Effect of oral and transdermal hormone therapy on hyaluronic acid in women with and without a history of intrahepatic cholestatics of pregnancy

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OBJECTIVE: Intrahepatic cholestasis of pregnancy predisposes women to liver disorders years after affected pregnancy. We compared the basal levels and responses of hyaluronic acid, a marker of liver fibrosis, and liver transaminases to postmenopausal hormone therapy in women with (n = 20) and without (n = 20) a history of intrahepatic cholestasis of pregnancy.

STUDY DESIGN: This was a randomized, double-blind, placebo-controlled, crossover trial.

RESULTS: Basal levels of hyaluronic acid were similar in both groups. Two weeks of oral estradiol 2.0 mg/day led to significant but similar (10.9% to 15.4%) rises in hyaluronic acid in both groups. Increasing the dose of oral estradiol to 4.0 mg/day resulted in normalization of the levels, whereas the addition of medroxyprogesterone acetate led to falls (11.0% to 10.7%) in hyaluronic acid. Transdermal estradiol 50 μg led to a rise (3.2%) in hyaluronic acid only in the control group. Other liver markers were normal at baseline and during hormone therapy.

CONCLUSION: Normal basal levels and/or normal responses of hyaluronic acid and other liver markers to hormone therapy in women with previous intrahepatic cholestasis suggest that this therapy does not predispose these women to liver diseases.

Key words: hormone therapy, hyaluronic acid, intrahepatic cholestasis of pregnancy, liver disease


Hyaluronic acid (HA), a primary glycosaminoglycan synthesized in the cellular plasma membrane of almost all cells contributes the membrane function and takes part (eg, in tissue water homeostasis). In humans, approximately 90% of HA is found in skeleton, skin, and muscles, which all present also estrogen receptors. HA released from cell membranes enters the bloodstream through lymphatic vessels and circulates to hepatic sinusoidal endothelial cells, which rapidly catabolize it by receptor-mediated endocytosis. If the hepatic elimination of HA is insufficient, as in some liver disorders, the HA level in serum rises. Indeed, a high level of HA is used for detecting the presence and severity of different hepatic disorders. Estrogen may be a factor in HA release and/or metabolism because in oophorectomized rats, estrogen stimulates the production of HA both in mammary glands and bladder/urethra, and this stimulation was blocked by progesterone or medroxyprogesterone acetate. No data exist on the effect of estrogen and/or progesterin on HA in women. Intrahepatic cholestasis of pregnancy (ICP) complicates approximately 1-2% of pregnancies in different countries. Symptoms and clinical signs of ICP normally resolve within the first weeks after delivery, and it was long thought that this condition is specific to pregnancy. However, a recent epidemiological study showed that the risk of nonalcoholic liver cirrhosis, pancreatitis, cholelithiasis, and some other hepatobiliary disorders was significantly elevated in women with a history of ICP. Thus, genetic or other factors predisposing women to ICP may later become activated and lead to liver diseases. Postmenopausal hormone therapy (HT) could be a risk factor. This hypothesis is supported by the data that the old-type oral contraceptives containing high amounts of synthetic steroids triggered an ICP-like condition in nonpregnant women with a previous cholestasis-affected pregnancy. Moreover, oral use of estradiol was shown to affect the serum levels of liver-derived proteins, such as C-reactive protein and sex hormone binding globulin, differently in postmenopausal women with and without a history of ICP, whereas transdermal estradiol, escaping enterohepatic circulation and liver metabolism, was without any effect. No epidemiolog-
ical evidence exists on the possible role of HT as a risk factor of liver disturbances in these women. In this study we compared the basal levels of HA and the responses of HA to increasing doses of oral and transdermal estradiol in postmenopausal women with or without a history of ICP. In addition, liver transaminases were measured as well.

Materials and Methods

This randomized, double-blind, placebo-controlled, crossover trial was conducted according to good clinical practice and the Declaration of Helsinki. With the permission of the local ethics committee (registration number 029/99), 40 postmenopausal women with (n = 20) and without (n = 20) a history of ICP were treated in a double-blind, crossover trial with increasing doses of oral estradiol (2.0 and 4.0 mg, both for 2 weeks) or with increasing doses of transdermal estradiol (50 and 100 µg, both for 2 weeks).

The study arrangement and basic clinical data have been presented before, but for clarity, we repeat the most relevant clinical characteristics also here (Table 1). Briefly, our volunteers had entered menopause 6-7 years prior to our trial and complained of hot flashes and other menopausal symptoms. Twenty-eight participants had used some HT regimen but ceased the use at least 4 weeks before recruitment. None of the women had suffered from other liver disease except ICP in the study group; this diagnosis was verified from hospital files. All participants had had at least 1 full-term pregnancy 26-33 years prior to this study.

ICP (n = 20) was diagnosed as typical skin itching and elevated liver enzymes in the third trimester of pregnancy. In 13 women ICP occurred repeatedly. All signs of ICP vanished during 6 weeks after delivery. The women were randomized to receive increasing doses of estradiol valerate (E2) either orally (2.0 mg followed by 4.0 mg) or transdermally (50 µg followed by 100 µg) from a patch in 2-week periods.

The study was blind in that during both treatments, each woman took tablets (active or placebo) and used patches (placebo or active). After using E2 for a total of 4 weeks, each subject took concomitantly with the highest E2 dose also 10 mg of medroxyprogesterone acetate (MPA) orally for 14 days to ensure endometrial safety. After a following 4-week washout period, the subjects crossed over to the other treatment, and thus, each woman used both oral and transdermal regimens. The women were seen at baseline and after the 4-week washout period (2 baseline assessments) and after each 2-week treatment period.

Serum samples collected after an overnight fast were stored at −20°C until analyzed for HA by an enzyme linked binding protein assay (HA quantitative test kit, Corgenix, Inc, Westminster, CO). To minimize the impact of interassay variation, all the samples of a given individual were analyzed in the same batch, and subjects with and without a history of ICP were evenly distributed in different batches of assay. The coefficients of intrassay and interassay variation were 4.7% and 7.0%, respectively.

The samples were also analyzed for the activities of alanine transaminase (ALAT), aspartate transaminase (ASAT), glutamyl transferase (GT), and total alkaline phosphatase (AFOS) by means of routine laboratory methods.

Serum samples collected before each of the 2 6-week treatment periods were used to calculate the true baseline level of HA, which was defined as the median HA level in these samples. Because of the skewed distribution of HA, nonparametric tests were used in the analyses of all variables. The baseline levels of HA and other liver tests were compared to see whether the sole history of ICP could have an influence on these levels.

To study the HT-induced responses in HA levels or other markers, both absolute and relative levels of HA (percent of initial level) were calculated. A possible period effect in the crossover study model was tested with the Mann-Whitney test. Because no such effect existed, the data were analyzed as a single oral and single transdermal regimen. The Friedman test was used to test variation among groups during treatment, and Wilcoxon’s signed-

### Table 1

Baseline clinical characteristics of women with a history of previous ICP (n = 20) and in control women without such a history (n = 20)

<table>
<thead>
<tr>
<th></th>
<th>Women with ICP</th>
<th>Control women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at index pregnancy (y)</td>
<td>25.9 (2.8)</td>
<td>26.7 (2.8)</td>
</tr>
<tr>
<td>Age at menopause (y)</td>
<td>48.0 (2.3)</td>
<td>50.3 (3.3)</td>
</tr>
<tr>
<td>Age at study (y)</td>
<td>55.8 (2.3)</td>
<td>56.3 (2.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5 (2.5)</td>
<td>25.9 (2.6)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130 (22)</td>
<td>134 (15)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>89 (13)</td>
<td>88 (9)</td>
</tr>
<tr>
<td>Smoking</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Previous use of HT</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Other diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>With oophorectomy</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Data are mean (SD) or number of women. There were no differences between the groups.

rank test was used to test changes between groups. Analyses were made by using StatsDirect statistical software version 2.5.5 (StatsDirect Ltd, Cheshire, UK). The HA data are expressed as medians with 95% confidence intervals and SEM. A two-sided \( P < .05 \) was considered statistically significant.

**RESULTS**

At baseline the study groups were comparable in regard to age and other pertinent clinical variables (Table 1). Approximately 30 years had elapsed from the ICP-affected or control pregnancy to our study. The basal levels of HA showed no difference between women with and without a history of ICP (Table 2). A history of previous use of HT, age, body mass index, smoking, or the use of antihypertensive drugs did not affect the HA levels in either group. The level of HA did not correlate with the levels of ALAT, ASAT, GT, or AFOS, which were fully comparable between the study groups (Table 2).

A daily oral dose of 2.0 mg of E2 led to a significant rise \( (P = .017) \) in HA in the ICP group (10.9% [SEM = 7.3] from baseline), but this rise did not differ from that (15.4% [SEM = 7.3]) in the control group (Figure 1). Increasing the daily dose of oral E2 to 4.0 mg resulted in the return of HA to the baseline level. The addition of MPA to oral E2 was accompanied by a further drop of HA from the baseline (–11.0% [\( P < .01 \), SEM = 9.6] and –10.7% [\( P < .001 \), SEM = 6.5], respectively) in both groups (Figure 1). Neither dose of transdermal E2 raised the level of HA in the ICP group, but in the control group, 50 \( \mu \)g of E2 led to a transient rise in HA level (3.2% [SEM = 10.6], \( P = .012 \)); this rise was lower than that seen during the oral treatment. MPA given concomitantly with transdermal E2 failed to affect the HA levels (Figure 1).

An oral or transdermal regimen did not affect the levels of ALAT, ASAT, GT, or AFOS in either group, although a trend toward a decrease in these levels during both regimens was seen (Figure 2).

**TABLE 2**

Baseline values of HA and those of other liver markers (median, 95% confidence interval) in women with and without a previous history of ICP

<table>
<thead>
<tr>
<th></th>
<th>Women with ICP (n = 20)</th>
<th>Control women (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>HA (( \mu )g/L)</td>
<td>27.0</td>
<td>(20.0 to 64.8)</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>10.0</td>
<td>(6.0 to 36.0)</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>27.0</td>
<td>(18.0 to 50.0)</td>
</tr>
<tr>
<td>GT (U/L)</td>
<td>23.0</td>
<td>(11.5 to 135.5)</td>
</tr>
<tr>
<td>AFOS (U/L)</td>
<td>100.3</td>
<td>(71.5 to 198.0)</td>
</tr>
</tbody>
</table>

There were no differences between groups.

**COMMENT**

Hyaluronic acid, a cell membrane-stabilizing glycosaminoglycan, is abundantly present in the connective tissue, skin, bones, and muscles.\(^1\) These tissues undergo atrophic changes in hypoestrogenic postmenopausal women. No human data exist on the possible connection of estrogen and HA in women, but oophorectomy in rats resulted in the drastic fall in HA content in mammary glands,\(^4\) bladder, and urethra\(^5\); these falls were prevented by intramuscular E2\(^4\) or oral conjugated equine estrogen administration.\(^6\) Progesterone\(^4\) or MPA\(^8\) given concomitantly with estrogen blocked the stimulating effect of estrogen on HA.

We show that the initiation of oral E2 in postmenopausal hypoestrogenic women causes a rise in the circulating HA level. Although our data cannot deduce whether this rise was a reflection of increased synthesis and release and/or reduced hepatic elimination of HA, we consider the first explanation more plausible. Oral E2 may cause a consistent stimulation in the synthesis of HA, but subsequent activation of hepatic sinusoidal cell function eliminates the excess of the circulating HA. Therefore, despite increasing the dose...

**FIGURE 1**

Responses of HA in women with and without ICP to doses of E2 and MPA

Responses of HA (percentual change from basal level) in women with and without ICP of pregnancy to increasing doses of A, oral and B, transdermal E2 followed by 10 mg oral (MPA). Data are expressed as medians.

of oral E2 to 4.0 mg daily, no further rise in HA was seen.

In this study, oral and transdermal E2 was given at doses that are thought to be clinically equipotent. However, transdermal E2 failed to affect the synthesis and/or elimination of HA almost entirely; only a minor rise in HA was seen following the first transdermal dose. Thus, oral E2 appears to be a stronger and more rapid stimulator of HA than transdermal E2. The cause of this difference is not known, but it may be related to a different estrogenic milieu in both HA-producing organs and the liver during oral and transdermal E2 use. Estrone, predominantly present during oral but not during transdermal regimens, may be needed for the stimulation of HA in women. This, however, is not supported by the animal data showing that intramuscular E2 and oral conjugated equine estrogens caused almost similar rises in HA content in oophorectomized rats. Alternatively, it is possible that the HA synthesis became activated also during the transdermal use, but this happened later at a time when the hepatic sinusoidal cells had already become activated and could eliminate the excess in circulating HA.

The addition of progesterone to estradiol or MPA to conjugated equine estrogens abolished the stimulating effect of estrogen on the production of HA in rats. Our data suggest a similar relationship in women; MPA, if given concomitantly with oral E2, caused a significant fall in the level of HA, whereas it was without any effect on HA during transdermal E2 use. Thus, a stimulated production of HA because of oral E2 was blocked by MPA, but a less stimulated or unstimulated HA production during transdermal E2 was not significantly affected by MPA. The possibility that MPA could have increased the hepatic elimination of HA and therefore caused falling HA levels appears less likely.

Recent guidelines on optimal use of postmenopausal HT do not list ICP as a contraindication to HT. Therefore, given the relatively high incidence of ICP, a large number of these women could become exposed to long-term HT. It has not been shown whether postmenopausal HT is a factor for the elevated risk of subsequent liver diseases in women with previous ICP. If this would be the case, oral HT could exhibit a stronger risk than transdermal HT because oral, but not transdermal, HT is subject to enterohepatic circulation and liver metabolism. Our data show that approximately 30 years after an ICP-affected pregnancy, the basal levels of HA and those of transaminases, GT, or AFOS or their responses to both oral and transdermal E2 followed by oral MPA are normal in women with a history of ICP. Thus, these women do not suffer from such a dysfunction of hepatic parenchymal or sinusoidal endothelial cells, which could be detected with the aid of HA or other liver markers.

Our study has limitations. First, the small sample size may weaken the power to detect any differences between the...
study groups at baseline. However, regarding the effect of HT, the crossover design of the study enabled us to find any differences in HT responses between the study groups. Second, HT was given for only 6 weeks, and this exposure is short when compared with the use of several years in postmenopausal women. Thus, a possibility exists that a longer therapy might have different effects. And third, we administered natural E2 and MPA; other HT regimens could behave differently in this regard. Therefore, an epidemiological study on ICP women with and without later liver complications is warranted to fully conclude whether HT is a liver risk factor for women with previous ICP.

REFERENCES