutic dose. Thus, the concentration required for effective and well tolerated use of a drug in humans was greater than the concentration that caused severe toxicity in rats!

The new upper dose limit (1500 mg/kg) for rodent bioassays seems to be unjustifiably conservative. In contrast to the lack of data supporting the use of systemic concentrations for inter-species predictions, there are data from 30 to 40 chemicals indicating that dose in terms of mg/kg bodyweight is a reasonable, even though still approximate, basis for inter-species comparison of carcinogenic potency of chemicals. Examination of the data from rodent bioassays for chemicals that are carcinogenic to humans (including those in table I) showed that these chemicals are all potent carcinogens in rodents, all giving a carcinogenic response at daily dose levels of 50 mg/kg or less.[38] Application of an arbitrary additional safety factor of 10 to allow for the possibility of uncovering some less potent rodent carcinogens led to the suggestion that an upper dose limit of 500 mg/kg/day would improve the relevance of results of rodent bioassays. A complementary analysis bioassay results for over 500 chemicals tested in rodent bioassays showed that only 5 of them required dose levels >1000 mg/kg/day to provoke a carcinogenic response.[39] The tumour types observed were considered to be of no relevance to humans, thus confirming that a dose limit of 1000 mg/kg/day would not result in the loss of useful information for human risk assessment.

In summary, this guideline on dose selection has at last opened up the possibility of using approaches other than the MTD. As further information becomes available in the future these alternative endpoints may undergo revision or refinement.

4. Conclusion

The last few years have seen a considerable shift in approaches for assessing the carcinogenic potential

of pharmaceuticals. There is now a shift away from dependence on the results of long term studies in rodents (carcinogenicity bioassays) towards a 'weight of evidence' approach that incorporates information from genotoxicity studies, pharmacology and pharmacokinetic studies in animals and in humans, and, above all, a search for carcinogenic risk factors in humans. Indeed, it would seem desirable that more resources be made available for the development of methodology in humans that can be tailored to the characteristic of a drug and its pattern of use.

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