SYMPOSIUM PRESENTATION

Carcinogenesis Risk Assessment of Chemicals Using Medium-term Carcinogenesis Bioassays

Seiko Tamano

Abstract

There is a pressing need for medium term models as alternatives for two year testing of environmental compounds for carcinogenicity and toxicity. Optimally these should be of short duration *in vivo*, readily performed in the laboratory without the need for specialist equipment, be based on *a priori* reasoning and scientific principles and use effective surrogates for malignancies. The two models developed in DIMS Institute of Medical Science, the medium-term liver carcinogenesis bioassay and the medium-term multi-organ carcinogenesis bioassay, fulfil these criteria and have the massive advantage of already being used for testing of large numbers of agents.

Key Words: Two-year bioassay - carcinogenesis screening - mutagenesis - cell proliferation

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Introduction

Carcinogens can be essentially divided into DNA-reactive genotoxic and non-genotoxic types on the basis of their mechanisms of action. They can cause initiated cells to develop into preneoplastic and neoplastic lesions by stimulating clonal proliferation. We have developed medium-term liver and multi-organ carcinogenesis bioassays for the detection of carcinogens in relatively short periods based on this property.

Materials and Methods

1.Medium-term liver carcinogenesis bioassay.

Six-week-old male F344/duCrj rats were initially given a single ip injection of diethylnitrosamine (DEN) (200mg/kg b.w.) dissolved in saline to initiate hepatocarcinogenesis (see Figure 1). After 2 weeks, they received test compounds and were subjected to two-thirds partial hepatectomy (PH) at week 3. The animals were killed for quantitative analysis of glutathione S-transferase placental form (GST-P) positive liver foci at week 8. Carcinogenic potential was scored by comparing the numbers and areas per cm2 of induced GST-P positive foci in the livers of groups of about 15 rats with those of corresponding control groups given DEN alone. A positive response was defined as a significant increase in the quantitative values of GST-P-positive foci, and a negative response as no change or a decrease. The results obtained were then compared with reported Salmonella/microsome and long-term carcinogenicity test findings for the same compounds.

2. Medium-term multi-organ carcinogenesis bioassay.

Six-week-old male F344/DuCrj rats were treated sequentially with three carcinogens (DEN, 100mg/kg b.w. in saline, ip, single dose at the commencement; N-methyl-N-nitrosourea, 20mg/kg b.w. in citrate-buffered solution, ip, 4 doses on days 2, 5, 8, 11; dihydroxy-di-N-propylnitrosamine, 0.1% in drinking water during weeks 3 and 4)(DMD treatment) or with five carcinogens (DMD treatment plus N-butyl-N-(4-hydroxybutyl)nitrosamine, 0.05% in drinking water during weeks 1 and 2; 1, 2-dimethylhydrazine, 40mg/kg b.w. in saline, sc, 4 doses on days 14, 17, 20, 23)(DMBDD treatment). After these treatments the animals were given test substances for 16 (DMD regime) or 24 weeks (DMBDD regime) from week 5. All animals were killed and subjected to complete necropsy and all organs/tissues were histopathologically and immunohistochemically examined.

Results

1. Medium-term liver carcinogenesis bioassay

Of a total of 327 chemicals examined , 61 out of 66 known hepatocarcinogens (92%) gave positive results (see Table 1). Four hepatocarcinogens which proved negative belonged to the peroxizome proliferator group that depresses GST-P expression. Therefore, the positive rate for hepatocarcinogens excluding these 4 peroxisome proliferators was in fact 98% (61 out of 62). Furthermore, a high positive rate (12 out of 14, 86%) was shown for liver carcinogens confirmed only in the mouse. Carcinogens tar-

DIMS Institute of Medical Science Inc. 64 Goura, Nishiazai, Azai-cho Ichinomiya, 491-0113 Japan URL: http://www.dims.co.jp

Table 1. Positive/Examined (%) Results for 327 Compounds in the Medium-term Liver Bioassay

Carcinogenic potential	Mutagenicity (Ames test) Positive	Negative	Unknown	Total
Liver	31/32 (97)[a]	29/33 (88)[b]	1/1 (100)	61/66 (92)
Other	7/26 (27)	2/15 (13)	1/2 (50)	10/43 (23)[d]
None	0/6 (0)	1/42 (2)[c]	0/2 (0)	1/50 (2)[d]
Unknown	4/14 (29)	32/90 (36)	15/64 (23)	51/168 (30)
Total	42/78 (54)	64/180 (36)	17/69 (25)	123/327 (38)

[a]Negative; 4,4-Diaminodiphenylmethane; [b]Negative; Clofibrate, Di(2-ethylhexyl)adipate, Di(2-ethylhexyl)phthalate, Trichloroacetic acid; [c]Positive; Malathione; [d]A total of 11 chemicals were positive in this model although hepatocarcinogenicity is not proven

Table 2. Validity of the Medium-term Liver Bioassay for Hepatocarcinogens

Parameter	Including Peroxisome Proliferators	Excluding Peroxisome Proliferators
Sensitivity	61/66 (92.4%)	61/62 (98.4%)
Specificity	49/54 (90.7%)	49/50 (98.0%)
Positive predictability	61/66 (92.4%)	61/62 (98.4%)
Negative predictability	49/54 (90.7%)	49/50 (98.0%)
False positive rate	5/54 (9.3%)	1/50 (2.0%)
False negative rate	5/66 (7.6%)	1/62 (1.6%)

geting organs other than liver gave fewer positive results (10 out of 43, 23%). One of the 45 chemicals reported as non-carcinogenic, was found to be positive in this assay, but this might suggest that it is liver tumor promoter rather than being a false-positive.

Table 2 shows the validity of the assay system based on correlations with medium-term liver carcinogenesis bioassay data obtained from long-term carcinogenicity testing. The evaluation was calculated using the formula for validity of carcinogen screening tests described by Cooper et al (1979). Five categories, sensitivity, specificity, predictive value (positive predictability), false positive, rate and false negative rate were calculated and all demonstrated excellent values. Although carcinogenic potential of four chemicals that are peroxisome proliferators could not be accurately detected, even when they were included, the sensitivity, specificity, positive predictability and negative predictability were found to be 92.4%, 90.7%, 92.4% and 90.7%, respectively. It is noteworthy that the false-positive and false-negative rates so far are 2.0% and 1.6%, respectively, clearly demonstrating that this medium-term liver bioassay is excellent for detection of liver carcinogens.

2. Medium-term multi-organ bioassay

Of 65 chemicals tested, 17/17(100%) of the hepatocarcinogens and 19/22 (86%) of the non-hepatocarcinogens were positive with the DMD/DMBDD protocols and five non-carcinogens were negative (see Table 3). For substances with unknown carcinogenicity, the positive rate was 11/21(52%).

Conclusion

These medium-term liver and multi-organ bioassay systems are very useful tools for detection of not only genotoxic but also non-genotoxic carcinogens. Positive results obtained in a relatively short period closely correlate with long-term carcinogenicity. A combination of liver and multi-organ bioassay systems is indicated for detection of potential hazard of chemicals to human. Rodent systems like those described here have in fact recently become regarded as appropriate alternatives for assessment of carcinogenic risk. Further confirmation of their potential applications is clearly warranted.

Table 3. Positive/Examined (%) Results for 65 Compounds in the Medium-term Multi-Organ Bioassay

Carcinogenic potential	Mutagenicity (Ames test) Positive	Negative	Unknown	Total
Liver	12/12 (100)	5/5 (100)	0/0 (0)	17/17 (100)
Other	10/11 (91)	8/10 (80)	1/1 (100)	19/22 (86)
None	0/1 (0)	0/4 (0)	0/0 (0)	0/5 (0)
Unknown	0/1 (0)	8/13 (62)	3/7 (43)	11/21 (52)
Total	22/25 (88	21/32 (66)	4/8 (50)	47/65 (72)

References

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