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Dose-effect and pharmacokinetics of estrogens given to correct bleeding time in uremia

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Dose-effect and pharmacokinetics of estrogens given to correct bleeding time in uremia. Conjugated estrogens have a significant and long-lasting effect in shortening bleeding time in patients with end-stage renal disease. The studies so far available indicate that repeated estrogen administrations are necessary to short bleeding time in uremia in a dose range of 95 to 325 mg. With the present study we wanted to establish whether single or repeated doses are required to induce a significant shortening of bleeding time in uremia, and the minimum cumulative dose of conjugated estrogens necessary to control bleeding time for a prolonged period of time, and to check whether the prolonged effect of estrogens on bleeding time in uremia is due to an accumulation of the drug or its metabolites in the blood. Fifteen uremics on chronic hemodialysis were studied. A pilot study carried out in five uremic patients indicated that single or repeated estrogen infusions of 0.3 mg/kg did not significantly influence bleeding time values. Therefore the subsequent studies have been carried out using daily infusion of 0.6 mg/kg. A single estrogen infusion of 0.6 mg/kg shortened bleeding time in all patients. The effect was transient and bleeding time returned to pre-infusion values within 72 hours. A 50% decrease of bleeding time or a shortening of bleeding time more than 30 to 15 minutes or less was obtained in all patients with four or five infusions (0.6 mg/kg) spaced 24 hours apart. The effect lasted for 14 days. At day 25 from the last infusion all the patients had bleeding time values comparable with the pre-infusion ones. Pharmacokinetic parameters of both estrone sulfate and equilin sulfate, the major components of the estrogen mixture we used, were comparable in both controls and uremics and can be described assuming a two compartmental model. After repeated administrations of conjugated estrogens no accumulation of estrone sulfate and equilin sulfate or their metabolites was detectable in blood in both uremics and controls.

Bleeding in chronic renal failure has been the subject of intensive investigation in the recent years. The most effective therapeutic measures to date are red cell transfusions [1, 2], cryoprecipitate [3], and desmopressin (1-deamino-8-D-arginine vasopressin) [4–6]. However, red cell transfusions and cryoprecipitate carry the risk of transmitting blood-associate infections. Moreover, both cryoprecipitate and desmopressin have a short duration of effect [3, 4]. Recently it has been found that conjugated estrogens shorten the prolonged bleeding time of uremics and stop severe hemorrhages [7, 8]. Estrogens, by virtue of their long duration of action, are particularly effective when long-lasting "hemostatic" correction is required.

Studies published to date have employed a wide range of estrogen dosages (95 to 325 mg) administered in a variety of different regimens. Whether repeated administrations are really necessary to obtain a long lasting effect on bleeding time is not known, nor information is available on the minimum dose of conjugated estrogens actually needed to correct the hemostatic defect of uremia. Given the potentially major side effects of estrogens [9, 10] the area is worth investigating. Moreover studies on estrogen pharmacokinetics in uremics in relationship to the estrogen effect on the bleeding time have not been done so far.

The aims of the present study were: 1) to investigate whether single or repeated doses of estrogens are required to induce a significant shortening of bleeding time in uremia; 2) to establish the minimum effective cumulative dose necessary to control bleeding time for a prolonged period of time; and 3) to check whether the reported long-lasting effect of estrogens in shortening bleeding time in uremia is due to an accumulation of the drug or of its metabolites in the blood.

Methods

Subjects

Fifteen patients with chronic renal failure (CRF) and three control subjects participated in the study. CRF patients (10 men and 5 women; median age, 52 years; range, 32 to 65) with diagnosis of chronic glomerulonephritis ($N = 8$), chronic pyelonephritis ($N = 5$) and interstitial nephritis ($N = 2$), were being treated in our renal unit and were admitted to the study on the basis of the concomitant presence of chronic renal failure with anuria and prolonged bleeding time (longer than 15 minutes). All the patients had normal results on coagulation screening tests (fibrinogen, prothrombin time, activated partial thromboplastin time, and thrombin time) before and after the study. During the study, no technical changes were done in dialytic treatment (that is, changing heparin dosing) and no significant changes were observed in hematocrit and blood urea nitrogen values for each patient (data not shown). The mean platelet count (± SD) was 275 ± 61 × 10^3 per cubic milliliter (range 190 to 370 × 10^3). None of the patients received transfusions or blood derivatives during the three months preceding the study

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or during the study. None of the patients had taken aspirin or other drugs known to affect platelet function for at least 20 days before the study. All patients were requiring therapeutic hemodialysis three times a week (12 hours per square meter parallel flow kidney with cuprophan membrane). All patients were aware of the nature and purposes of the study and all gave informed consent in accordance with the Declaration of Helsinki.

Healthy subjects (2 men and 1 woman; median age, 46 years; range 34 to 61) had not received drugs during the three months preceding the study or during the study. All were aware of the nature and purposes of the study and all gave informed consent in accordance with the Declaration of Helsinki.

Design of the study

Since the first study carried out in five uremic patients indicated that single or repeated estrogen infusions of 0.3 mg/kg did not significantly influence bleeding time values, all the subsequent studies were carried out using daily infusion of 0.6 mg/kg. Patients (N = 10) received cycles of conjugated estrogens infusion (Emopremarin, Ayerst Laboratories, New York, New York, USA) followed by a period of observation. The goal of estrogen therapy was to reduce the bleeding time to at least 50% of the baseline value, or if more than 30 minutes, to a value of 15 minutes or less. The experimental design was as follows: the first infusion was given 24 hours after dialysis and a baseline bleeding time determination. Bleeding times were repeated at 6, 24, 48, 72, and 96 hours and seven days after infusion. If the prescribed reduction in bleeding time was not achieved within seven days, the cycle was repeated with infusions on two consecutive days followed by five days of observation. If the second cycle was also ineffective, then a third cycle with infusion on three consecutive days was performed followed by a fourth and fifth cycle when necessary. A wash-out period of at least one month elapsed between each cycle of estrogen therapy. Bleeding time was also monitored 14, 21, and 30 days after the first infusion of the cycle.

Conjugated estrogens were added to 50 ml of physiologic saline and infused over a period of 40 minutes in the arm contralateral to the vascular access for hemodialysis. Blood pressure and pulse rate were monitored during the infusion. Pharmacokinetic parameters were determined by the noncompartmental pharmacokinetic method according to Gibaldi and Perrier [11]. Blood samples were taken immediately before (time 0) and 40, 50 minutes, and 1, 2, 4, 6, 12, 24, 48, 72, and 96 hours after the first conjugated estrogen infusion plus 6 hours after the start of each infusion.

Healthy subjects received five consecutive estrogen infusions of 0.6 mg/kg each following the same schedule as for patients. Bleeding time was determined before (time 0) and at 6, 24, 48, 72, 96 hours and seven days. Blood samples for pharmacokinetic parameters were taken immediately before (time 0) and 40, 50 minutes, and 1, 2, 4, 6, 12, 24, 48, 72 and 96 hours after the first conjugated estrogen infusion and six hours after the start of each infusion.

Blood collection

Venous blood samples (5 ml) were collected through a 19-gauge needle into heparinized syringes. Platelet poor plasma was obtained after centrifugation (4000 × g for 20 min) at room temperature, was quick-frozen, and kept at −80°C until tested.

Bleeding time measurement

Template bleeding time was performed by the same investigator with the Simplate II device (General Diagnostic, Milan, Italy) on the antecubital surface of the forearm, under a counter pressure of 40 mm Hg. The blood was blotted every 30 seconds until no more appeared at the site of tranverse standard incisions. The time of bleeding was recorded for a maximum of 30 minutes. Results were expressed as the average bleeding time from the two incisions. The normal range with this method in 10 healthy women and 10 healthy men was three to seven minutes.

Pharmacokinetic assay

Plasma concentrations of estrone sulfate (E_1S) and its major metabolites, estrone (E_1) and estradiol (E_2), and of equilin sulfate (EQS) and its major metabolite equilin (EQ), were determined by high-performance liquid chromatography (HPLC). Briefly: 1 ml of plasma was added to a centrifuge tube containing 50 μl of tetrabutylammonium hydrogen sulfate (TBA), 2.5 mM adjusted to pH 3.0 ± 0.1 with NaOH, and was vortexed for few seconds. Seven ml of chloroform were added to each tube, and the samples were vortexed for 30 seconds. After centrifugation, the organic layer was separated and evaporated to dryness. The residue was dissolved in 200 μl of mobile phase and exactly 50 μl was injected into the liquid chromatograph. Using a model 334 liquid chromatograph equipped with a model 163 variable wavelength detector (Beckman Instruments, Inc., Fullerton, California, USA) operating at 215 nm and a reversed-phase column (Ultrasphere^{tm}, ODS, 5 μm, 250 × 4.6 mm, Beckman), the samples were eluted in isocratic conditions with a mobile phase of acetonitrile:methanol:TBA (12.5 mM adjusted to pH 3.0 ± 0.1 with NaOH) at 30:30:40, and at a 1.0 ml/min flow rate. The retention times of E_1S, E_1, E_2, EQS, EQ were 6.7, 9.4, 7.9, 6.1, and 8.8 minutes, respectively. Internal calibration curves of E_1S, E_1, E_2, EQS, EQ, were prepared for each set of samples. Linearity was found over the investigated concentration range. The detection limit was 10 ng/ml for each compound.

Statistical analysis

The values for bleeding time were not normally distributed and differences were compared by means of the Friedman test [12].

Pharmacokinetic parameters were analyzed by one-way analysis of variance, using Duncan test for multiple comparisons [13]. Results are expressed as means ± SD. P values less than 0.05 were considered significant.

Results

Bleeding time

A single estrogen infusion of 0.3 mg/kg did not influence bleeding time (basal: 19.6 ± 3.8 min; 6 hr after: 18.1 ± 3.1 min; 24 hr after: 19.1 ± 3.1 min). Two infusions of 0.3 mg/kg (spaced 24 hr apart) did not affect bleeding times (data not shown). Three repeated infusions of 0.3 mg/kg shortened bleeding times, but values did not reach statistically significant differences

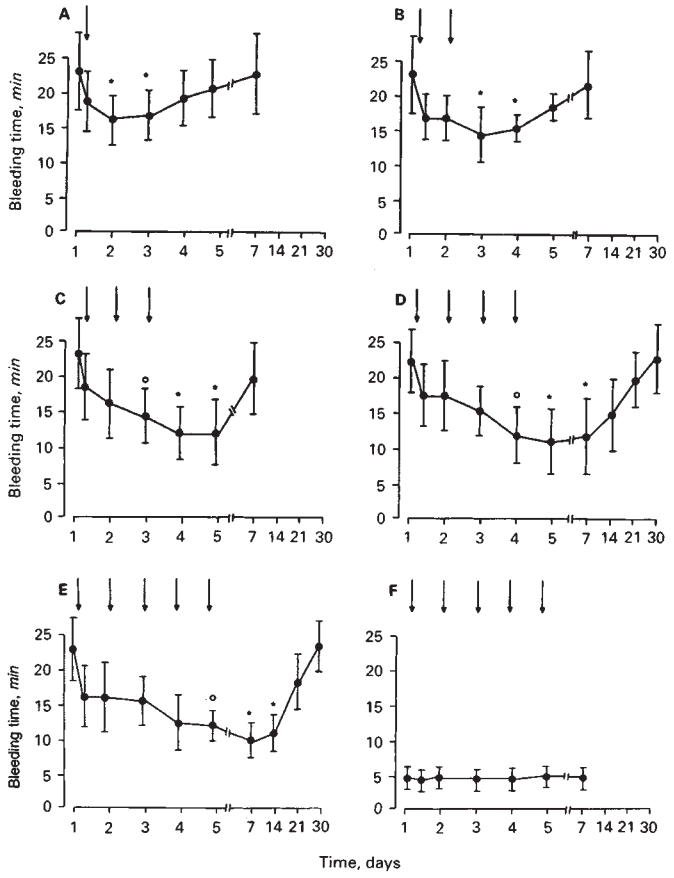

Fig. 1. *Panels* **A to D** *show the effect on bleeding time of single and repeated doses of estrogens (0.6 mg/kg) in uremic patients.* Panels **E** and **F** show the effect on bleeding time of 5 infusions in 7 uremic patients and 3 healthy controls, respectively. Arrows indicate estrogen infusions ($^{\circ}P < 0.05$ and $^{*}P < 0.01$ vs. basal value). Data are expressed as means ± SD.

(basal: 18.4 ± 2.7 min; 24 hr: 17.7 ± 2.1 min; 48 hr: 16.7 ± 2.3 min; 72 hr: 16.3 ± 2.5 min; 96 hr: 17.1 ± 2.4 min; day 7: 18.0 ± 2.1 min). On the basis of these data, we performed the subsequent studies using estrogen infusions at a dose of 0.6 mg/kg.

A single estrogen infusion of 0.6 mg/kg did shorten the bleeding time (Fig. 1A) in all patients. Bleeding time shortening was not statistically significant at six hours after estrogen infusion (second solid circle, Fig. 1A). At the two subsequent measurements, 24 and 48 hours after estrogen infusion, bleeding times were significantly shorter ($P < 0.01$) than basal values. Within 72 to 96 hours, bleeding times returned to pre-infusion values (Fig. 1A).

The time course of bleeding time observed after repeated estrogen infusions is represented in Figure 1. After two infusions of 0.6 mg/kg, bleeding times were significantly shortened at 48 and 72 hours ($P < 0.01$). Five to seven days after the first infusion bleeding times returned to pre-infusion values (Fig. 1B). After three infusions of 0.6 mg/kg a significant shortening of bleeding times was observed at 48 ($P < 0.05$), 72 and 96 hours ($P < 0.01$). Seven days after the first infusion bleeding times returned to the pre-infusion values (Fig. 1C). After four infusions of 0.6 mg/kg bleeding times were significantly shortened at

Table 1. Effect of cumulative doses of conjugated estrogens (CE) on bleeding time

Patient no.	Administration no.	Cumulative dose of CE *mg*	Bleeding time *min*	
			pre-	14 days
1	4	98	21	10
2	4	156	15	7
3	5	180	23	11
4	5	195	>30	15
5	5	153	25	11
6	5	180	25	10
7	5	228	20	9
8	5	138	>30	15
9	4	132	21	9
10	5	195	17	8

72 ($p < 0.05$) and 96 hours, and at day 7 ($P < 0.01$). Within 30 days bleeding times returned to basal values (Fig. 1D).

Table 1 shows that in patients number 1, 2 and 9, daily infusion for four consecutive days prolonged the control of bleeding time for 14 days, while the remaining seven patients required five infusions to reach the same end point. Indeed, when this latter group of patients received five infusions of 0.6 mg/kg, the bleeding times significantly shortened at 96 hours ($P < 0.05$) and at days 7 and 14 ($P < 0.01$). The bleeding time at day 21 was shorter than basal but the difference was not significant. All these patients had bleeding time values comparable to basal ones at day 30 (Fig. 1E).

Figure 1F shows that five consecutive estrogen infusions in healthy subjects did not influence bleeding times.

Pharmacokinetics

Emopremarin is a mixture of conjugated equine estrogens derived from urine of pregnant mares. In the preparation used in this study the content in sodium estrone sulfate and sodium equilin sulfate was determined by HPLC method, and accounted for 59% and 29%, respectively.

Mean E_1S and EQS plasma concentrations after the infusion of 0.6 mg/kg of Emopremarin (equivalent to 0.354 mg/kg E_1S and 0.174 mg/kg EQS) over a period of 40 minutes showed a biexponential decline in both patients (Fig. 2) and controls. Initial plasma concentrations of E_1S were higher than those of EQS, but about one hour after administration plasma levels of EQS exceeded those of E_1S. Individual and mean values for the noncompartmental pharmacokinetic parameters of E_1S and EQS are shown in Table 2. The elimination half-life of $E_1S(t_{1/2})$ varied widely and was similar in patients and controls (1.87 ± 1.05 and 2.58 ± 0.80 hr, respectively). A large variability was also found for total body clearance (CL), 0.273 ± 0.181 in patients and 0.132 ± 0.048 $l\ hr^{-1} \cdot kg^{-1}$ in controls, and for apparent volume of distribution (V), 0.614 ± 0.347 in patients and 0.480 ± 0.208 $l\ kg^{-1}$ in controls, but these parameters were not statistically different. A similar variability in the pharmacokinetic parameters was observed also for EQS. No statistically significant differences were found in $t_{1/2}$ between patients and controls (2.79 ± 1.05 and 2.79 ± 0.80 hr, respectively); in all the subjects but one (patient 8) $t_{1/2}$ for EQS was longer than for E_1S. Total body clearance was 0.118 ± 0.078 in patients and 0.086 ± 0.040 $l\ hr^{-1}\ kg^{-1}$ in controls (NS). Apparent volume of

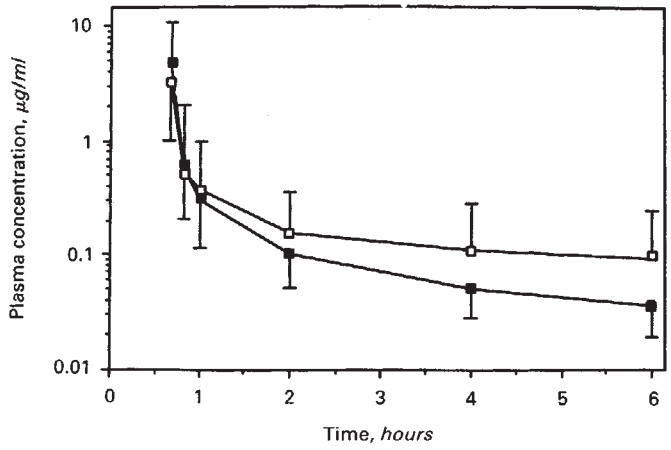

Fig. 2. *Semilogarithmic plot of plasma estrone sulfate (■) and equilin sulfate (□) concentrations in uremic patients after 40-minute infusion of 0.6 mg/kg of conjugated estrogens.* Data are expressed as means ± SD.

distribution was 0.421 ± 0.246 in patients and 0.345 ± 0.186 l kg^{-1} in controls (NS).

Table 3 shows the E_1S and EQS plasma levels in patients and controls measured six hours after each infusion. Trough levels measured immediately before the second, third, fourth and fifth infusion were always under the detection limit (<10 ng/ml) even at the fifth day of infusion (data not shown). These findings suggest that the administered drug did not accumulate in the blood stream. E_1, E_2, the major metabolites of E_1S, and EQ, the major metabolite of EQS, were always under the detection limit of the method (data not shown). No side effects were noted during or after infusion. There were no significant changes in blood pressure or pulse rate during the treatment.

Discussion

Our present results confirm that conjugated estrogens effectively shorten the bleeding time in uremia. The potential clinical relevance of this finding is based on two considerations. First a prolonged bleeding time in uremia correlates with clinical bleeding [14]. Second, in addition to shortening the bleeding time, estrogens are effective in inducing cessation of bleeding [8] and maintenance of a prolonged hemorrhage-free period in patients with chronic renal failure and recurrent gastrointestinal bleeding [7]. Two previous studies, one uncontrolled [7] and one controlled [8], have documented that estrogens effectively reduce bleeding time in uremia and that the effect lasts for several days. In these studies estrogens have been used in repeated administrations, that is, four to twenty infusions spaced 12 or 24 hours apart. The present study was primarily designed to comparatively evaluate the effect of single or repeated doses of estrogens in determining a long-lasting effect on bleeding time in chronic renal failure patients. The data indicate that a single dose of 0.6 mg/kg was effective in shortening the bleeding time in all patients. However, the effect of 0.6 mg/kg as a single administration was transient, reaching its maximum 24 hours and ending 72 hours after the beginning of the infusion in all patients. Two to three daily infusions of 0.6 mg/kg estrogen resulted in a consistent, more pronounced and more prolonged shortening of the bleeding time (maximum effect between 72 and 96 hours; return to pre-infusion values at day 7). A long-lasting effect of estrogens on bleeding time was only observed in the present study when four to five consecutive administrations of 0.6 mg/kg were given at 24 hour intervals. Actually in three out of ten patients the bleeding time was controlled for 14 days with four infusions and in seven out of ten was controlled for the same time with five infusions. Between 16 and 25 days after the last infusion the bleeding time returned to pre-treatment levels in all patients. This study documented a cumulative effect of repeated dosing of conjugated estrogens. It would seem that if six doses were given, a longer effect may have been obtained. This possibility has to be taken into account when a "hemostatic" competence longer than 20 to 30 days is required in patients at particular risk of bleeding such as those with subdural hematoma or who undergo major surgery [15].

Studies in healthy subjects have shown that E_1S has a relatively short-half life (4 to 6 hr) [16]. Moreover, E_1S has been identified in human urine as itself or converted to estradiol glucuronide and estriol [16, 17]. It is therefore conceivable that in chronic renal failure patients with anuria estrogens or their metabolites accumulate in uremic blood after repeated administrations, thus accounting for the prolonged effect on bleeding time. The second purpose of the present study was to verify the above-mentioned hypothesis examining estrogen pharmacokinetics in uremics as compared to normal subjects. Information on E_1S and EQS pharmacokinetic parameters in uremia are not available in the published literature. Our present results indicate that pharmacokinetic parameters of both E_1S and EQS were comparable in uremics and controls; plasma concentration of both compounds can be described assuming a two-compartment model. Ruder, Loriaux and Lipsett [16] found that E_1S pharmacokinetics after rapid i.v. injection could be described assuming a one-compartment model. These authors' sampling was not frequent enough during the first hour after estrogen administration, thus the early distributive phase might have been missed. This interpretation is supported by the work of Longcope [17], who according to our present findings described the time course of E_1S after intravenous injection using a two-compartment model. Our present data showed a $t_{1/2}$ for E_1S slightly shorter than that previously reported [16, 17], which explains the lack of accumulation of the drug into the blood stream. Our data indicate that for EQS also accumulation did not occur, even though this compound is cleared more slowly from plasma than E_1S. As far as E_1S major metabolites, E_1 and E_2, are concerned, failure to identify measurable levels of these two compounds in chronic renal failure patients even after repeated estrogen administrations indicates that the lack of estrogen metabolite urinary excretion is not an explanation for the long lasting effect on bleeding time. Since estrogens can be converted to estriol and oxidated to nonestrogenic substance [17] in the enterohepatic circulation, one has to assume that this is the major estrogen degradation pathway in uremia.

That repeated administrations of estrogens are necessary in our patients to obtain a long-lasting "hemostatic" effect is not surprising taking into account that estrogens are considered to act via a receptor mechanism and that the magnitude of the response is a function of the degree of occupation of the receptor [18, 19]. An interesting finding of the present study is the dissociation between short plasma half-life of estrogens or

Table 2. Pharmacokinetic findings of estrone sulfate (E_1S) and equilin sulfate (EQS) after conjugated estrogens infusion

	Body wt *kg*	λ_z hr^{-1}		$t_{1/2}$ hr		CL $l\ hr^{-1} \cdot kg^{-1}$		V $l\ kg^{-1}$	
		E_1S	EQS	E_1S	EQS	E_1S	EQS	E_1S	EQS
Patient									
1	41	0.45	0.26	1.52	2.72	0.228	0.107	0.502	0.420
2	65	0.85	0.47	0.82	1.48	0.267	0.128	0.315	0.273
3	60	0.67	0.25	1.04	2.79	0.457	0.223	0.682	0.895
4	65	0.67	0.30	1.04	2.33	0.601	0.201	0.903	0.670
5	51	0.19	0.15	3.71	4.75	0.110	0.042	0.592	0.288
6	60	0.39	0.23	1.79	3.00	0.457	0.098	1.179	0.426
7	76	0.59	0.42	1.18	1.67	0.074	0.049	0.127	0.118
8	46	0.20	0.37	3.51	1.89	0.204	0.240	1.034	0.654
9	55	0.26	0.17	2.62	4.20	0.058	0.037	0.218	0.224
10	65	0.48	0.23	1.44	3.04	0.281	0.055	0.586	0.238
Mean		0.48	0.29	1.87	2.79	0.273	0.118	0.614	0.421
± SD		0.22	0.11	1.05	1.05	0.181	0.078	0.347	0.246
Control									
1	67	0.21	0.20	3.29	3.54	0.085	0.058	0.407	0.298
2	70	0.41	0.36	1.71	1.94	0.129	0.067	0.318	0.188
3	53	0.25	0.24	2.74	2.89	0.181	0.132	0.714	0.548
Mean		0.29	0.27	2.58	2.79	0.132	0.086	0.480	0.345
± SD		0.11	0.08	0.80	0.80	0.048	0.040	0.208	0.186

Abbreviations are: λ_z, elimination rate constant; $t_{1/2}$, elimination half-life; CL, total body clearance; V, apparent volume of distribution.

Table 3. Estrone sulfate (E_1S) and equilin sulfate (EQS) plasma levels (μg/ml) after repeated conjugated estrogen infusions

	Time after the first dose *hr*							
	24 + 6		48 + 6		72 + 6		96 + 6	
	E_1S	EQS	E_1S	EQS	E_1S	EQS	E_1S	EQS
Patient								
1	ND	ND	ND	ND	ND	ND		
2	ND	ND	ND	ND	ND	ND		
3	ND	0.017	ND	ND	ND	ND	ND	ND
4	0.019	0.026	ND	ND	ND	ND	ND	ND
5	0.072	0.158	NA	NA	NA	NA	0.068	0.068
6	ND	ND	ND	ND	ND	ND	ND	ND
7	0.099	0.140	0.111	0.133	0.075	0.055	0.098	0.175
8	0.028	0.063	0.172	0.180	0.254	0.190	0.080	0.063
9	0.074	0.168	ND	ND	ND	0.101		
10	0.035	0.060	0.021	0.038	0.035	0.041	ND	ND
Control								
1	0.104	0.125	0.123	0.131	0.120	0.112	0.133	0.128
2	ND	ND	0.022	ND	ND	ND	ND	ND
3	0.025	ND	0.028	ND	0.022	ND	0.025	ND

Abbreviations are: ND, not detectable (<10 ng/ml); NA, not available.

their metabolites, which is matter of few hours, and the effect on bleeding time which, after repeated administrations, lasts for 14 days or more. The interpretation of this finding lies on the proposed models of estrogen action [19, 20] which suggest that estrogens enter the cell and bind with high affinity receptor proteins on the cytosol. The binding of estrogens to estrogen-responsive cells induces a change in the receptor which promotes the translocation of the complex into the nucleus and its retention in the chromatin. The time necessary for the initiation of estrogen response and the duration of the response can well be related to the retention of the estrogen-receptor complex in the nucleus. Apparently platelets do not have specific binding sites for estrogens [21]; at variance, endothelial cells have been found to possess estrogen receptors [22]. Whether the long lasting effect of estrogens on bleeding time in our experimental condition is mediated via endothelial cell receptors remains to be investigated.

In summary our results show that: a) single infusion of conjugated estrogen at a dose of 0.6 mg/kg consistently and significantly shortens bleeding time; the effect is detectable within six hours of the beginning of the infusion, but is short lasting; b) four or five consecutive infusions of conjugated estrogens (cumulative dose ranging from 98 to 228 mg) are necessary to induce a long lasting and more pronounced shortening of bleeding time in uremics; c) the effect of conjugated estrogens on bleeding time in uremics is always reversible, and after 25 days all patients had a bleeding time value comparable to the basal one; d) the pharmacokinetics of conjugated estrogens in uremics is comparable to that of normal subjects. In particular, no accumulation of E_1S and its metabolites is

detectable in the blood following repeated administrations to uremics.

Further studies are necessary to elucidate estrogens' mechanism of action in shortening bleeding time in chronic renal failure patients.

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