3. Studies of Cancer in Experimental Animals

Only one study on the carcinogenicity of conjugated equine estrogens plus progestogens was reviewed in the previous monograph (IARC, 1999; Sakamoto *et al.*, 1997a).

3.1 Oral administration

3.1.1 *Mouse*

In a study to compare estrogen therapy with combined estrogen–progestogen therapy that used conjugated equine estrogens or conjugated equine estrogens plus medroxyprogesterone acetate, three groups of 14 female SHN mice (a strain that has a high spontaneous rate of mammary tumours and uterine adenomyosis), 71 days [about 10 weeks] of age, were fed 0 (controls) or 1.875 mg/kg of diet conjugated equine estrogens (Premarin®) with or without 7.5 mg/kg of diet medroxyprogesterone acetate (Provera®) for 230 days. Based upon a daily dietary intake of 2–3 g per mouse weighing 20–30 g, the daily intakes of conjugated equine estrogens and medroxyprogesterone acetate were calculated to be 0.19 and 0.75 mg/kg bw per day, respectively. Mice were killed 20 days after the appearance of a palpable mammary tumour or at 300 days of age. The significance of differences was evaluated by the χ^2 test. The incidence of mammary tumours [of unspecified histopathology] did not differ in the three groups (control, 4/14; estrogen alone, 6/14; estrogen–progestogen, 5/14). However, treatment with estrogen–progestogen shortened the latent period of mammary tumorigenesis by 44 days ($p < 0.05$ versus controls; not statistically significantly different from estrogen only). Treatment with estrogen–progestogen completely suppressed the development of uterine adenomyosis (0/14 versus 5/14 controls or 6/14 estrogen only-treated mice, $p < 0.01$) (Sakamoto *et al.*, 1997b). [These results are somewhat confounded by the potential influence of endogenous ovarian hormones that are reduced in the postmenopausal state. Endogenous estradiol levels in controls (3.91 ± 1.16 pg/mL) were significantly lower ($p < 0.01$) than those in estrogen only-treated (28.15 ± 2.91 pg/mL) and estrogen–progestogen-treated (20.15 ± 1.37 pg/mL) mice. However, the levels in hormone-treated mice were physiological and did not exceed that observed on day 1 of the estrus cycle (Raafat *et al.* 1999).]

3.1.2 *Monkey*

In one study, ovariectomized cynomolgus monkeys (*Macaca fascicularis*), 5–13 years of age, were treated for 2.5 years with either conjugated equine estrogen alone (Premarin®) (equivalent to 0.625 mg per woman per day; 22 animals) or in combination with medroxyprogesterone acetate (Cycrin®) (equivalent to 2.5 mg per woman per day; 21 animals) in

the diet or were untreated (26 animals). Determination of serum hormone levels of estradiol and medroxyprogesterone acetate confirmed the completeness of ovariectomy. The experiment was terminated at the end of the treatment phase. Mammary gland atrophy was seen in control animals. Eighty-six per cent (18/21) of the estrogen–progestogen-treated animals had mammary hyperplasia, defined as greater mammary gland development than that seen in animals with normal cycles ($p = 0.0065$). Forty-one per cent (9/22) of the animals given estrogen only had mammary hyperplasia. No neoplasms were observed (Cline *et al*., 1996).

In a subsequent, similar study, a progestogen-only group was added and the treatments were administered in the diet for 3 years. Ovariectomies were carried out 3 months before the start of treatments. The treatment groups were controls (no treatment; 27 animals), conjugated equine estrogen-treated (0.625 mg per woman per day equivalent; 27 animals), medroxyprogesterone acetate-treated (2.5 mg per woman per day equivalent; 26 animals) and estrogen–progestogen-treated (0.625 mg per woman per day equivalent conjugated equine estrogen plus 2.5 mg per woman per day equivalent medroxyprogesterone acetate; 26 animals); mean age at the end of the study was 7.5 years. The effective number of control animals was 20. Mammary gland lobuloalveolar hyperplasia was increased with estrogen-only treatment (effective number of animals, 25) and the effect was further increased with estrogen–progestogen treatment (effective number of animals, 26) [incidence not provided]; this development exceeded that usually seen in premenopausal animals with normal cycles. Progestogen-alone treatment (effective number of animals, 19) did not increase hyperplasia. No neoplasms, ductal hyperplasia or atypia were observed (Cline *et al*., 1998).

In a third study (Cline *et al.*, 2002a) designed to assess the effect of tibolone, a similar experimental protocol and the same doses were used as those described by Cline *et al.* (1996). Twenty-eight to 31 ovariectomized monkeys (6–8 years of age) per group were treated for 2 years. Mammary lobuloalveolar hyperplasia was observed in 19/30 (63%) control, 27/28 (96%, $p < 0.001$) estrogen only-treated and 28/29 (97%, $p < 0.001$) estrogen–progestogen-treated animals. No neoplasms were observed.

3.2 Administration with a known carcinogen

Rat

7,12-Dimethylbenz[*a*]anthracene

Female Sprague-Dawley rats, 48 days [about 7 weeks] of age, were divided into four groups of seven rats per group and were administered: 7,12-dimethylbenz[*a*]anthracene (DMBA) alone (as a single intravenous injection of 5 mg); DMBA and were oophorectomized; DMBA plus conjugated estrogens (Premarin®) at a concentration of 1.875 mg/kg of diet and were oophorectomized; or DMBA plus Premarin® plus medroxyprogesterone acetate (Proveza®) at a concentration of 7.5 mg/kg of diet and were oophorectomized. The animals were autopsied at 285 days [about 41 weeks] of age. Mammary tumours were found in 6/7 rats given DMBA, 0/7 given DMBA plus oophorectomy, 5/7 given DMBA plus Premarin® plus oophorectomy and 5/7 given Premarin® plus medroxyprogesterone acetate

plus oophorectomy. Thus, oophorectomy completely inhibited mammary tumour development, but conjugated estrogen with or without medroxyprogesterone acetate markedly stimulated mammary carcinogenesis in the ovariectomized rats (Sakamoto *et al*., 1997a).