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The Carcinogenicity Debate

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Factors affecting the duration of carcinogenicity studies: when should the study end?

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Summary

1. The duration of long-term studies cannot be discussed in isolation. It is necessary to consider a number of fundamental questions as to why the tests are being conducted, and whether genotoxic or non-genotoxic agents are involved.
2. Tests on chemicals that are clear-cut potent mutagens are of doubtful value. However, given the state of current knowledge, tests on pharmaceuticals for non-genotoxic carcinogenicity should be regarded as necessary unless adequate reassurance of safety from metabolism, pharmacokinetic and general toxicity studies is already available.
3. Carcinogenicity tests should be continued until 50% of the animals in the control group have died, and the termination of the experiment should not be delayed if survival in the treated groups exceeds the controls. Furthermore, males and females should be regarded as belonging to separate experiments for the purpose of deciding how long they should last.

Introduction

Before addressing the issue posed by the question in the title of this paper, it is important to point out that during the last few years there has been a major revolution in the concepts underlying carcinogenicity testing. Ten years ago the term "non-genotoxic carcinogenicity" would not have been mentioned by anyone addressing a meeting on the evaluation of any class of chemical for possible carcinogenicity. By contrast, most of the contributors to this workshop have used this term quite freely and Dr Purchase, in his opening paper, provided a definition of it which no participant has thought necessary to challenge.

A second point is that, whereas ten years ago there would have been much dependence on the assumption that carcinogenesis is a two-stage process with an initiating phase involving DNA damage (mutation) followed by a proliferation stage (tumour promotion) brought about by irritants that cause cell replication, hardly any contributor to this workshop has made this assumption or used these terms.

Personally, I particularly welcome the phasing out of the use of the terms "initiation" and "promotion", stemming as they do from the two-stage concept of carcinogenesis. The concept itself has undeniably played an important role in reaching our present level of insight into the mechanisms underlying carcinogenesis. However, most of the experimental evidence on which the two-stage theory was based was obtained under highly contrived laboratory conditions which rarely have any counterpart in real life. Furthermore, there is considerable circumstantial evidence, which has been touched on by Dr Purchase, that more than two stages are commonly involved in carcinogenesis and that multiple sequential mutations may be implicated.

The duration of long-term studies cannot therefore be discussed in isolation. It is necessary to consider a number of fundamental questions as to why the tests are being conducted and whether genotoxic or non-genotoxic agents are involved.

New concepts in carcinogenesis

Underlying the changes during recent years in relation to knowledge of the mechanisms of carcinogenesis are four facts.

1. An increasing number of substances have been found that, usually under conditions of high dosage, increase cancer risk in laboratory animals, but which in sensitive tests for genotoxicity, do not cause DNA damage, either directly or as a consequence of their conversion within the body to electrophilic metabolites.

2. It has been realised that where hormones or disturbance of hormonal homeostasis predispose to cancer development, the sequence of events in target organs is cellular proliferation first, and evidence of genotoxic damage second (i.e. the opposite way round to that postulated in the two stage hypothesis).

3. It has been realised that extensive DNA damage is occurring all the time in ordinary body cells because of the endogenous production of electrophiles (e.g. oxidants) during the conversion of food substances (especially fats) to energy.

4. Evidence has been produced that the process of cell division impairs the normally effective mechanisms by which damaged DNA is repaired so that there is an increased risk that mutations will be "fixed" in daughter cells. Although such mutations may be caused by exposure to exogenous xenobiotic substances, they are probably far more often caused by electrophiles produced endogenously during the metabolism of ordinary foodstuffs.

These four facts have led to the devaluation of genotoxicity tests as predictors of carcinogenicity. At the same time they have served to stress the need for testing non-genotoxins for carcinogenicity in long term animal tests.

Non-genotoxic carcinogens

Some of the hallmark effects of non-genotoxic carcinogens will now be discussed. Clearly, there are numerous non-genotoxic mechanisms of carcinogenesis, many of them complex and involving tissues other than the eventual target for tumour development, and a large number still awaiting discovery. Many of the mechanisms that have so far been characterised in laboratory animals have been associated with very high and often wholly unrealistic levels of exposure to test substances. Some of the mechanisms have depended on the use of particular species and particular strains of animals (ie. species-specific and strain-specific effects) and some have affected animals of only one sex (ie. sex-specific effects). These characteristics of non-genotoxic mechanisms serve to diminish concern about them in relation to man. However, it would not, in my opinion, be safe to assume that species barriers are never crossed or that there are invariably threshold levels below which non-genotoxic carcinogens pose no hazard for man. Evidence to support such a conclusion needs to be obtained for each chemical and in each situation.

One factor which seems to lead to increased risk of cancer development in the case of all known non-genotoxic carcinogens is increased cell replication. This may be brought about by hormonal imbalance, by chronic inflammation, by recurrent necrosis associated with regenerative hyperplasia, or by other observable disturbances of one or other aspect of tissue homeostasis. I personally am not aware of any example of non-genotoxic carcinogenesis in which there has not been evidence of one or more of these kinds of disturbance of normal physiological status. It is probably true, therefore, that if, in a sub-chronic toxicity study, there is no evidence from the histological examination of tissues or from clinical chemistry or haematology measurements etc, of any kind of disturbance of physiological status, then there is no risk of non-genotoxic carcinogenesis. However, in the case of pharmaceutical agents, which are designed to have pharmacological effects, there is always a good chance that evidence of disturbance of physiological status will be observed in animal tests. Furthermore, evidence of such disturbance may be seen even at doses that are pharmacokinetically similar to human use levels. This being so, it will often be necessary, where tumours arise in excess incidence in a chronic toxicity test in rodents, to undertake further studies to, as far as possible, ensure that the mechanism involved does not operate in man.

Selection of dose levels

There has been much discussion at this Workshop of the folly of being required to test chemicals – in particular non-genotoxic chemicals – for carcinogenicity in rodents at maximum tolerated doses (MTD). I agree that such practice is often foolish, provided that comparative pharmacokinetic data indicate that the MTD dose in rodents is vastly in excess of proposed clinical use levels. The more important point to be made, however, is that evidence of carcinogenicity at or near the MTD should not, by itself, be regarded as a basis for rejecting a drug for clinical use. Judgments in this respect should be based on information on the mechanism involved and on a consideration of the risk:benefit ratio.

How long should carcinogenicity tests in rodents last?

Finally, it is my opinion that, to be meaningful, tests need to last for the major part of the life-span of the test species and strain. Furthermore, tests should not be conducted in strains of animal that have genetic faults which predispose to early deaths. Nor should they be conducted under conditions of overfeeding, which significantly shorten life. Thus, I essentially agree with the view

expressed by many regulatory authorities that tests should be continued until 50% of the animals in the control group have died. I further believe that the two sexes should be regarded as belonging to separate experiments for the purpose of deciding how long they should last.

It is not uncommon for groups of rodents exposed to toxic levels of test chemicals to eat less, put on less weight and to live longer than controls in consequence. In such circumstances should the termination of the experiment be delayed until 50% of the high-dose animals have died? The answer is usually "No" since one needs to be able to compare dosed and control animals of the same age and if the experiment was prolonged there may not be a quorum of control animals for meaningful comparison.

Underlying these views is the fact that the risk of virtually all forms of cancer in all species increases logarithmically during the last third of the life-span. One of the reasons for this may be that defence mechanisms which are effective earlier in life cease to be so later in life. Consequently, we need to test whether, under realistic conditions of exposure, a drug weakens or strengthens these defence mechanisms.

How should carcinogenicity tests in rodents be conducted? What is their purpose?

In discussing how tests should be designed and conducted, I strongly hold the view that neoplasia is simply one manifestation both of aging and of chronic toxicity. Tests should, therefore, be designed in such a way that data relating to aging, chronic toxicity and carcinogenicity are collected in parallel. Chronic toxicity which involves hormonal disturbance, chronic persistent increased cell turnover, or premature aging is directly relevant to the assessment of non-genotoxic cancer risk, while the absence of such effects provides substantial grounds for dismissing the possibility of non-genotoxic carcinogenicity in the species and strain of animal used for the test. Of course, it is theoretically possible that there may be species-specific mechanisms for non-genotoxic carcinogenicity in man which do not operate in laboratory animals. Happily, to my knowledge, no such human-specific mechanism has so far been discovered. In any case, animal tests will not, by themselves, lead to the identification of such mechanisms. For this purpose studies directly in humans will be needed.

A factor which greatly increases the risks of (a) premature death, (b) the early onset of aging related diseases, (c) the development of benign and malignant cancers of virtually all sites, is over nutrition. This is illustrated in Table 8.1, where the incidences of malignant (potentially fatal) neoplasms in Wistar rats

fed on the same diet either *ad libitum* (ie. overfed) or restricted to 80% of *ad libitum* are compared. None of the animals was deliberately exposed to any genotoxic carcinogen. In Table 8.2, data reported by Lok *et al* (1990) show how diet restriction reduces cell-turnover rates as measured by thymidine-labelling. The data in these two tables, taken together, support the hypothesis that overnutrition predisposes to oxidative damage and increased cell turnover rates, and that the combination of these effects predisposes to increased cancer risk.

Table 8.1: Effect of calorie restriction to 80% of *ad libitum* in Wistar rats on the life-long incidence of fatal or potentially fatal malignant neoplasia at all body sites

	Males		**Females**	
	ad lib	80% of *ad lib*	*ad lib*	80% of *ad lib*
Number of rats	100	100	100	100
% malignant neoplasm[1]	39	13***	33	18***

***p < 0.001

1. The lower incidence of malignant neoplasms in the calorie-restricted rats occurred despite highly significantly better survival

Table 8.2: Effects of calorie restriction on thymidine labelling in various tissues in Female Swiss Webster mice (from Lok *et al* 1990)

Tissue	**% inhibition in mice restricted to 75% of *ad libitum* food intake**	**Significance**
Mammary gland	72	<0.01
Urinary bladder	43	<0.05
Oesophagus	49	<0.001
Colon (crypt cells)	54	<0.01

The seriousness of the effects of overnutrition is at long last beginning to be taken seriously, and at this Workshop it seems to be almost the consensus view that we should move as quickly as possible towards conducting all carcinogenicity tests under conditions of dietary restriction. One urgent reason for this is that, in the case of many rat and mouse strains, longevity has been getting shorter and shorter whilst the mean bodyweights of mature

animals have been getting higher and higher. Whether genetic drift is wholly responsible for this or whether overnutrition has trans-generation effects is not known. The argument that diet-restricted animals may be more susceptible to carcinogens is still put forward by some. However, the counter-argument that it is easier to see weak carcinogenic effects against a low background incidence in controls than against a high one is holding increasing sway. Furthermore, comparisons of the responses to test agents including some genotoxic and some non-genotoxic carcinogens have, so far, revealed no example in which a carcinogenic effect would have been missed if testing had been conducted only under conditions of dietary restriction.

Concluding remarks

Personally, I doubt the value of carrying out tests on chemicals that are clear-cut potent mutagens. It is only when the interpretation of tests, particularly *in vivo* tests for genotoxicity, have given equivocal results that long-term animal tests on known or possible genotoxins are needed. By contrast, in the present state of our knowledge, tests for non-genotoxic carcinogenicity on pharmaceuticals should be regarded as necessary unless adequate reassurance of safety from metabolism, pharmacokinetic and general toxicity studies is already available.

In this paper I have refrained from citing references to published work and from citing supportive data, as most of the ground I have covered here has been reviewed by me before (Roe, 1989; Roe 1991; Roe, *et al,* 1991).

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