The Use of the Tg.rasH2 Mouse in Carcinogenicity Studies

Joseph Younan and Lev Kolodzieyski

ITR Laboratories Canada Inc., General Toxicology Department, 19601 Clark Graham, Baie d'Urfe (Montreal), Quebec, Canada H9X 3T1

ABSTRACT

The objective of the study was to evaluate the response of the Tg.rasH2 mouse test system to two known carcinogens and to demonstrate its utility as a quicker and more cost-effective alternative to the conventional two-year mouse bioassay.

Fifteen (15) male and 15 female Tg.rasH2 mice were assigned to each of 3 dose groups. Animals of Group 1 received purified water by oral gavage once daily for 26 consecutive weeks. Animals of Group 2 received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5), while animals of Group 3 received a single intraperitoneal injection of MNU (75 mg/kg). Parameters monitored during the study included mortality, clinical observations, including examinations for the presence of palpable masses, body weights and food consumption. Upon completion of the 26-week treatment/holding period (Groups 2 and 3), all surviving animals were euthanized and subjected to a necropsy examination. Subsequently, tissues collected from all animals were examined histopathologically.

A total of 2 Control, 28 Urethane-treated and 23 MNU-treated animals died or were preterminally euthanized due to poor clinical condition during the study. Bronchiolo-alveolar carcinoma of the lungs and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity in the 2 Control females. Bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma were considered to be the cause of mortality/morbidity in the majority (26/28) of the Urethane-treated animals, whereas malignant lymphoma of the hemolymphoreticular system was the cause of death/morbidity in the in the majority (17/23) of the MNU-treated animals.

INTRODUCTION

For several decades the two-year rodent bioassay in rats and mice has been an integral part of safety testing for the carcinogenic potential of pharmaceutical agents. Unfortunately, the two-year conventional rodent bioassay is long, expensive and frequently provides ambiguous results for human risk assessment. These limitations have led to the creation of transgenic animal models, which carry genetic constructs specially designed to enhance the detection of carcinogens. Regulatory agencies now agree that the carcinogenic potential of pharmaceuticals can be evaluated from data collected from one long term conventional rat study plus data from one short-term carcinogenicity study using transgenic animals. In fact, the US FDA (CAC) has recommended the use of the rasH2 mouse for non-dermal, non-genotoxic drugs, the p53 for non-dermal, genotoxic drugs and the Tg.AC for dermal drugs. In addition, validation studies conducted in a number of laboratories around the world indicate that these transgenic lines provide a quicker and more cost-effective alternative to the traditional two-year mouse bioassay, in addition to being more susceptible to carcinogens in comparison to wild-type mice.

EXPERIMENTAL DESIGN

Fifteen (15) male and 15 female Tg.rasH2 mice were assigned to each of 3 dose groups.

Group Number and Designation	Test/Control articles	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
					Male	Female
1. Control	Purified water	0	0	10	15	15
2. Test Article 1	Urethane	1000	100	10	15	15
3. Test Article 2	MNU	75	7.5	10	15	15

Animals of Group 1 received purified water by oral gavage once daily for 26 consecutive weeks.

Animals of Group 2 received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5), while animals of Group 3 received a single intraperitoneal injection of MNU (75 mg/kg).

Parameters monitored during the study included mortality, clinical observations, including examinations for the presence of palpable masses, body weights and food consumption. Upon completion of the 26-week treatment/holding period (Groups 2 and 3), all surviving animals were euthanized and subjected to a necropsy examination. Subsequently, tissues collected from all animals were examined histopathologically.

RESULTS & DISCUSSION

A total of 2 Control, 28 Urethane-treated and 23 MNU-treated animals died or were preterminally euthanized due to poor clinical condition during the study. The deaths and preterminal sacrifices occurred between Days 39 and 173 of the study. Mortality rates were as follows:

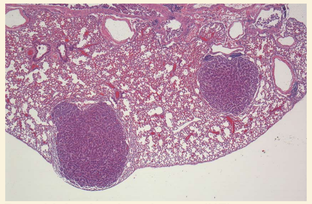
Group Number and Designation	Test/Control articles	Number of Animals		Mortality Rates	
		Male	Female	Male	Female
1. Control	Purified water	15	15	0 (0%)	2 (13.3%)
2. Test Article 1	Urethane	15	15	15 (100%)	13 (86.7%)
3. Test Article 2	MNU	15	15	12 (80%)	11 (73.3%)

Bronchiolo-alveolar carcinoma of the lungs and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity in the 2 Control females.

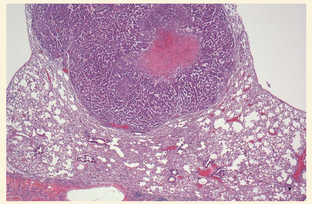
The 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5) to Tg.rasH2 mice resulted in the death or the preterminal sacrifice of all males (15/15) and 13/15 females between Days 39 and 173 of the study. Microscopic examinations performed on tissues obtained from these animals revealed a high incidence of bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma. These findings were considered to be the cause of mortality/morbidity in the majority (26/28) of these animals.

The single intraperitoneal injection of MNU (75 mg/kg) to Tg.rasH2 mice resulted in the death or the preterminal sacrifice of 12/15 males and 11/15 females between Days 72 and 170 of the study. Microscopic examinations performed on tissues obtained from the animals revealed a high incidence of malignant lymphoma of the hemolymphoreticular system. These findings were considered to be the cause of mortality/morbidity in the majority (17/23) of these animals, while hemangiosarcoma was considered to be the second most frequent tumor resulting in the death of 3/23 animals.

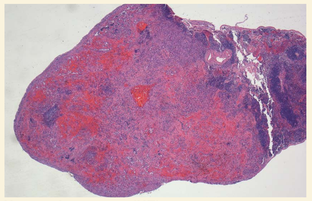
PICTURES



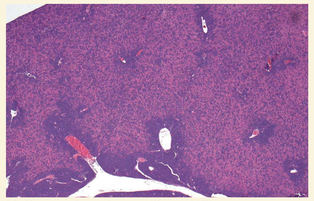
Bronchiolo-alveolar adenoma (HE)



Bronchiolo-alveolar adenocarcinoma (HE)



Hemangiosarcoma (HE)



Malignant lymphoma (liver; HE)

The cause of demise within each of the dose groups is summarized as follows:

Group	1 (Control)		2 (Urethane)		3 (MNU)	
Sex	M	F	M	F	M	F
Number of dead/sacr. Animals	0	2	15	13	12	11
Blood vessel tumors	0	1	12	5	2	1
Lung tumors	0	1	3	6	0	0
Malignant lymphoma (hemolymphoreticular system)	0	0	0	0	8	9
Subcutaneous tumors	0	0	0	0	1	0
Stomach tumors	0	0	0	1	1	0
Unclear	0	0	0	1	0	1

There were no changes in body weights or food consumption that could clearly be attributed to the administration of MNU. The administration of Urethane however, resulted in a slight and reversible decrease in body weights and food consumption during the first week of the study (Days 1 to 8) in the animals of both sexes.

CONCLUSION

In conclusion, the intraperitoneal administration of 2 known carcinogens (Urethane and MNU) to Tg.rasH2 mice followed by a 26-week holding period produced clearly higher incidences of benign/malignant tumors and tumor bearers resulting in significantly higher mortality rates among the animals treated with the carcinogens (Urethane and MNU) in comparison to Controls.

Consequently, the use of the Tg.rasH2 mouse model for carcinogenicity studies is considered a suitable quicker and more cost-effective alternative to the conventional two-year mouse bioassay and is now validated at ITR Laboratories Canada Inc.

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