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Confounding carcinogenicity studies —

Decreasing life span of rats poses problems in labs

By Matthew J. Palazzolo, PhD

For more than a decade, concern has been mounting over the declining survival of rats used in carcinogenicity studies, as well as over gains in average weight and an increased incidence of spontaneous cancer and other diseases in these animals. Efforts to understand the causes of the drop in survival, and to reduce its impact on studies evaluating the safety of drugs and other compounds, are under way in a number of laboratories (see sidebar on page 2).

Guidelines, many of them adopted by regulatory agencies throughout the world in the 1970s and 1980s, typically specify that chronic carcinogenicity tests for new pharmaceutical products be conducted with two species, usually mice and rats. The duration of mouse studies is 18 to 24 months, while the length of rat studies is 24 months, or 104 weeks. These times represent a significant portion of the animals' expected life span.

The standard carcinogenicity bioassay includes 50 animals of each sex for each species. Many countries have regulations that require, or strongly recommend, a 50% survival, or 25 animals per sex per group, at the end of a 2-year study with rats if the results are to be accepted (see examples on page 4). This target may no longer be obtainable with certain strains of laboratory rats. A number of laboratories are now reporting survival rates below 50% (see table on page 3), and survival rates as low as 7% at the end of 104-week studies have been reported. As a result, some completed studies may face challenges by regulatory agencies.

The survival problem centers on the CD rat, perhaps the most widely used animal for toxicology studies in the U.S. The CD strain accounted for 85% of rats used for product registration studies in 1990. The observed changes in CD rats affect treated and control animals alike, to the point where the data from long-term (chronic) studies may have reduced statistical significance and hence an ever-decreasing value in risk assessment.

Problems are reported

Diminishing life spans of CD rats were documented in the early 1980s, but the problem gained considerable attention in 1988 when the Pharmaceutical Manufacturers Association (PMA) distributed a questionnaire to member companies. Results of the questionnaire showed that 9 of 30 responding companies had encountered regulatory difficulties due to low survival rates in rodent studies.

The PMA findings were bolstered in 1989 when Charles River Labo-

ratories published information indicating unexpectedly low survival of CD rats at the end of 2-year studies in many laboratories in the U.S. and suggesting that the problem was inherent in the CD strain.

About 5 years ago, decreasing life spans of other rat strains (such as the Fischer 344) were detected, but since these strains always have lived longer than CD rats, the drop in their survival rates did not pose an immediate regulatory concern.

Changes in breeding practices, including selection for rapid growth and reproductive performance, are the primary factors that have led to the current situation. Possible contributing influences include ad libitum feeding and individual housing of the animals. These two environmental factors may be the cause of increasing average body weight in CD rats, a circumstance that has led them to be dubbed "fat rats."

Correcting the situation

A variety of remedies have been proposed to the longevity dilemma, and some are being tried. Still, a definitive method of dealing with declining survivability has yet to be developed.

Restricting or controlling the animals' diet. One possible solution to the problem of "fat rats" with reduced life spans is to adjust their dietary intake. When the original guidelines on using rats in carcinogenicity studies were drawn up, the importance of eliminating impurities in the animals' diet was recognized, but no mention was made of the optimum number of calories. Recent studies have shown that restricting or controlling the diet, rather than allowing animals to eat as much as they like, can increase longevity and decrease age-related diseases, including cancer.

If diets are controlled and the incidence of cancer is thereby reduced, a new question arises: Does decreased occurrence of disease also mean decreased sensitivity of the assay to detect carcinogenicity? Other potential limitations to dietary restriction include the possibility that this solution would not be accepted by regulatory authorities and that it would increase the effort required to maintain the animals. Even if dietary restriction is successful in improving longevity, the underlying problem is almost certainly genetic, so just changing the diet may still not offer the ultimate solution.

Group housing. The decrease in longevity of CD rats has not been observed universally. Some reports indicate that no significant reduction in the life span occurs if these animals are housed in groups of three or more, rather than individually. Group housing, however, may simply be producing a behavioral food restriction.

Rederiving the CD strain. In 1993, Charles River Laboratories addressed the concern over the reduced longevity of its so-called CD rats. The CD variety, the company noted, was developed decades earlier, having been derived from an outbred stock created in the 1920s by Sprague Dawley. The CD animals of the 1990s, however, most likely have changed considerably from their ancestors. The company concludes that these changes in longevity have come about gradually since the mid-1970s. Charles River maintains over 20 colonies of CD rats around the world, and differences most likely occur from colony to colony as well as between the current and the ancestral stocks.

As a long-term solution to the longevity problem with CD rats, Charles River plans to institute "a

Rat longevity, tumors, weight being studied at Corning Hazleton

A variety of studies have been and are being conducted in laboratories throughout the U.S. to determine the longevity and incidence of tumors for various strains of rodents used in carcinogenicity bioassays. In a 2-year study under way at Corning Hazleton, 50 animals per sex of each of four strains of rats are being housed under identical conditions to determine comparative survival, incidence of tumors, frequency of nonneoplastic lesions, food consumption, and body weight fluctuations. Halfway through the study, the CD group ("fat rats") shows an increase in food consumption and body weight over the SD group. Other changes being monitored are ocular opacities in the Fischer 344 rats and detectable masses, especially in the CD and SD females.

program to modify them in such a way as to reduce selection pressures which may be linked to the reported problems in longevity." The goal, the company stresses, is not to replace the old strain with a new strain of rats, "but to maximize and stabilize the existing genetic diversity" within the CD rats.

Choosing a different strain of rat. A number of other rat strains are available for use in carcinogenicity studies, but most do not have the years of historical background data that are available for the CD rat strain. Historical data are valuable for determining, for example, the incidence of spontaneous tumors in a specific strain of rats.

The SD rat, an outbred strain originated in 1925, and the Fischer 344 rat, an inbred strain, both have better survival rates than CD rats in chronic bioassays but also have drawbacks that keep them from being more widely used in carcinogenicity studies.

Increasing the size of groups. To improve the chance of having 25 surviving CD rats per sex in each group after 104 months, the original number could be increased. The FDA is considering allowing the number of rats in control and treated groups to rise to 60 per sex. This change would increase the cost of carcinogenicity bioassays and may not satisfy the regulatory requirements of the European Union (EU).

Reducing the length of carcinogenicity bioassays. Based on statistical considerations, the FDA appears to be giving some thought to allowing a 50% survival rate among CD rats after 80 to 90 weeks of a chronic bioassay, rather than after 104 weeks. An official guidance on this point has not been issued, however. Furthermore, it is far from certain that EU and Japanese regulatory bodies are considering a similar course of action.

Regulatory directions

Representatives of the National Center for Toxicological Research, a branch of the U.S. Food and Drug Administration (FDA), have indicated recently that more emphasis will be placed on controlling and restricting the diets of rodents used in bioassays. The aim will be to reduce the variability of test results and to enhance survival in chronic studies. The FDA plan may call for two control groups, one in which the diet matches that of the test animals and another in which the diet is manipulated so that body weights remain similar to those of the dosed animals.

Published survival rates of CD rats at 18 and 24 months in carcinogenicity studies at one laboratory, 1979-1990

Study	*Year*	*Sex*	*Percent surviving*	
			18 months	**24 months**
1	1979	M	92	70
		F	86	56
2	1980	M	92	63
		F	91	61
3	1981	M	96	58
		F	96	58
4	1981	M	96	70
		F	84	60
5	1981	M	90	67
		F	91	68
6	1982	M	95	67
		F	91	68
7	1983	M	93	69
		F	87	60
8	1984	M	93	75
		F	85	53
9	1985	M	93	74
		F	93	54

1979-1985 means at 24 months: males - 68%; females - 60%

The VAF variant of the CD strain was used after 1987.

10	1988	M	75	33
		M	71	27
		F	84	45
		F	76	51
11	1989	M	80	47
		M	62	27
		F	69	36
		F	67	35
12	1990	M	84	56
		M	86	56
		F	82	46
		F	92	50

1988-1990 means at 24 months: males - 41%; females - 44%

Source: Nohynek et al. 1993

Harmonization

For a number of years, the FDA has been taking part in meetings designed to promote harmonization of technical requirements for drug development among regulatory agencies in various countries. The International Conference on Harmonisation (ICH) has been focusing its attention on registration requirements for pharmaceutical products in the EU, Japan, and the U.S. Eventually, the ICH may be able to produce uniform guidelines to be followed when conducting rodent bioassays for carcinogenesis. The issue of carcinogenicity bioassays is scheduled for consideration at the November 1995 meeting of the ICH in Yokohama, Japan. Currently, however, fundamental differences exist between nations, and even between different agencies within a single country, over the acceptable rate of survival among rats in chronic bioassays.

Some regulatory guidelines for rat survival in chronic studies

FDA (1993 draft of "Red Book II"): "FDA guidelines no longer require 50% survival (25/50 animals per sex per group) for carcinogenicity bioassays. . . . The petitioner is encouraged to begin bioassays with more than 50 animals per sex per group." The goal is "to ensure that at least 25 rodents per sex per group survive at the end of the study."

EPA (Federal Insecticide, Fungicide, and Rodenticide Act - FIFRA): For rats, 50 males and 50 females should be used at each dose level and concurrent control. The number of survivors in any group should not fall below 50% at 18 months or 25% at the termination of a study at 24 months.

Europe (Organization for Economic Cooperation and Development Guidelines): Survival in each group of rats should be no less than 50% at 24 months for a negative test to be acceptable.

Japan (Agricultural Chemicals): The number of rats in any group should not fall below 50% at 18 months or below 25% at the termination of a study at 24 months.

Adapted from: Lang 1991 & FDA 1993

Alternatives to current bioassays

Our understanding of carcinogenesis has progressed significantly during the past half century. This new knowledge has led some researchers to conclude that the basic 2-year rodent bioassay for detecting potential carcinogens is no longer sufficient for providing the complex information needed to make decisions about risk management. Traditional rodent bioassays, critics contend, are fine for detecting overtly carcinogenic chemicals (genotoxic compounds), such as nitrosamines, but are inappropriate for nongenotoxic substances that do not necessarily cause cancer as a result of obvious genetic damage.

The need, therefore, is to devise specific methods for testing pharmaceuticals and other compounds for their potential to produce cancer by genotoxicity, immunosuppression, hormone disturbance, or chronic irritation. Methods for obtaining this information may include rodent studies of shorter duration and involving only one species. Such studies could yield toxicokinetic and mechanistic data needed to make predictions of a compound's carcinogenic potential early in the evaluation process of the chemical.

One possible change in the current bioassay methodology is to use transgenic animals, which are genetically controlled to be sensitive to genotoxic carcinogens. Transgenic mice are already being tested as models in cancer studies, although a transgenic rat model has yet to be created. The sensitivity of transgenic animals means that they will show the effects of a cancer-causing compound sooner than other animal models, and the effects of age, immune status, and hormone levels will be lessened. If and when a transgenic rat model is developed, more compounds can be tested more quickly with fewer animals and at less cost than with traditional bioassays. It will certainly be several years, however, before researchers validate transgenic models and accumulate enough historical data to make these animals attractive substitutes in carcinogenicity bioassays.

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Future directions in carcinogenicity testing

Since the incorporation of the National Cancer Institute bioassay in the 1970s, we have not progressed in developing a model that takes into consideration all the scientific advances in cancer research over the past 25 years. In fact, the current bioassay was designed to be a screening tool for compounds, and not to be the definitive study. We have depended on the current bioassay, and it has served us well in most situations. However, it has forced us to rely on low dose extrapolation of high dose conditions in animal models that have oncogenic endpoints dissimilar to those in humans.

In the next several years, changes will occur in the way we test new compounds for oncogenicity. These methods will depend on our understanding of the carcinogenic processes of initiation, promotion, and progression. Animal models will become more sensitive and have well characterized genetic composition. New carcinogenic models will make use of the quantitative assessments of dose and will incorporate endpoints that reflect mechanisms that relate to the oncogenic response. Some advances have begun with the use of transgenic animal models, but we are a long way from regulatory acceptance of these new models.

Conclusion

Eventually, additional sources of data, including human epidemiology and in vitro studies, will play greater roles in hazard assessments. Currently, however, no other tests offer more useful results than chronic rodent bioassays. Most toxicologists agree that rodent bioassays are the only proven tool for detecting carcinogens. After all, virtually all human carcinogens also cause cancer in one or more rodent species. With careful attention paid to the genetic condition of the rodents and to their diet and housing, these animals will continue to play a prominent role in protecting humans from exposure to carcinogens for many years to come.

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FDA publishes ICH guideline on validation of analytical procedures

Earlier this year, the FDA announced the publication of a final guideline on topics that should be considered during the validation of analytical procedures included as part of registration applications for pharmaceuticals. The guideline was prepared under the auspices of the International Conference on Harmonisation (ICH) and endorsed at the ICH meeting in October 1994. The guideline presents terms and definitions that "are meant to bridge the differences that often exist between various compendia and regulators of the European Union, Japan, and the United States."

The guideline describes types of analytical procedures, each with different objectives.

- Identification tests are intended to ensure the identity of any analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc.) to that of a reference standard.
- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample.
- Assay procedures are intended to measure the analyte present in a given sample. In the context of the guideline, the assay represents a quantitative measurement of the major component(s) in the drug substance.

The validation characteristics that need to be evaluated depend on the objective of the analytical procedure, and these characteristics are defined in the guideline.

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present, such as impurities, degradants, and matrix.

Accuracy expresses the closeness of agreement between the value which is accepted either as the true value or an accepted reference value and the value found.

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision is usually expressed as the variance, standard deviation, or coefficient of variation of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time.

Reproducibility expresses the precision between laboratories.

Detection limit is the lowest amount of analyte in a sample that can be detected.

Quantitation limit is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Linearity is the ability the achieve test results that are directly proportional to the concentration of analyte in the sample.

Range is the interval between upper and lower concentrations of analyte in the sample for which the procedure has suitable precision, accuracy, and linearity.

Robustness is a measure of a procedure's capacity to remain unaffected by small, but deliberate, variations in method parameters and indicates reliability during normal usage.

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