

Armen K. NERSESYAN¹,
Victor A. KOUMKOUMADJIAN²

The comparative mutagenic action of 7,12-dimethylbenz(a)anthracene in the Armenian hamsters and mice

The comparative mutagenic action of 7,12-dimethylbenz(a)anthracene (DMBA) on mice and the Armenian hamsters (AH) was studied by means of micronucleus (MN) test in bone marrow polychromatic erythrocytes. It was shown that MN level in bone marrow cells of the AHs caused by DMBA was significantly lower than in mice although the carcinogen was used at equitoxic doses. Our data show that the AHs are much more resistant to mutagenic action of DMBA than mice. This may be due to the unique resistance of the AHs to the toxic action of chemical agents.

¹DEPARTMENT OF GENETICS, STATE UNIVERSITY,
YEREVAN, ARMENIA

²LABORATORY OF CARCINOGENESIS, CANCER
RESEARCH CENTER, YEREVAN, ARMENIA

KEY WORDS: *Micronucleus tests; 7,12-Dimethylbenz(a)anthracene; Cricetulus; Rats; Mice*

Archive of Oncology 2001;9(3):193-194 ©2001, Institute of Oncology Sremska Kamenica, Yugoslavia

The Armenian hamster (AH), *Cricetulus migratorius*, was first used in biomedical research by Yerganian and Papoyan (1). This rodent has a low diploid number of 22 chromosomes, like the Chinese hamster, and was analyzed using routine and differential staining (1,2).

The AHs are successfully used in mutagenesis studies such as detection of reciprocal translocations in the germ cells (3), chromosomal aberrations induced by cyclophosphamide (CP) and thiotepa in bone marrow cells (4,5). These experiments show the increased resistance of AHs to clastogenic action of drugs and metaphase arresting ability of colchicine compared to mice, rats, guinea pigs and the Chinese hamsters.

The aim of our work was to study comparative mutagenic action of strong rodent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) in AHs and mice.

The experiments were performed with AHs, trapped in the region of Yerevan (Armenia) and maintained in our animal room (20 male AHs, weighing 25 g). We used also 20 male random-bred albino mice (22 g b.w.) from animal room of Cancer Research Center, Yerevan (Armenia). The animals were maintained in our animal room on standard rodent diet (specific for each species) and water *ad libitum*.

To study the comparative mutagenic action of DMBA (Fluka,

Buchs, Switzerland), the carcinogen was dissolved in olive oil and was injected twice apart intraperitoneally into rodents at doses equal to 1/10 of LD50 (22 mg/kg and 40 mg/kg for mice and AHs, respectively) (6). As a negative control the vehicle of DMBA was used (olive oil). For positive control we used the CP (Mosmedpreparati, Russia) dissolved in saline and injected intraperitoneally at single dose of 25 mg/kg b.w. for mice, and 50 mg/kg for AHs. Bone marrow sampling was done 24 h after the last chemical agent injection and processed as described earlier (7). Slides were stained with acridine orange according to Tinwell and Ashby (8). We used the LM-4 fluorescence microscope (USSR) to observe the slides. Each slide was assessed for micronucleated (MN) polychromatic erythrocytes (PEs) among 1000 erythrocytes. In addition, the per cent content of PE was calculated.

Statistical analysis was performed by using Student's *t* tests. The data concerning MN inducing action of DMBA are presented in Table 1.

Table 1. Comparative mutagenic action of DMBA in the Armenian hamsters and mice

Species	Number of rodents	Treatment (dose in mg/kg)	MN PE ±S.E. (%)	Range	PE among 1000 erythrocytes(%)
AHs	5	-	0.6±0.4*	0-1	58.4
	10	DMBA (40)	9.7±1.6*	3-18	54.4
	5	CP (50)	12.2±2.2*	6-16	52.2
Mice	5	-	1.8±0.4	1-3	53.6
	10	DMBA (22)	16.8±1.5	12-26	47.8
	5	CP (25)	19.8±1.9	15-24	51.8

* significant difference with corresponding mouse data

Address correspondence to:

Dr. Armen K. Nersesyan, Department of Genetics, State University, 1 Alex Manoukian Street, Yerevan 25, Armenia

The manuscript was received: 17. 08. 2001.

Provisionally accepted: 24. 09. 2001.

Accepted for publication: 28.09. 2001.

We observed that at equitoxic doses the carcinogen induced significantly lower level of MN in bone marrow polychromatic erythrocytes than in mice ($p<0.05$). Hence, AHs are significantly

Nersesyan A.

more resistant to the mutagenic action of DMBA than mice, because 1.8-fold greater dose of carcinogen induced significantly lower level of MN in PEs of mice than in AHs. As in earlier experiments, the AHs were significantly resistant to the mutagenic action of CP (4) because two-fold more dose of drug induced significantly lower level of MN in PEs in bone marrow of the AHs than in mice. It is noteworthy that spontaneous level of MN in the AHs is significantly lower than that of mice ($p < 0.01$). In our previous experiments we had also shown that spontaneous level of chromosomal aberrations in bone marrow cells of the AHs was significantly lower than in rats and mice (4,5). In all cases there were no differences in the number of PE before and after carcinogen treatment, as evidenced by non-toxicity for haemopoietic cells.

We suggest that the resistance of the AHs to the MN-inducing action of chemical agents may be due to the unique resistance to their toxic action. It has been shown that AHs are much more resistant to the toxic action of CP, 5-fluorouracil, melphalan, DMBA and poisons used to kill the rodents, than mice and rats (6, 9). For example, the LD₅₀ of DMBA for the AHs is about 400 mg/kg b.w. after intraperitoneal injection, much more than this value for random-bred mice (220 mg/kg b.w.).

Hayashi et al (10) studied the toxicity and MN-inducing activity of 17 chemical agents in two strains of inbred mice. They showed that in most cases CD-1 mice were more resistant to the toxic and MN-inducing activity of chemicals than MS/Ac mice were. It is noteworthy that AHs have unique sensitivity to carcinogenic and toxic action of estrogen (11). This hepatocellular carcinoma hamster model is unique because estrogen alone without any other known mutagen is responsible for induction of tumors. In addition, the AHs are also extremely sensitive to tularemia microbes including live vaccine (12).

In conclusion we suggested that AHs are much more resistant to mutagenic action of DMBA than mice, and most likely it is due to their unique resistance to the toxic action of chemicals. Further investigations are certainly warranted to explain the reasons of resistance of the AHs to the toxic and mutagenic action of chemicals.

- and 4 other species of rodents. *Mutation Res* 1992;268:211-5.
5. Nersesyan AK. Clastogenic action of thiotepa on bone marrow cells of the Armenian hamster. *Mutation Res* 1994;322:287-90.
 6. Koumkoumadjian VA. Toxicity of some chemicals for the Armenian hamsters. *J Exper Clin Med* 1976;16:425-9.
 7. Nersesyan AK, Muradyan RE. The influence of immunization with tularemia vaccine on the clastogenic action of cyclophosphamide in mice. *Mutation Res* 1996;356:225-8.
 8. Tinwell H, Ashby J. Comparison of acridine orange and Giemsa stains in several mouse bone marrow micronucleus assays - including a triple dose study. *Mutagenesis* 1989;4:476-81.
 9. Koumkoumadjian VA. The use of the Armenian hamsters in biology and oncology (dissertation). Yerevan: State Medical University; 1974.
 10. Hayashi M, Sutou S, Shimada H, Sato S, Sasaki Y and Wakata A. Difference between intraperitoneal and oral gavage application in the micronucleus test. *Mutation Res* 1989;223:329-44.
 11. Coe JE, Ishak KG, Ross MJ. Estrogen-induced hepatic toxicity and hepatic cancer: differences between two closely related hamsters. *Liver* 1998;18:343-51.
 12. Nersesyan AK. Modification of mutagenesis and carcinogenesis by means of some biologically active agents (dissertation). Yerevan: Institute of Biochemistry; 1997.

REFERENCES

1. Yerganian G, Papoyan SA. Isomeric sex chromosomes, autosomal heteromorphism and telomeric associations in the gray hamster of Armenia. *Cricetulus Migratorius Pall Hereditas* 1964;52:307-19.
2. Lavappa KS. Chromosomal banding patterns and idiogram of the Armenian hamster, *Cricetulus migratorius Pall*. *Cytologia* 1977;42:6572-9.
3. Lavappa KS, Yerganian G. Latent meiotic anomalies related to an ancestral exposure to a mutagenic agent. *Science* 1971;172:171-4.
4. Nersesyan AK, Zilfian VN, Koumkoumadjian VA. Comparative investigation of cyclophosphamide action on bone marrow cells of the Armenian hamster