

Xenoestrogens: Do They Lower Survival after Thermal Injury?

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ABSTRACT. The effect of hormone disruptors on human health is an area of recent concern. The authors measured heptachlor epoxide and oxychlordan—the body storage forms of estrogenic insecticides—in the sera of patients with major burns (i.e., 7 survivors and 10 age- and burn-size-matched nonsurvivors) on days 1, 3, 5, 7, and 11 after they had been burned, as well as in 12 age-matched normal controls. During the hypermetabolic phase, serum concentrations of heptachlor epoxide and oxychlordan were greater in nonsurvivors than in controls, and heptachlor epoxide concentrations in nonsurvivors exceeded those in survivors on postburn day 5. The postburn alterations in heptachlor epoxide and oxychlordan concentrations could not be accounted for by changes in concentrations of circulating lipid. These findings, which indicate that xenoestrogens are released from fat depots after thermal injury, suggest a possible contribution to mortality, especially in older patients.

<Key words: burn injury, heptachlor epoxide, organochlorines, oxychlordan, survival, xenoestrogens>

COMPARED WITH MEN, women reportedly have less risk of cardiovascular disease,¹ sepsis following trauma,² and major postoperative infection.³ The incidence of cardiovascular disease in postmenopausal women, however, approaches that of men of similar age.¹ Recently, results in various models have indicated that treatment with 17 β -estradiol (E₂) improves outcome after ischemic reperfusion injury,⁴⁻⁶ carotid artery injury,⁷ inflammatory response to foreign material,⁸ and response to trauma hemorrhage.⁹ In each of these injury models, the beneficial effect of E₂ is likely receptor-mediated because it can be blocked by E₂ receptor antagonists (ICI182,780 or tamoxifen)⁷⁻¹⁰ and may not be mediated by vascular effects.^{6,10}

Acting as E₂ receptor agonists/antagonists, xenoestrogens may inhibit the beneficial effects of E₂ by altering the transcription of E₂-regulated genes,^{11,12} thus inducing steroid hydroxylases that convert E₂, as well as progesterone, testosterone, and glucocorticoids, into inactive metabolites¹³⁻¹⁶ or inhibiting the expression or activity of aromatase, which converts androgens into E₂ in tissues.¹⁷ One class of estrogenic insecticides—cy-

clodienes (chlordane, heptachlor, and aldrin)—was introduced in the mid-1950s. Use of these insecticides peaked in the late 1960s, but by the mid-1970s they had been banned for agricultural use.¹⁸

In humans and domesticated animals, these cyclodienes are metabolized to their respective epoxides—oxychlordan (OC), heptachlor epoxide (HE), and dieldrin—all of which accumulate in lipid-containing compartments (*within the body?*).¹⁹⁻²¹ Serum concentrations of HE and OC have declined minimally in humans since the agricultural use of these compounds was banned, a result that possibly reflects the continued inhalation of chlordane and heptachlor in an estimated 30 million homes in the United States that were treated for termites with these chemicals between the mid-1950s and late 1980s.^{18,22,23} In contrast to cyclodienes, agricultural use of another estrogenic insecticide, dichlorodiphenyltrichloroethane (DDT), peaked a decade earlier and residue concentrations of its main metabolite, dichlorodiphenyldichloroethylene (DDE), declined in meat, dairy products, and human adipose tissues during the 1970s and 1980s.¹⁸

Since World War II, the treatment of trauma patients has improved steadily, with concomitant increases in survival. However, one major burn center reported incremental decreases in overall survival during the 1950s and 1960s²⁴—a trend that was reversed in the late 1970s and 1980s.²⁵ Similarly, assessing mortality in the patient population at this burn center on the basis of age and burn size showed that the size of the burn injury (percentage of body surface area) resulting in a 50% probability of death (LA₅₀) decreased greatly (indicating lower survival) in older patients during the 1960s—the period during which chlordane and heptachlor were used most widely in agriculture (Fig. 1).²⁶ In contrast, survival in young adults, who presumably would have had greater preinjury amounts of sex steroids than would older patients, *increased* during the 1950s and 1960s. Interestingly, for coronary heart disease—currently recognized as an inflammatory disease that mainly affects older people—overall survival reportedly decreased in the 1950s and 1960s, and then increased after the mid-1970s.²⁷ In addition, patients with coronary heart disease reportedly had higher serum concentrations of DDE and HE than did normal subjects.²⁸

To ascertain whether environmental factors may be linked to survival in a more recent cohort of burn patients, we tested whether survival correlated with serum concentrations of cyclodiene insecticides. We limited our study to the presumably most susceptible patients—older men—who are known to have a higher body burden of cyclodienes²² and lower concentrations of sex steroids.

Materials and Method

Subjects. Analyses of xenoestrogens were performed on archived serum samples from burn patients treated at the institute between 1991 and 1994. Patients were selected if they had survived for at least 7 days, had a total burn surface area (TBSA) greater than or equal to 25% of body surface area, were 40–70 yr of age, were male, had provided adequate serum samples on a specified postburn day (PBD), and had no other major trauma at the time of injury. We took this approach to match approximately the mean and range for TBSA and age between survivors and nonsurvivors. This process was used to select 10 nonsurviving burn patients and 7 surviving ones, which formed the basis for our analyses, along with 12 healthy men of similar mean age whose blood was sampled during 1995 and who served as controls. Informed consent was obtained from control subjects, whereas the burn patients had already consented to general research use of excess volume in specimens taken for their regular clinical care. This protocol was approved by the Institute's Human Use Committee.

Cyclodienes and lipids in serum. The concentrations of these insecticides (*cyclodienes?*) in serum taken from burn patients on PBDs 1, 3, 5, 7, and 11 and stored at –75 °C were determined in accordance with a previously reported method.¹⁶ Lab personnel used a Dynamic Thermal Stripper (Environchem [Kemblesville, Pennsylvania]) to sparge the insecticides from 50 µl of serum for collection on Tenax solid sorbent (Envirochem). The tube of solid sorbent which contained the adsorbed insecticides was subsequently thermal-desorbed with a Unicon 810 (*what type of eqpt?*) (Environchem) into an HP 5890 gas chromatograph (Hewlett Packard [Palo Alto, California]) equipped with a SPB-608 30 M fused silica capillary column (J & W Scientific [Folsom, California]) and an electron capture detector. The analytical procedure was optimized for the semivolatile HE and OC, with an overall coefficient of variation of less than 20%. We determined insecticide concentrations in the subjects' sera by comparing responses for the samples with standard curves constructed from certified standards (Ultra Scientific [Kingstown, Rhode Island]) added into a composite sample of serum from 3 subjects with previously measured low levels of each insecticide. A reference serum pool from 3 patients who had medium concentrations of each insecticide was measured 1–2 times during each day of analyses. The mean concentration of HE in samples from the reference pool measured concurrently with burn-survivor samples was almost identical to that of the reference samples measured along with burn-nonsurvivor samples. The same was true for reference sample OC concentrations. Serum cholesterol and triglyceride concentrations were analyzed with a Monarch Clinical Chemistry System (Instrumentation Laboratory, Inc. [Lexington, Massachusetts]).

Data analysis. Analyses were conducted with SPSS software (SPSS [Cary, North Carolina]). We used 1-way analysis of variance (ANOVA) as an overall test for any differences among the groups, and 2-tailed post hoc tests for a difference between 2 specified groups. Post hoc tests (identified in the Results section and in figure legends) were chosen after testing was performed for normality and variance homogeneity, and accounted for multiple comparisons. We considered $p < 0.05$ (2-tailed for post hoc tests) as significant. Where indicated, serum concentrations were expressed as the within-patient mean over PBDs 3–7, which enabled inclusion of the unburned control subjects (with single samples) in comparisons and allowed for performance of Pearson correlations and multiple linear regressions without inflating the degrees of freedom. Separate analyses were also conducted in which we excluded results for 4 burn nonsurvivors who had prior major medical conditions or personal beliefs that severely altered patient care. We refer to these collectively as “preinjury conditions.” These conditions, listed by patient number in Table 1,

were as follows: *Patient 3*: Laennec's cirrhosis, chronic pleural effusion, and splenectomy; *Patient 5*: refusal of blood transfusion; *Patient 9*: treated for renal cell carcinoma (4 yr) and ascites (2 yr), with metastasis not currently demonstrated; and *Patient 17*: alcoholism, drug abuse, and homelessness.

Results

Cyclodienes and lipids in serum. Characteristics of the burn patients admitted in 1991–1994 are given in Table 1. Burn survivors and nonsurvivors did not differ significantly with respect to mean age (55 yr and 52 yr, respectively), TBSA (42% and 47%), or proportion with inhalation injury (6 of 7 and 6 of 10). The mean age of the 12 nonburned control subjects was 53 yr (range 39–66 yr), which was not different from that in the burn patients.

The concentration profiles for serum cyclodienes are shown in Figure 2, and profiles for serum lipid concentrations and body weights appear in Figure 3. On PBD 5, the mean HE concentration in sera of burn nonsurvivors (186 ng/l) significantly exceeded that in burn survivors (114 ng/l [$p < 0.05$]). Averaging the serum concentrations of cyclodienes during the onset of the hypermetabolic phase in burn patients on PBD 3, 5, and 7 yielded means \pm standard errors in control, burn survivor, and nonsurvivor groups of 96 ± 15 , 122 ± 11 , and 171 ± 19 ng/l, respectively, for HE (ANOVA, $p < 0.01$), and 245 ± 46 , 384 ± 74 , and 501 ± 73 ng/l, respectively, for OC (ANOVA, $p < 0.05$). For both HE ($p < 0.01$) and OC ($p < 0.05$), concentrations in nonsurvivors were significantly higher than in controls (Tukey's Honestly Significantly Different [HSD] tests). Reanalysis following exclusion of data from 4 patients with various preinjury conditions indicated persistence of significance (p equal to or less than the respective values cited above) for all the earlier comparisons. In this reanalysis, the serum concentrations of HE on PBD 3–7 were also significantly greater ($p < 0.05$) in nonsurvivors (193 ± 27 ng/l) than in survivors (122 ± 11 ng/l).

Serum cholesterol concentrations (Fig. 3a) were lower in both groups of burn patients, for each day on and after PBD 3, than in the control group (Bonferroni-corrected t tests, $p < 0.001$), but the concentrations did not differ between survivors and nonsurvivors. Triglyceride concentrations (Fig. 3b) did not differ detectably among the controls and the 2 groups of patients.

In testing correlations and regressions, we used serum concentrations of HE, OC, and lipids as the within-patient mean (*vs.* "means"?) for PBDs 3–7 in burn patients. Single (bivariate) correlations indicated a strong positive relationship between the concentration of HE and the concentration of OC in burn patients and, separately, in the control subjects ($p < 0.001$, results not shown). Among the burn patients, multiple linear-re-

gression analysis of the mean HE concentration for PBDs 3–7 against TBSA, age, triglycerides, and cholesterol indicated a positive relationship of HE only with cholesterol ($p < 0.05$, Fig. 4). A similar regression of OC in burn patients indicated simultaneous positive relationships of OC concentrations with cholesterol ($p < 0.05$, Fig. 4) and age ($p < 0.01$, results not shown). Inclusion of results for the controls in the regressions showed no relationship of HE or OC with cholesterol; however, serum concentrations of both HE ($p < 0.05$) and OC ($p < 0.01$) increased with age (results not shown) after accounting for the effects of burn. The correlation and regression results remained the same after excluding measurements from the 4 patients with preinjury conditions, except that the relationship with age was significant for only OC ($p < 0.01$, results not shown).

The gain in body weight in burn patients over the first 4 days (Fig. 3c) was the same in burn survivors and nonsurvivors, which indicated similarity of initial body weight and of fluid resuscitation in both groups.

Discussion

Survival after severe burns,^{24–26} as with other types of trauma,^{29,30} is age-dependent and biphasic, peaking in young adults but decreasing in prepubescent and markedly decreasing in older adult patients. The biphasic, age-dependent survival in trauma patients mirrors plasma concentrations of E_2 ,³¹ testosterone,³² and dehydroepiandrosterone (DHEA)³³ in women, and of testosterone³⁴ and DHEA³³ in men. DHEA and testosterone are readily converted to E_2 by aromatase, the expression and activity of which in tissues are increased by inflammatory cytokines^{35,36} and glucocorticoids³⁷ after trauma. Xenoestrogens mimic E_2 by inducing steroid hydroxylases that convert not only E_2 but also progesterone, testosterone, and glucocorticoids to inactive metabolites.^{13–16} Simultaneously, xenoestrogens may act as antagonists, blocking the E_2 receptor-mediated processes^{11,12} that dampen inflammation, such as inhibiting migration of leukocytes into injured tissues,^{38–41} inhibiting release of inflammatory cytokines^{5,42,43} and oxidants,^{44–46} and blocking aromatase¹⁷—the enzyme that increases tissue content of E_2 .^{35,36} Xenoestrogens may reduce the beneficial effects of E_2 in several ways: by increasing degradation of E_2 , by decreasing aromatase conversion of E_2 precursors, or by blocking access of E_2 to its receptors.

The serum concentrations of 2 xenoestrogens, HE (means: 0.25–0.5 nmol/l) and OC (means: 0.5–1.0 nmol/l), increased after injury in our patients; by PBD 5, the concentrations were twice those of normal controls. Even though the activity of HE and OC on E_2 receptors may be less than that of E_2 , the serum concentrations of HE and OC in our patients were 5- to 10-fold greater

than those of E₂ (total) reported in normal men (0.1 nmol/l). In male burn patients, concentrations of E₂ will increase after injury.⁴⁷ Whether xenoestrogens can interfere or interact with E₂ to alter immune and metabolic processes in patients after injury is still uncertain. However, one of us (RAC) has determined that HE, at the concentrations found in the sera of nonsurviving burn patients, induces intracellular oxidants and deoxyribonucleic acid strand breaks in isolated human granulocytes by an E₂ receptor-mediated process (inhibited by ICI182,780 and tamoxifen).⁴⁸

Because these xenoestrogens accumulate in lipid stores with age,²² just as preinjury quantities of E₂ and its precursors (i.e., DHEA and testosterone) are expected to decrease, one might expect that the potential for altering E₂ receptor-mediated effects would increase with patient age. The concept that an age-related increase in the ratio of xenoestrogens to E₂ (**ok?**) might contribute to decreased survival in older burn patients is an hypothesis worthy of further examination.

Organochlorine insecticides are distributed evenly in the lipid phase of the body, with tissue concentrations of these chemicals being proportional to the lipid content of the tissue.⁴⁹ During the postinjury catabolic and lipolytic response, one would expect insecticides, as well as other lipophilic chemicals to be released from fat depots. Once released from fat, the availability of these previously sequestered xenoestrogens to bind to receptors may increase as the serum concentrations of lipid carriers (e.g., cholesterol) decrease.⁵⁰ In this study, serum cholesterol in burn patients decreased 2- to 3-fold following injury. Interestingly, in burn patients, even though concentrations of circulating cholesterol were depressed, HE and OC contents were increased. Considering these increased serum concentrations and possibly increased bioavailability, we suggest that the direct toxic effects of HE on the patients' immune and nervous systems after thermal injury may contribute to an adverse outcome.^{16,23}

Increasing concentrations of xenoestrogens might inhibit the reported E₂-dampening effect on lipolysis,⁵¹ which raises the possibility of a positive feedback loop that accelerates the release of cyclodienes from fat depots. Whether the greater concentrations of HE in nonsurviving patients than in the survivors resulted solely from increased lipolysis of fat stores that contained equivalent HE contents with coincident increased fat utilization, or from lipolysis of fat containing increased insecticide concentrations, is unknown. However, given that the known major factors that account for mortality (i.e., burn size, inhalation injury, and age) and for transporting xenoestrogens in serum (i.e., cholesterol and triglycerides) were similar, and that body weights were almost identical in the burn survivors and nonsurvivors in which HE was measured, the possibility of a causal relationship between HE serum concen-

trations and mortality cannot be dismissed. Additional research is needed to assess the effects of serum lipids (e.g., cholesterol, triglycerides) on the ability of xenoestrogens to interact with steroid receptors, and to determine how these interactions alter immunological response after injury and after rapid weight loss induced by other causes.

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Fig. 1. Effects of age and year of injury on survival in burn patients. Survival indexed by the 50%-lethal burn-size area (LA_{50}) in patients at the U.S. Army Institute of Surgical Research.²⁶ LA_{50} was calculated from logistic regressions of mortality on burn size and age (in yr) (3rd-order polynomial) performed on sequential 5-yr periods, advancing 1 yr at a time, and plotted at the midpoint of each period. (?Au: The left 2 graphs in this figure appear to be identical. (A check of input reveals the same.) As intended?)

Fig. 2. Concentrations of (a) heptachlor epoxide and (b) oxychlor-dane cyclodiene insecticides in serum of □ surviving ($n = 7$) and ■ nonsurviving ($n = 10$) burn patients (shown as means \pm standard errors) and normal control subjects ($n = 12$; mean \pm standard deviation depicted with horizontal lines). Notes: * $p < 0.05$, results for nonsurvivors vs. survivors on postburn day (PBD) 5 (Student t test); # $p < 0.05$ and ## $p < 0.01$, results for nonsurvivors (mean data for PBDs 3–7) vs. normal control subjects, respectively (analysis of variance, followed by Tukey's Honestly Significantly Different test).

Fig. 3. Serum concentrations of (a) cholesterol and (b) triglycerides, and (c) body weight in □ surviving and ■ nonsurviving burn patients (means \pm standard errors) and in normal control subjects (mean \pm standard deviation depicted with horizontal lines). Serum cholesterol concentrations in nonsurvivors and survivors (separately) at postburn days 3, 5, 7, and 11 were significantly different from that in normal control subjects ($p \leq 0.001$; 2-tailed Bonferroni-corrected tests).

Fig. 4. Serum concentrations of ■ heptachlor epoxide and □ oxychlor-dane in burn patients, plotted against the cholesterol concentration in each sample. Data are the means for postburn days 3–7. Heptachlor epoxide and oxychlor-dane increased with cholesterol ($p < 0.05$) in separate multiple-regression analyses.

