Serum Lipid and Estrogenic Effects of Manvene, a New Estrogen Analog

Comparison with Premarin in Men with Coronary Heart Disease

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Estrogenic side-effects and serum lipid changes were studied in middle-aged men with coronary heart disease during a 6-month period with varying dosage levels of Manvene, a new estrogen analog. Although significant lipid-shifting effects were noted at 5 and 10 mg. daily, these were not comparable with those of 10 mg. of Premarin. Analysis of scattergrams of individual estrogenic and lipoprotein responses of both Manvene-treated and Premarin-treated patients revealed a favorable dissociation of these effects in 5 of the 29 men in the Manvene groups.

The profound changes in the serum lipids resulting from estrogen administration to men and women with coronary heart disease have been documented in several laboratories.1-6 But these presumably "favorable" alterations have been offset by undesirable estrogenic side-effects, especially in middle-aged men.5,6 Among many measures suggested to avoid the side-effects, including very low estrogen dosages,7 intermittent estrogen administration,8 and various androgen plus estrogen combinations,9 the most promising appeared to be the use of synthetic analogs of steroidal and nonsteroidal estrogens, which might retain significant lipid-shifting potency and yet lack estrogenicity. Among many estrogen analogs synthesized in the laboratories of G. D. Searle & Co. one, 3-methoxy-16α-methyl-1, 3, 5(10)-estra-triene-16β, 17β-diol (Manvene), showed lipid-shifting effects in the rabbit and the chick, anti-atherogenic effects in the chick, but only slight estrogenic activity when tested in mouse uterine growth or rat vaginal smear assays.10 The results of subacute and chronic toxicity studies in the rat showed this compound to be devoid of serious toxic side-effects,10 thus allowing its use in clinical trials.

Such a trial was designed to test whether this dissociation remained valid in men on long-term therapy. Previously reported data for relatively brief treatment periods suggested such a dissociation.11 The outcome of a 6-month period of therapy with oral Manvene at 3 dosage levels is the basis of this report.

Material and Methods

Clinical Material. Forty-nine middle-aged ambulatory men, who had recovered from proved myocardial infarction, were selected for study. These patients were seen in private practice and were largely business, professional and white-collar workers, 60 per cent of whom were of Northern European or Anglo-Saxon descent.

Clinical evaluations were done prior to therapy, together with at least 2 complete serum lipid studies. Twenty-nine patients were divided into 3 groups and placed on 2.5, 5.0, or 10.0 mg. of Manvene daily for 6 months, with monthly clinical and serum lipid studies. For comparison, 20 patients were placed on 10 mg. daily of oral, mixed conjugated equine estrogens, N.N.R. (Premarin) on a long-term basis, and were re-evaluated at 1, 2, and 6 months of therapy. The structural formulas of Manvene and sodium estrone sulfate, the chief constituent of Premarin, are shown in figure 1.

Estrogenic side-effects in both the Premarin and Manvene groups were graded according to the schema of table 1.
TABLE 1.—Scoring of Estrogenic Side-effects

<table>
<thead>
<tr>
<th>Side Effects</th>
<th>2 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast tenderness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Breast hypertrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Depression of sex functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight to moderate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Complete</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Maximum possible score</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Structures of Manvene and sodium estrone sulfate, the principal active estrogen of Premarin.

The reason for the assignment of more weight to breast hypertrophy than to the other categories is the objectivity of this effect, ruling out any misinterpretation due to the bias of either the examiner or the patient.

Biochemical Methods. Total serum cholesterol and lipid phosphorus were determined by the standard methods of this laboratory, and phospholipids and cholesterol/phospholipid (C/P) ratios were calculated in the conventional way. Ultra-centrifugal separation of the $\alpha$- and $\beta$-lipoproteins, determination of the cholesterol content of the 2 fractions, and calculation of the $\beta$/$\alpha$-lipoprotein cholesterol ratios were done as described in an earlier communication.

RESULTS

The average changes of the C/P ratios in the 4 treatment groups are shown in figure 2. At 1 month of therapy, both 5 and 10 mg. dosage levels of Manvene and 10 mg. of Premarin had shown similar lowering effects on the C/P ratio. But from the second month the Premarin group showed a sustained decrease, nearly double that of the 5- and 10-mg. Manvene groups. There was a smaller decrease in the 2.5-mg. Manvene group, reaching statistical significance ($p \geq .05$) only at 4 and 6 months. Post-treatment control studies in the Manvene groups showed...
a return to approximate pretreatment levels. Off-treatment data are not available for the Premarin group because therapy was continued beyond the 6-month period of observation.

Similar changes were seen in the β/α-lipoprotein cholesterol ratios during treatment (fig. 3). Again, the Premarin-treated group showed a greater average response than did the Manvene-treated groups, even at 1 month. Maximum sustained response was attained by 2 months of therapy. This was least in the 2.5-mg Manvene group. All changes were statistically significant (p < .01). Pretreatment levels were attained within 1 month when Manvene therapy was discontinued.

The graded side-effects, derived according to table 1, are shown in figure 4 for all groups. Since both the extent of side-effects and the degree of serum lipid changes were closely akin in the 5- and 10-mg Manvene groups, these were combined. The mean side-effects score of the Premarin group was 10.8, range 8-14. The mean score of the 2.5-mg Manvene group was 2.4, and that of the 5- and 10-mg group, 6.6. Ten of these 29 subjects suffered no side-effects. Analysis of the individual responses to Manvene was carried out by constructing a scattergram to determine whether this substance is in fact a non-estrogenic lipid-shifting agent.

In the upper portion of figure 5 the individual responses of the Premarin group are shown in a scattergram; the sum of the percentage decreases of the β/α-lipoprotein cholesterol ratios at 2 and 6 months of therapy was used as a measure of lipid-shifting effect and was plotted against the sum of the estrogenic side-effects scores evaluated at the same time. In all but 1 patient, there was excellent serum lipid response, accompanied by significant side-effects.

The corresponding scattergram of the Manvene-treated groups is shown in the lower portion of figure 5. In contrast to the Premarin group, only 9 Manvene-treated patients showed both extensive serum lipid changes and severe side-effects. Twelve patients had no or slight side-effects accompanied by minimal serum lipid changes. There were 8 patients showing a dissociation, 5 of whom were considered to be favorable because of significant serum lipid response with slight side-effects.
Nausea was absent in the Premarin group; only 1 patient in the Manvene groups reported this complication. During the 6-month period of observation no patients in either the Premarin or Manvene groups suffered migraine or nocturnal cramps of leg muscles. One patient developed sodium and water retention followed by pulmonary congestion after 2 months of Premarin. Omission of the drug resulted in his recovery.

**Discussion**

Manvene is clearly a potent lipid-shifting agent in dosages above 2.5 mg. daily. This is shown in its effects on both the C/P and the β-1/α-lipoprotein cholesterol ratios. But even at 10 mg., the effects are not as dramatic as those of an equal dosage of Premarin. Analysis of data from a preliminary study of higher Manvene dosages\(^{11}\) showed that 50 mg. of this drug produced serum lipid changes very similar to those obtained with 10 mg. of Premarin (fig. 6). This Premarin/Manvene "potency ratio" of 5 is similar to that of estrone/Manvene in terms of the C/P ratio lowering effect in cholesterol-fed chicks, also found to be about 5.\(^{10}\)

Comparative studies of different estrogens using the immature mouse uterus test revealed that Manvene had only 0.3 to 3 per cent of the estrogenic activity of Premarin.\(^{10}\) This relative lack of estrogenicity has not been borne out by the results in men, since the side-effects scores of patients on 5 or 10 mg. of Manvene daily were often similar to those of the 10-mg. Premarin group. The high incidence of estrogenic side-effects in the 50-mg. Manvene group prevented the continuation of this dosage beyond 2 months. Furthermore, when 10 mg. of Manvene daily were given to 10 postmenopausal women for 7 days, comparison of pretreatment and post-treatment Papanicolaou smears showed uniform cornification of the vaginal epithelial cells, indicating an estrogenic effect. A typical sequence is illustrated in figure 7.

The conventional biologic tests for estrogenic activity have failed to predict the estrogenic potency ratio of Premarin/Manvene in men. It is suggested that the response of the vaginal epithelium of healthy postmenopausal women might be used for the crude estimation of estrogenicity of new lipid-shifting compounds related to the steroidal estrogens before these substances are administered to men. A similar lack of correspondence between estrogenic potency in human beings and in experimental animals has been reported by Brown and Bradbury.\(^{12}\)

Analysis of the scattergrams of the individual results offers an explanation for the initial impression that Manvene was a practical "weak" estrogen. The similarity of results in 9 Manvene-treated subjects to those seen in the Premarin group implies that these subjects handled Manvene metabolically as another estrogen. In contrast, the 12 men with both minimal lipid changes and side-effects were probably unable to utilize this compound as an estrogen. The favorable dissociation, i.e., significant serum lipid changes and minimal estrogenic side-effects, seen in
Fig. 7. Vaginal smears of a postmenopausal subject (Papanicolaou stain) (Left) before administration of Manvene showing numerous basophilic epithelial cells without cornification, and (right) after 10 mg. of Manvene daily for 7 days, showing large polyhedral, cornified epithelial cells, typical of estrogen stimulation.

5 Manvene-treated patients, is encouraging. Thus, a continuing search for other compounds related to both natural and synthetic estrogens is worthwhile, in an attempt to find a substance that uniformly produces a favorable separation of lipid-shifting and estrogenic effects.

SUMMARY AND CONCLUSIONS

A long-term clinical trial of varying dosages of Manvene, a new estrogen analogue, was undertaken to evaluate its usefulness as a lipid-shifting agent without significant estrogenicity in men with coronary heart disease. Similar patients were treated with Premarin, a conventional potent estrogen, for comparison. Serum lipid studies and clinical evaluations were done on all patients during a pretreatment control period and after 2 and 6 months of therapy.

At dosages above 2.5 mg. daily, Manvene produced significant serum lipid changes, lowering both the C/P and $\beta$-/a-lipoprotein cholesterol ratios. However, 50 mg. of Manvene were required to match the lipid changes produced by 10 mg. of Premarin.

Manvene produced changes similar to those of adequate doses of conventional estrogens in some subjects, while in others these changes were slight. However, significant serum lipid changes with minimal estrogenic side-effects were seen in 5 of the 29 patients. This observation suggests the feasibility of continuing the search for a truly ‘‘nonestrogenic’’ estrogen derivative with maintained lipid-metabolic effects.

ACKNOWLEDGMENT

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SUMMARIO IN INTERLINGUA

Esseva interprendite un studio clinie a longe vista in le uso de Manvene que es un nove analogo de estrogeno. Le droga esseva administrate in varie doses, e le objectivo del
studio esbeva evalutar le utilitate de Manvene como agente capace a modificar le stato lipidic sin producere grados significative de estrogenicitate in masculos con morbo cardiac coronari. Pro objectivos de comparation, un gruppo de patientes simile a illes tractate con Manvene esbeva subjecite a un curso de Premarina que es un potente estrogeno conventional. Studios del lipidos seral e evaluaciones clinica esbeva effectuate pro omne le patientes durante un periodo de controlo ante le tractamento e post 2 e 6 menses de medication.

A dosages supra 2,5 mg per die, Manvene produceva significative alterationes del lipidos seral, abassante tanto le proportion C/P como etiam le proportion lipoproteinie beta/alpha. Tamen, 50 mg de Manvene esbeva requirite pro attingere le alterationes lipidic producete per 10 mg de Premarina.

In certe subjectos Manvene produceva alterationes simile a illos producete per adequate doses de estrogens conventional, sed in altere subjectos le effectes de Manvene esbeva leve. Tamen, significative alterationes del lipidos seral con minimal effectes lateral estrogenic esbeva observate in 5 del 29 patientes. Iste constatation justifica le continuation del cerca de un vermente "non-estrogenic" derivato de estrogeno in que le effectes super le metabolismo lipidic remane intacte.

REFERENCES

At the outset I would like to emphasize the fact that the student of internal medicine cannot be a specialist. The manifestations of almost any one of the important diseases in the course of a few years will 'box the compass' of the specialties.—WILLIAM OSLER, M.D. Internal Medicine as a Vocation. Med. News (N.Y.), 1897.
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