Eur J Cancer Clin Oncol, Vol. 24, No. 4, pp. 803–805, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

Letter to the Editor

Stimulation by Caffeine of Spontaneous Mammary Tumorigenesis in Mice

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IN 1983, Welsch *et al.* [1] reported that caffeine acted as a promoter of carcinogen-induced mammary tumors in rats. We [2, 3] also found in mice that the growth of mammary hyperplastic alveolar nodules (HAN), a major preneoplastic state of mammary tumors of this species, was significantly enhanced by chronic ingestion of caffeine. Based on these observations, the effects of caffeine on spontaneous mammary tumorigenesis in a low mammary tumor strain of C3H/He mice [4] were studied in this communication. The development and the progression of uterine adenomyosis [5–8] were also examined in these mice, since the relationship between the incidences of these mammary and uterine lesions were seen in four strains of mice with different mammary tumor potentials [9].

At weaning on day 20 of age, half of each female litter were given caffeine (Sigma Co., St Louis, MO, U.S.A.) in drinking water at a concentration of 0.05% (500 mg/l tap water) throughout the experiments and the other half received tap water only as a control. All mice were bred on day 63 and retired after the first lactation [10]. These were regarded as 'Breeders' and female litters from 'Breeders' were used as 'Virgins', which were continued to receive tap water containing caffeine (0.05%) or tap water only. Each mouse was palpated for mammary tumors every 7 days until 3 weeks after the first tumor appearance, death or 25 months of age. At autopsy, the bilateral third

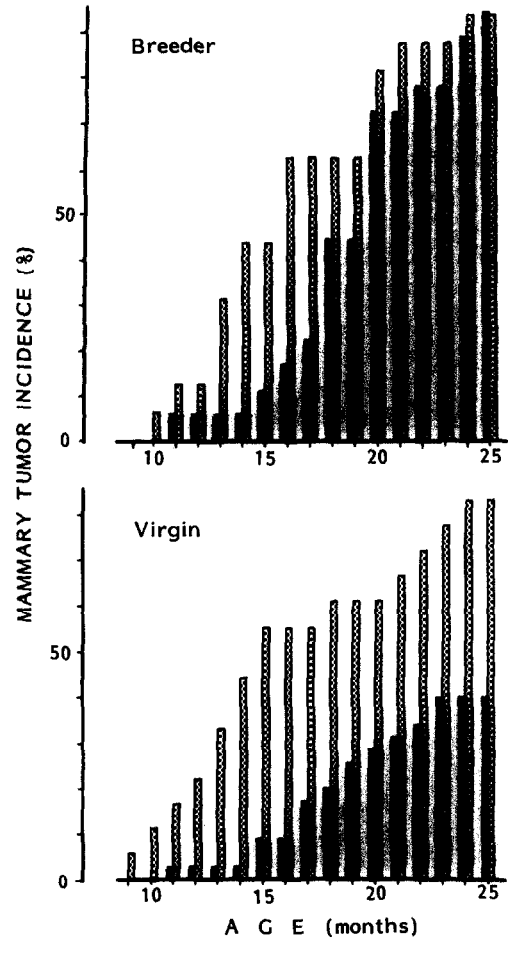

Fig.1. Effects of caffeine on spontaneous mammary tumorigenesis in C3H/He mice. Mammary tumorigenesis was significantly higher in caffeine treated mice (▣) than in the control (■) in both 'Breeder' and 'Virgin' at $P < 0.01$ *by analysis of variance considering simultaneously the incidence and the onset age of tumors. Numbers of mice examined in the control and caffeine-treated groups are* 18 *and* 16 *in 'Breeder' and* 36 *and* 18 *in 'Virgin', respectively.*

Accepted 8 December 1987.
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Table 1. Effects of caffeine on normal and preneoplastic mammary gland growth and uterine adenomyosis in C3H/He mice

Group		Mammary gland		Adenomyosis		No. and age (months) of mice which died developing no tumors
		End-bud rating*	Number of HAN†	Incidence (%)	Grade‡	
Breeder	Control	1.4 ± 0.2§ (18)	18.0 ± 2.4 (18)	83.3 (15/18)"	2.1 ± 0.3 (15)	0 —
	Caffeine	1.7 ± 0.1 (14)	28.9 ± 3.5** (14)	93.8 (15/16)	2.0 ± 0.2 (15)	1 17.0
Virgin	Control	1.3 ± 0.1 (30)	12.6 ± 2.3 (30)	41.7 (15/36)	1.7 ± 0.2 (15)	2 14.0, 21.0
	Caffeine	1.2 ± 0.1 (16)	19.5 ± 3.1¶ (16)	55.6 (10/18)	1.3 ± 0.2 (10)	2 7.0, 9.0

*Rated from 1 to 7 in increments of 1 as an index of end-bud formation. Mean of the bilateral glands.
†Sum of the bilateral glands.
‡Rated from 1 to 5 in increments of 1 as an index of progression.
§Mean ± S.E.M.
"Number of mice with adenomyosis/total number of mice examined.
¶ or ** Significantly different from the control at $P < 0.05$ or 0.01.

thoracic mammary glands were mounted for preparation to be checked for the formation of normal end-buds and HAN and uteri were examined adenomyosis histologically. All procedures were the same as detailed previously [9].

As shown in Fig. 1, spontaneous mammary tumorigenesis was significantly stimulated by caffeine, especially in virgin mice. Caffeine-treated mice were also higher than the control in the number of HAN in both breeders and virgins, but not in normal mammary end-bud formation (Table 1). Caffeine showed no effects on the number and the growth of mammary tumors (data not shown).

The effects of caffeine on benign and malignant breast lesions in humans are still far from understood [11, 12]. However, the present results in mice are in good agreement with those in rats [1, 13]. Furthermore, Welsch *et al.* [14] have most recently reported that chronic ingestion of caffeine resulted in a stimulation of spontaneous and carcinogen-induced mammary tumorigenesis in C3H and BALB/c mice, respectively.

The development and the progression of uterine adenomyosis were affected little by caffeine treatment in either breeders or virgins.

According to Welsch *et al.* [1], daily consumption of caffeine in rats was 15 mg and it was equivalent to the consumption of 16 cups of coffee per day for 60 kg women if corrected to metabolic weight. In this study, each mouse received a daily dose of 2.5 mg caffeine based on the volume of water intake. The dose was about 1.7 times as high as that in rats when adjusted by body weight.

Acknowledgements—We thank T. Naito and N. Sakagami in our laboratory for their help.

REFERENCES

1. Welsch CW, Scieszka KM, Senn ER, DeHoog JV. Caffeine (1,3,7-trimethylxanthine), a temperate promoter of DMBA-induced rat mammary gland carcinogenesis. *Int J Cancer* 1983, **32**, 479–484.
2. Nagasawa H, Sakurai N, Ishida M. Estimation by caffeine of the onset time of precancerous mammary hyperplastic alveolar nodules in mice. *IRCS Med Sci* 1985, **13**, 29.
3. Nagasawa H, Konishi R. Precancerous mammary hyperplastic alveolar nodules in four strains of virgin mice with different mammary tumor potentials: influence of chronic caffeine ingestion. *Eur J Cancer Clin Oncol* 1987, **23**, 1019–1023.
4. Nagasawa H, Yanai R, Taniguchi H, Hayashi S. Mammary tumour incidence in relation to the pattern of oestrous cycles in mice. *Hormone Res* 1979, **10**, 123–129.
5. Mori T, Nagasawa H, Takahashi S. The induction of adenomyosis in mice by intrauterine pituitary isografts. *Life Sci* 1981, **29**, 1277–1282.
6. Mori T, Nagasawa H, Nakajima Y. Strain-difference in the induction of adenomyosis by intrauterine pituitary grafting in mice. *Lab Anim Sci* 1982, **32**, 40–41.
7. Mori T, Nagasawa H. Alteration of the development of mammary hyperplastic alveolar nodules and uterine adenomyosis in SHN mice by different schedules of treatment with CB-154. *Acta Endocrinol* 1984, **107**, 245–249.

8. Ohta Y, Mori T, Nagasawa H. Ultrastructural changes of endometrium and myometrium during development of adenomyosis in mice with ectopic pituitary transplants. *Zool Sci* 1985, **2**, 239–247.
9. Nagasawa H, Konishi R, Naito T, Ohmiya S, Mori T. Relationship between mammary tumorigenesis and uterine adenomyosis in four strains of mice. *In Vivo* 1987, **1**, 141–146.
10. Nagasawa H, Sakurai N. Effects of chronic ingestion of caffeine on mammary growth and reproduction in mice. *Life Sci* 1986, **39**, 351–357.
11. Ernster VL. Epidemiologic studies of caffeine and human health. In: Spiller GA, ed. *The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects*. New York, Alan R Liss, 1984, 377–400.
12. Pozniak PC. The carcinogenicity of caffeine and coffee: a review. *J Am Dietetic Assoc* 1985, **85**, 1127–1133.
13. Minton JP, Abou Issa H, Foecking M, Sriram MG. Caffeine and unsaturated fat diet significantly promotes DMBA induced breast cancer in rats. *Cancer* 1983, **51**, 1249–1253.
14. Welsch CW, DeHoog JV, O'Conner DH. Caffeine consumption and development of the carcinomatous and normal mouse mammary gland. *Proc Am Assoc Cancer Res* 1987, **28**, 158.