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Serum and Tissue Levels of Estradiol During Estrogen-induced Renal Tumorigenesis in the Syrian Hamster

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The estrogen-induced renal tumor in the hamster has emerged as a major animal model in hormonal carcinogenesis. However, a fundamental aspect of this experimental model has as yet not been investigated. In the present study, comparisons between the serum and tissue 17β-estradiol (E_2) levels in cyclic female hamsters and corresponding hormone levels in E_2-treated castrated male hamsters have been made. Data is provided concerning the concentration of estrogenic hormones in the serum and target tissue typically required to elicit renal tumorigenesis in this species. Serum E_2 levels in the cyclic female hamster average 79 pg/ml on days 1–2 and 311 pg/ml on days 3–4, attaining a maximum of 358 pg/ml on day 4 of the cycle. Elevation in uterine, renal and hepatic E_2 tissue levels during days 3–4 of the cycle reflect increases in serum E_2 levels which were 3.0-, 2.0-, and 2.6-fold higher when compared to day 1 of the cycle in these tissues. As expected, serum E_2 levels of untreated castrated male hamsters did not appreciably vary over a 6 month period of aging and averaged about 32 pg/ml. Under conditions which produced essentially 100% renal tumor incidence, a rapid rise in serum E_2 levels, averaging 71.0-fold higher than untreated castrated levels, was seen. A steady state serum E_2 level of 2400 to 2700 pg/ml was maintained from 45–180 days of continuous estrogen treatment. Compared to kidneys of untreated hamsters, renal E_2 levels in E_2-treated hamsters rose only on average 5.4-fold between 15–180 days of hormone exposure. Serum levels of E_2-treated hamsters were 5.7- to 8.0-fold higher than those observed in cyclic female hamsters on days 3 and 4. However, at these higher E_2-treated serum levels there was no apparent effect either on weight loss or mortality of the animals.

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INTRODUCTION

In the past two decades a causal association has been made between estrogens, either natural or synthetic, and tumor development in man [1, 2] and animals [3]. Many mammalian species, including primates [4], are susceptible to neoplastic transformation in a variety of sites after continuous exposure solely to estrogenic hormones. Causal associations between estrogens and endometrium, breast, vagina/cervix, and liver cancer have been made [1]. In the absence of any identifiable chemical or physical intervening agent(s) associated with the etiology of prevalent sex-hormone related human cancers, hormones themselves, whether exogenous or endogenous in origin, become probable etiologic agents. To study this phenomena, the estrogen-induced renal tumor in the hamster described by Kirkman and Bacon [5], has emerged as a major experimental animal model in hormonal carcinogenesis. Briefly, multiple bilateral renal tumors are induced by both natural and synthetic estrogens in both intact and castrated male Syrian hamsters with an incidence approaching 100%. Intact female hamsters do not generate renal tumors in response to carcinogenic estrogens, and ovariectomized females exhibit either low or no renal tumor incidence. Spontaneous kidney tumor incidence have not been found in this species in numerous large colonies [6–8]. Recently, this hormonally-induced kidney tumor model system has been reviewed by us [9, 10]. Based on considerations in those reviews [9, 10], estrogens are regarded as complete weak non-genotoxic carcinogens. Despite intensive study [11–17], a fundamental aspect of this

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experimental model has not as yet been investigated; i.e. information concerning the concentration of estrogenic hormones in the serum and in the target tissue typically required to elicit renal tumorigenesis in this species. It has been established that 17β-estradiol (E_2) and other potent estrogens are highly carcinogenic in the hamster kidney [18]. In the present study, we have used RIA to determine the concentration of E_2 in the serum, kidney and liver of castrated male hamsters treated with E_2, and compared these data to appropriate tissues of cyclic female hamsters.

EXPERIMENTAL

Animals and treatment

Adult castrated male and virgin intact female Syrian golden hamsters weighing 90–100 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in facilities certified by the American Association for the Accreditation of Laboratory Animal Care. They were acclimated for at least 1 week prior to use and were maintained on a 12-h light:12-h dark cycle, fed certified rodent chow (Ralston-Purina 5002) and tap water *ad libitum*. The animal studies were carried out in adherence to the guidelines established in the *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Resources (NIH 1985). In male castrated hamsters, pure E_2 pellets (20 mg) were implanted in the shoulder region as described previously [19]. Pellets were implanted every 45 days to maintain hormone levels. Groups of 4–6 castrated male hamsters were treated for 15–105 days with E_2, and killed after 15 days and, thereafter, at 30 day intervals. The mean daily absorption of E_2 pellets, estimated as described previously, was 120 ± 18 μg [20].

Estrous cycle

Employing the protocol of Scharmann *et al.* [21], estrous cycles in female hamsters were monitored daily, between 10:00 a.m. and 12:00 p.m. The appearance of post-estrous vaginal discharge was assumed as day 1 of the estrus cycle, estrus/metestrus; day, 2 was early diestrus; day 3, mid diestrus; and day 4, late diestrus [20]. Only those hamsters that displayed three consistent and consecutive 4-day cycles were used in this study. Groups of four hamsters were killed in each day of the cycle between 14:00 and 15:00 h, to obtain the highest serum E_2 values as reported previously [22].

Serum and tissue collection

In all animals, blood was collected under ether anesthesia via the abdominal aorta. Clots were removed and the serum was centrifuged, decanted, pipetted, and frozen for later assay. After blood collection, the hamsters were perfused via the inferior vena cava with 0.9% NaCl. Following the perfusion, kidneys and liver were rapidly removed. In female hamsters, uteri were also removed.

Extraction of E_2

All tissues were rinsed, minced, weighed and homogenized in chilled glass–glass tissue homogenizers with 3 vol/g in 0.9% saline solution. Homogenate aliquots (1 ml) were extracted 3 times with ether (12 ml total). The extracts were separated by centrifugation, collected in round bottomed flasks and evaporated under nitrogen. The residues were reconstituted in 1 ml of absolute ethanol and transferred to 5 ml polypropylene tubes. After evaporation of the ethanol, the samples were stored at -20°C together with the serum samples until RIA analyses.

Radioimmunoassay

E_2 levels were determined in serum and tissue extracts using the double antibody RIA kit designed for the quantitative measurement of E_2 from Diagnostic Products Co. (Los Angeles, CA). Extracted tissue samples and serum from E_2-treated hamsters were dissolved and diluted with charcoal-treated bovine serum. In recovery experiments, 50 or 100 pg of E_2 were added to serum or tissue sample extracts and the recovery rate was between 90 and 95%.

Protein determination

The homogenates of the tissue samples were analyzed for protein content by the method of Lowry *et al.* [23] using bovine serum albumin as the standard. Data obtained for the hormone levels in hamster tissues are expressed as pg of E_2 per mg of protein.

RESULTS

Serum and tissue levels of E_2 in cyclic female hamsters

Table 1 summarizes the E_2 levels in the serum and in the uterus, kidney and liver of female hamsters during various stages of the estrous cycle. There was a significant rise in E_2 levels in the serum and in all tissues studied on days 3 and 4, compared to E_2 levels found on day 1 of the cycle. The elevated serum levels of E_2 were reflected in concomitant rises in the tissue concentration of this hormone (Table 1). While a slight rise in E_2 serum levels was detected on day 2, a substantial 4.2- to 5.7-fold increase in E_2 serum levels was observed in days 3 and 4 of the cycle. On days 3

Table 1. *Levels of E_2 in serum, uterus, kidney and liver of cycling female hamsters*[a]

Day of the cycle[b]	Serum (pg/ml)	Uterus (pg/mg P)	Kidney (pg/mg P)	Liver (pg/mg P)
1	63 ± 6	4.2 ± 2.4	5.7 ± 0.1	5.7 ± 2.1
2	95 ± 14	4.1 ± 0.7	6.0 ± 0.9	5.9 ± 0.4
3	264 ± 26*	11.4 ± 1.2*	10.5 ± 1.2*	13.5 ± 0.8*
4	358 ± 35*	14.3 ± 4.2*	12.3 ± 4.6*	15.4 ± 2.5*

[a]The numbers represent the mean $\pm$ SEM of 8 individual determinations performed in duplicate.

[b]The days of the cycle were determined by examination of vaginal discharge. After 3 consecutive cycles, groups of 4 animals were killed in each day of the cycle.

*Statistically significantly different from day 1 ($P < 0.05$).

Table 2. E_2 concentration in serum, kidney and liver of castrated male hamsters treated with E_2 for different periods of time[a]

Treatment (days)	Serum (pg/ml)		Kidney (pg/mg P)		Liver (pg/mg P)	
	Control[b]	Treated[c,d]	Control	Treated[c,e]	Control	Treated[c,e]
15	27 ± 7	1672 ± 80	0.81 ± 0.34	4.3 ± 0.9	0.3 ± 0.1	3.5 ± 0.9
30	32 ± 2	1860 ± 63	0.78 ± 0.24	4.8 ± 1.0	0.4 ± 0.2	4.2 ± 1.1
60	32 ± 3	2166 ± 98	0.80 ± 0.40	4.5 ± 0.8	0.5 ± 0.1	3.9 ± 1.2
90	36 ± 5	2392 ± 98	0.73 ± 0.44	5.5 ± 0.7	0.3 ± 0.1	3.5 ± 1.0
120	31 ± 4	2556 ± 90	0.72 ± 0.31	4.1 ± 0.4	0.4 ± 0.1	3.7 ± 1.0
150	34 ± 8	2736 ± 85	0.92 ± 0.22	3.9 ± 1.8	0.5 ± 0.2	3.7 ± 1.4
180	30 ± 5	2561 ± 99	1.33 ± 0.30	4.9 ± 0.2	0.5 ± 0.1	4.1 ± 0.1

[a]The numbers represent the mean ± SEM of 4 to 6 individual samples measured in duplicate.
[b]Control animals were age-matched castrated untreated animals.
[c]Treated animals received 20 mg pellets of E_2 every 75 days.
[d]All treated values were statistically significantly different from control values ($P < 0.001$).
[e]All treated values were statistically significantly different from control values ($P < 0.005$).

and 4 of the estrous cycle, uterine E_2 levels averaged 3.0-fold higher than day 1 whereas liver and kidney E_2 levels averaged 2.6- and 2.0-fold higher, respectively.

Serum and tissue levels of E_2 in E_2-treated hamsters

As anticipated, serum E_2 levels of untreated castrated male hamsters did not vary appreciably throughout the experimental period, remaining at about 32 pg/ml (Table 2). When castrated hamsters were implanted with E_2 pellets, a rapid rise in serum E_2 levels was seen as early as 2 weeks after hormone implantation. During the 15 to 90 days of renal tumor induction, E_2 serum levels averaged 71.0-fold higher than untreated castrated serum levels (Table 2). Animals treated with a single 20 mg pellet showed an average concentration of 1672 to 2166 pg/ml of serum. After a second pellet was implanted at 45 days, the serum levels of E_2 attained a steady state that maintained a range of 2392 pg/ml at 90 days to 2736 pg/ml of serum after 150 days of treatment. A third pellet implanted after 150 days of treatment did not increase the serum hormone levels any further, as seen in the serum levels after 180 days of treatment. On the other hand, kidney E_2 levels in hamsters treated with E_2 pellets for 15–180 days only rose on the average 5.4-fold over the treatment period within a narrow limit compared to E_2 kidney levels in untreated castrated hamsters (mean 0.87 pg/mg protein). Interestingly, E_2 levels in E_2-treated livers averaged 2.1-fold lower than in corresponding kidneys. However, E_2 levels in the liver rose 10.0-fold on average over the 15 to 180 days E_2 treatment period.

Comparing E_2 average serum levels from days 3 and 4 in normal females to serum levels of E_2-treated hamsters, there was a 5.7- and 8.0-fold increase of E_2 levels between 15–30 days and 30–180 days of E_2-treatment, respectively. In contrast, kidney E_2 levels during days 3 and 4 of the cycling female were about 2.0- and 3.6-fold higher than in E_2-treated hamsters, respectively, throughout the treatment period.

Serum and tissue levels of E_2 after estrogen withdrawal

Table 3 shows the serum and tissue levels of E_2 in hamsters treated for 90 days and sacrificed with the pellets in or with the pellets out at different time intervals. The results show that the E_2 concentration in kidney and liver returned to age-matched untreated control levels 24 h after pellet withdrawal. The serum levels, however, fell dramatically after 24 h, but required at least 48 h to return to control untreated levels. The rapid clearance of E_2 from the serum, kidney and liver of E_2-treated hamsters was similar to that found in these tissues of similarly E_2-exposed castrated male mice (CD) and rats (SD). Thus, all species examined clear estrogens equally well (data not shown).

DISCUSSION

Greenwald and his associates [22, 24, 25] have extensively investigated peripheral blood levels of gonadal steroids in cyclic female hamsters. In these studies, day 1 E_2 serum levels ranged between 14–25 pg/ml; day 2 ranged between 15–46 pg/ml; day 3 ranged between 47–101 pg/ml, and day 4 ranged between 38–187 pg/ml. These values are all well below E_2 values reported herein for respective days of the cycle. In contrast, Shaikh [26] reported considerably higher plasma E_2 levels by cannulating the ovarian vein during the estrous cycle of the hamster. E_2 plasma levels in cyclic female hamsters in this study ranged 11.4- to 14.2-fold higher for days 1 and 2 and 8.0- to 7.0-fold higher for days 3 and 4, respectively, compared to values presented herein and were comparable to E_2 serum levels

Table 3. Levels of E_2 in serum, kidney and liver in castrated male hamsters treated with E_2 for 90 days[a]

Treatment	Serum (pg/ml)	Kidney (pg/mg P)	Liver (pg/mg P)
Control untreated	31 ± 2	0.7 ± 0.4	0.3 ± 0.1
90 Days pellet in	2595 ± 85*	5.5 ± 1.0**	3.5 ± 1.0**
90 Days pellet out, 24 h	64 ± 16**	0.4 ± 0.3	0.4 ± 0.1
90 Days pellet out, 48 h	34 ± 5	0.5 ± 0.4	0.3 ± 0.1
90 Days pellet out, 72 h	27 ± 1	0.5 ± 0.4	0.3 ± 0.1

[a]The numbers represent the mean ± SEM of 4 individual duplicated determinations.
*Statistically significantly different from control ($P < 0.001$); **statistically significantly different from control ($P < 0.05$).

found in 60 to 180 day E_2-treated castrated males. The differences from the present data and those of Greenwald may be due to the greater sensitivity of the E_2 double-antibody procedure currently available. Nevertheless, the relative concentrations of E_2 in days 1–4 in the cycling hamster in the present study is proportional to those reported by these investigators.

The minimum effective oncogenic dose (MEOD) of estrogen required to induce renal tumors in hamsters is not known, although Kirkman and Bacon [27] estimated that the MEOD for estrogen needed to induce renal tumors was between 30–90 μg per day. Data presented herein extend this early estimation. Under conditions which produce essentially 100% kidney tumor incidence in castrated male hamsters, a serum E_2 level of 1.8–2.5 μg/ml or a renal E_2 level of 0.0046 μg/mg protein is evidently required. However, it is likely that somewhat lower E_2 levels, both in the peripheral blood and in the target tissue may also be equally oncogenic since slightly lower doses also result in a 100% renal tumor incidence, but appreciably longer latency periods are required. In any event, the 5.7- to 8.0-fold higher serum E_2 levels in E_2-treated male hamsters compared to days 3–4 of cyclic females is within an appropriate range where there is no significant effect either on hamster weight or mortality, even after 9.0- to 10.0-months of incessant E_2 exposure [5, 6, 18, 19, 27].

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