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CARCINOGENIC MONITORING DATA ON RASH2 MICE TO GUARANTEE A LONG-TERM BIOLOGIC STABILITY

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Introduction

Laboratory mice, which are used for the regulatory purposes such as carcinogenicity tests, are required long-term biological stability during the mass production and over successive generations. CByB6F1-Tg(HRAS) 2Jic (rasH2) mice have been known for having a high susceptibility to human carcinogens and mainly used for the short-term carcinogenicity test for pharmaceuticals.

RasH2 mice are now produced by two major breeding facilities, Taconic (Germantown, NY, USA) and CLEA Japan (Fuji, Shizuoka, Japan). In order to guarantee the biological equivalence and stability of the phenotype of rasH2 mice, mainly carcinogenic susceptibility produced by both facilities, we have periodically compared the carcinogenic response of these mice to the standard positive control compound *N*-methyl-*N*-nitrosourea (MNU).

Full-volume monitoring in which whole organs had been studied histopathologically at 26 weeks after MNU administration was performed approximately every 5 years at the time when the breeding colonies were replaced. In between the full-volume monitoring, simple histopathological monitoring of the forestomach, the most sensitive organ to MNU, has been performed every year (Figure 1).

In this study, we present the latest results of full-volume monitoring performed in 2013 and compared with the results obtained in 2006.

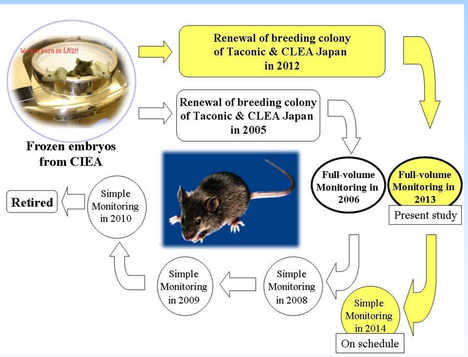

Figure 1. Standard schedule of the phenotypic monitoring in rasH2 mice

Full-volume monitoring (histopathological study of the whole organs) is performed at the time when the breeding colonies are replaced. Simple monitoring (histopathological study of the forestomach only) is performed in the other years until the renewal of breeding colonies.

Materials & Methods

Thirty male and female rasH2 mice introduced from Taconic and CLEA Japan at 6 weeks of age were divided into 15 mice / group. The mice were given a single intraperitoneal injection of vehicle or 75 mg/kg MNU.

The animals were observed until 26 weeks after vehicle or MNU administration. When they become moribund, mice were necropsied under anesthetized condition.

Weight of thymus, liver, spleen and lung were measured at necropsy.

Histopathological studies were performed for all organs to compare carcinogenic susceptibility of rasH2 mice derived from the two facilities.

The results of present study were compared to the results obtained in 2006 to investigate long-term biological stability .

Results

1. In present study, rasH2 mice produced by Taconic were slightly smaller than mice produced by CLEA Japan (Figure 2).
2. RasH2 mice produced by Taconic and CLEA Japan showed similar clinical findings during the study (Data not shown) .
3. Survival rate of MNU treated female rasH2 mice from Taconic in 2013 was shorter than mice from CLEA Japan (Figure 3).
4. Spleen and thymus weights of MNU treated mice were heavier than that of vehicle treated mice for both colonies (Figure 4).
5. In full-volume monitoring performed in 2006 and 2013, no significant difference was observed between the mice from Taconic and CLEA Japan for the incidence of major MNU-induced tumors such as forestomach papilloma / carcinoma, malignant lymphoma, skin papilloma and lung adenoma, except for male lung adenoma in 2013 (Table 1).
6. Most of non-neoplastic lesions observed in present study were similar to the background data (Data not shown) .

Table 1. Incidence of neoplasm after MNU administration in full-volume monitoring performed in 2006 and 2013

Male

Year	Treatment	Study site/ Breeder	Forestomach: Squamous cell carcinoma	Forestomach: Squamous cell papilloma	Forestomach: Papilloma/ carcinoma	Hematopoietic system: Malignant lymphoma	Skin: Squamous cell carcinoma	Skin: Squamous cell papilloma	Skin: Papilloma/ carcinoma	Skin: Kerato- acanthoma	Lungs: carcinoma	Lungs: Adenoma	Lungs: HP of Br alv.	Lungs: HP of methothelial cell	Lungs: hemangioma
1997-2000 (ILSI/HESI Int. Act.)[3]	*Control*	*12 facilities*	*0.6 % (0.0-6.7)*	*0.6 % ✱ (0.0-6.7)*	*0.0 % (0.0)*	*0.0 % (0.0)*	*0.0 % (0.0)*	*2.2 % (0.0-12.3)*	*0.0 % (0.0)*	*0.0 % (0.0)*	*0.6 % (0.0-6.7)*	*7.2 % (0.0-20.0)*	*2.2 % (0.0-6.7)*	*0.0 % (0.0)*	*0.0 % (0.0)*
	MNU	*12 facilities*	*16.3 % (0.0-40.0)*	*91.3 % (85.7-100)*	*86.2 % (86.6-100)*	*76.0 % (53.3-86.7)*	*0.0 % (0.0)*	*36.5 % (0.0-73.3)*	*36.5 % (0.0-73.3)*	*0.0 % (0.0)*	*0.0 % (0.0)*	*20.2 % (6.7-33.3)*	*19.2 % (0.0-50.0)*	*2.9 % (0.0-20.0)*	*0.0 % (0.0)*
2006 [1]	Control	CLEA Japan	0 (0/15)	0 (0/15) ✱✱	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	6.7 (1/15)	20.0 (3/15)	0 (0/15)	0 (0/15)
		Taconic	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	20.0 (3/15)	20.0 (3/15)	0 (0/15)	0 (0/15)	0 (0/15)	20.0 (3/15)	0 (0/15)	0 (0/15)
	MNU	CLEA Japan	6.7 (1/15)	100 (15/15)	100 (15/15)	93.3 (14/15)	0 (0/15)	73.3 (11/15)	73.3 (11/15)	13.3 (2/15)	0 (0/15)	6.7 (1/15)	40.0 (6/15)	0 (0/15)	0 (0/15)
		Taconic	13.3 (2/15)	93.3 (14/15)	100 (15/15)	93.3 (14/15)	0 (0/15)	73.3 (11/15)	73.3 (11/15)	33.3 (5/15)	0 (0/15)	20.0 (3/15)	20.0 (3/15)	0 (0/15)	0 (0/15)
2013	Control	CLEA Japan	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	13.3 (2/15)	0 (0/15)	0 (0/15)	0 (0/15)
		Taconic	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	6.7 (1/15)	6.7 (1/15)	0 (0/15)	0 (0/15)
	MNU	CLEA Japan	13.3 (2/15)	80.0 (12/15)	86.7 (13/15)	80.0 (12/15)	0 (0/15)	60.0 (9/15)	60.0 (9/15)	53.3 (8/15)	6.7 (1/15)	40.0 (6/15)##	6.7 (1/15)	0 (0/15)	6.7 (1/15)
		Taconic	6.7 (1/15)	73.3 (11/15)	73.3 (11/15)	93.3 (14/15)	0 (0/15)	46.7 (7/15)	46.7 (7/15)	46.7 (7/15)	0 (0/15)	0 (0/15)	13.3 (2/15)	0 (0/15)	0 (0/15)

Female

Year	Treatment	Study site/ Breeder	Forestomach: Squamous cell carcinoma	Forestomach: Squamous cell papilloma	Forestomach: Papilloma/ carcinoma	Hematopoietic system: Malignant lymphoma	Skin: Squamous cell carcinoma	Skin: Squamous cell papilloma	Skin: Papilloma/ carcinoma	Skin: Kerato- acanthoma	Lungs: carcinoma	Lungs: Adenoma	Lungs: HP of Br alv.	Lungs: HP of methothelial cell	Lungs: hemangioma
1997-2000 (ILSI/HESI Int. Act.)[3]	*Control*	*12 facilities*	*0.0 % (0.0)*	*1.7 % ✱ (0.0-13.3)*	*0.0 % (0.0)*	*3.4 % (0.0-26.7)*	*0.0 % (0.0)*	*3.4 % (0.0-7.1)*	*0.0 % (0.0)*	*0.0 % (0.0)*	*1.7 % (0.0-7.1)*	*8.4 % (0.0-20.0)*	*2.2 % (0.0-13.3)*	*0.0 % (0.0)*	*0.0 % (0.0)*
	MNU	*12 facilities*	*16.2 % (0.0-33.3)*	*90.5 % (80.0-100)*	*98.1 % (93.3-100)*	*76.2 % (53.3-100)*	*4.8 % (0.0-13.3)*	*41.0 % (0.0-93.3)*	*36.5 % (0.0-73.3)*	*4.8 % (0.0-13.3)*	*1.0 % (0.0-6.7)*	*24.8 % (13.3-33.3)*	*24.8 % (0.0-46.7)*	*8.6 % (0.0-60.0)*	*0.0 % (0.0)*
2006 [1]	Control	CLEA Japan	0 (0/15)	6.7 (1/15) ✱✱	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)
		Taconic	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	6.7 (1/15)	0 (0/15)	0 (0/15)
	MNU	CLEA Japan	13.3 (2/15)	86.7 (13/15)	86.7 (13/15)	80.0 (12/15)	6.7 (1/15)	60.0 (9/15)	60.0 (9/15)	33.3 (5/15)	0 (0/15)	20.0 (3/15)	20.0 (3/15)	0 (0/15)	0 (0/15)
		Taconic	26.7 (4/15)	93.3 (14/15)	100 (15/15)	93.3 (14/15)	6.7 (1/15)	60.0 (9/15)	66.7 (10/15)	40.0 (6/15)	0 (0/15)	26.7 (4/15)	53.3 (8/15)	0 (0/15)	0 (0/15)
2013	Control	CLEA Japan	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	6.7 (1/15)	0 (0/15)	0 (0/15)	6.7 (1/15)	0 (0/15)	0 (0/15)
		Taconic	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)
	MNU	CLEA Japan	0 (0/15)	86.7 (13/15)	86.7 (13/15)	86.7 (13/15)	6.7 (1/15)	20.0 (3/15)	26.7 (4/15)	26.7 (4/15)	6.7 (1/15)	13.3 (2/15)	46.7 (7/15)#	6.7 (1/15)	0 (0/15)
		Taconic	0 (0/15)	73.3 (11/15)	73.3 (11/15)	86.7 (13/15)	6.7 (1/15)	20.0 (3/15)	26.7 (4/15)	6.7 (1/15)	0 (0/15)	26.7 (4/15)	13.3 (2/15)	0 (0/15)	0 (0/15)

*: Upper and lower values indicate the mean and range, respectively
✱✱: Incidence (Number of animals induced the tumor / Number of animals examined)
HP: hyperplasia, Br alv.: Bronchiolo-alveolar
#: p<0.05, ##: p<0.01 vs corresponding group of another breeder

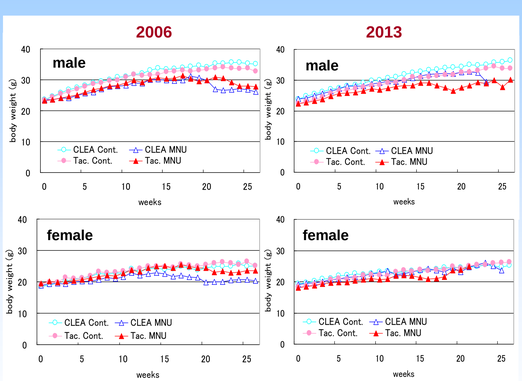

Figure 2. Body weight gain of rasH2 mice produced by Taconic and CLEA Japan

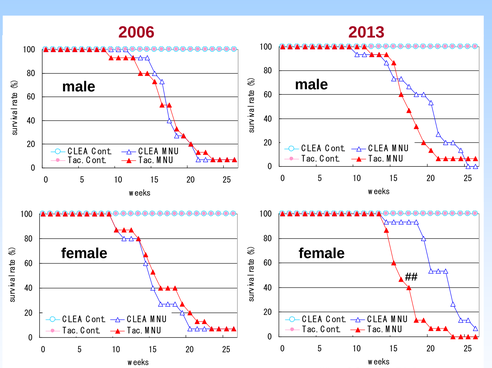

Figure 3. Survival rate of rasH2 mice produced by Taconic and CLEA Japan

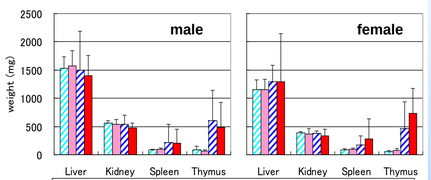

Figure 4. Organ weights of rasH2 mice produced by Taconic and CLEA Japan

Discussion and Conclusion

Although rasH2 mice produced by Taconic were smaller than mice produced by CLEA Japan in present study, they showed similar clinical findings during the study.

Survival curve after MNU treatment in female produced by Taconic in present study was shorter than that of female from CLEA Japan, but they started to become moribund or died simultaneously and final survival rates were similar.

The most of well known MNU-induced tumors were similarly observed in both colonies and they coincided with the background data including the study in 2006. Incidence of lung adenoma induced in MNU treated male from CLEA Japan was higher than that in mice from Taconic and the background data. The manufacturing company of MNU was changed in present study since the former company stopped producing MNU. It may be a reason for a fewer tumor incidences observed in present study that were different from the background data.

Incidences of spontaneous neoplasm in present study were also as low as the background data.

Considering these results, we concluded that no difference was recognized in carcinogenic susceptibility of rasH2 mice produced by Taconic and CLEA Japan and their carcinogenic susceptibility have been well maintained since the ILSI/HESI International Act. project.

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