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34. Pharmacology of Sex Steroids on Liver

ESTROGEN RECEPTORS AND ANDROGEN RECEPTORS IN THE MAMMALIAN LIVER

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Summary—An estrogen receptor and an androgen receptor are present in the mammalian liver. In the liver of the rat, the estrogen receptor concentration increases markedly at puberty and this change correlates with enhanced estrogen stimulation of plasma renin substrate synthesis. High doses of estrogen are required for nuclear binding in liver when compared to doses for the uterus. The high dose requirement appears to be predominantly due to extensive metabolism in the hepatocyte of the estrogen to inactive derivatives. Furthermore, estradiol is much weaker than ethinyl estradiol for promoting nuclear binding in the liver. This is due to extremely rapid and extensive metabolism of estradiol. In human liver the concentration of estrogen receptor is low.

An androgen receptor is present in high concentration in rabbit liver and is located predominantly in the nucleus after androgen administration. High concentrations of a putative androgen receptor are also present in human liver cytosol. Preliminary studies indicate that synthetic progestins can attach to the human liver androgen receptor. To date, a progesterone receptor has not been found in the mammalian liver.

Thus, it appears that extensive steroid metabolism in liver preferentially diminishes sex steroid interaction with liver receptors and that androgen receptors may mediate progestin effects in liver. These observations provide a scientific basis for improved safety of oral contraceptives. Lowering the estrogen and progestin doses in oral contraceptives will decrease the major side-effects, which are liver mediated, and still maintain the desired effects at the hypothalamic–pituitary axis and uterus. Furthermore, it is likely that by selecting which estrogen, progestin or androgen is administered as well as by utilizing a parenteral route of administration that sex steroid effects on the liver could be minimized.

INTRODUCTION

It is now recognized that the mammalian liver contains estrogen receptors and androgen receptors. In the past, the mammalian liver was not usually considered to be a classic target organ for sex steroids since early studies were unable to demonstrate sex steroid receptors in the mammalian liver.

Sex steroids have multiple effects on human liver function [1, 2]. Some of these effects are of clinical importance. In particular, such effects may strongly contribute to oral contraceptive side-effects by changing the synthesis of certain plasma proteins, by changing the composition of bile and by increasing the risk of developing liver tumors [3].

In addition, androgen effects on liver contribute to the side-effects of anabolic androgens [4]. The most serious side-effect of anabolic androgens is the development of liver tumors which are usually malignant [4, 5].

In this review we will describe what our laboratory has ascertained about the mammalian liver sex steroid receptor systems. We will indicate that these systems in mammalian liver have some unusual properties which could be utililized to diminish adverse liver effects of administered sex steroids.

RESULTS

Cytosol estrogen receptor in the rat liver and correlation with plasma renin substrate response

In earlier studies other laboratories often used the rat liver cytosol as an estrogen receptor negative control. Most of these studies used the liver of the prepubescent rat. Although the prepubescent rat liver cytosol has very little estrogen receptor we found that adult female rat liver cytosol has substantial levels of an estrogen receptor. This receptor has a K_d of 1×10^{-10} M for estradiol and an estrogen specificity consistent with the receptor detected in other target organs [6–8]. Since 1973 the presence in rat liver cytosol of estrogen binding consistent with estrogen receptor has been widely confirmed [9–12].

The level of this estrogen receptor in adult rat liver is 58 fmol/mg cytosol protein. This is estimated to represent 10,000 sites per hepatocyte which is about 1/3 of the level in the uterus. The level in the adult liver is more than 5 times higher than the level in prepubescent liver (Fig. 1, top) [8]. The increase in estrogen receptor in the adult liver correlates with an enhanced response by adult liver to estrogens. Plasma renin substrate, which is synthesized in the liver, only increases substantially in adult rats treated with estrogens: after administration of high doses of ethinyl estradiol, the plasma level of renin substrate in the adult is increased to 3 times control level, while in the prepubescent rat, there is no change. Although ethinyl estradiol is only effective in the adult, administration of a glucocorticoid, dexamethasone, is equally effective for increasing plasma renin substrate in both adult and in prepubescent rats [13].

Unlike the estrogen receptor of the uterus, the estrogen receptor in the liver is highly dependent on the presence of the pituitary. There are extremely low levels of the estrogen binding protein

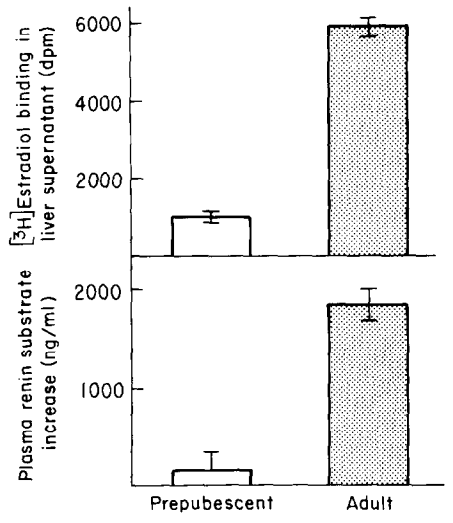

Fig. 1. Developmental correlation of estrogen binding in female rat liver with estrogen induction of plasma renin substrate. Macromolecular binding of 2 nM [^{3}H]estradiol was measured by gel filtration after incubation in ice for 1 h in 0.2 ml of cytosol from 27-day-old and 200 g adult female rats without estrogen treatment (top). Groups of 5 animals each of prepubescent and adult rats received s.c. injections of 100 μg of ethinyl estradiol or the vehicle alone at 0 and 24 h. At 48 h plasma renin substrate was measured by radioimmunoassay. The control levels of plasma renin substrate were 1040 ng/ml for the prepubescent group and 1100 ng/ml for the adult group. The graph indicates the increase above control in the estrogen-treated groups (bottom). The bars represent the SEM. [Reprinted from Eisenfeld *et al.*, *Science* **191** (1976) 862. Copyright 1976 by the AAAS.]

in the adult female rat liver following hypophysectomy [8]. Consistent with the interpretation that the liver estrogen binding protein is an estrogen receptor is our finding that estrogen administration to hypophysectomized adult rats does not increase plasma renin substrate [13]. A recent study shows that the low levels of estrogen receptor in liver of hypophysectomized adult rats can be restored to normal levels by administration of growth hormone and dexamethasone [14].

Nuclear retention—high doses required for liver

Recent studies provide evidence that the estrogen receptors are located in cell nuclei and that cytosol localization of unoccupied receptors is the result of leaching of the receptor subsequent to cellular disruption [15, 16]. Attachment of an estrogen to the receptor enhances the affinity of the receptor to remain in the nucleus following cellular disruption. Thus, our studies in which we observed apparent translocation of the estrogen receptor from cytosol to nucleus we now interpret as representing an increase in nuclear retention of receptor.

The liver receptor requires higher doses of estrogen *in vivo* for nuclear retention compared to uterus [17]. In adult female rat liver the level of estrogen receptor is high in the cytosol and low in purified nuclei. After administration of a high dose of ethinyl estradiol, 100 μg, the receptor level is markedly diminished in cytosol and more than half of the initial cytosol level is found in highly purified nuclei (Fig. 2). When 5 μg of ethinyl estradiol is injected, only low levels of receptor complex are detected in the liver nuclei. In contrast to liver, administration of 5 μg is as effective as 100 μg in increasing the receptor levels in the nuclear fraction of the uterus (Fig. 2).

High concentrations of EE2 are also required to promote the detection of nuclear complexes in isolated rat hepatocytes [18]. The estrogen receptor system is present in these cell preparations which are enriched in parenchymal cells relative to the Kupfer and endothelial cells which are also present in whole liver. The half-maximal concentration of EE2 is 5×10^{-7} M for nuclear retention of the estrogen receptor in the isolated hepatocytes.

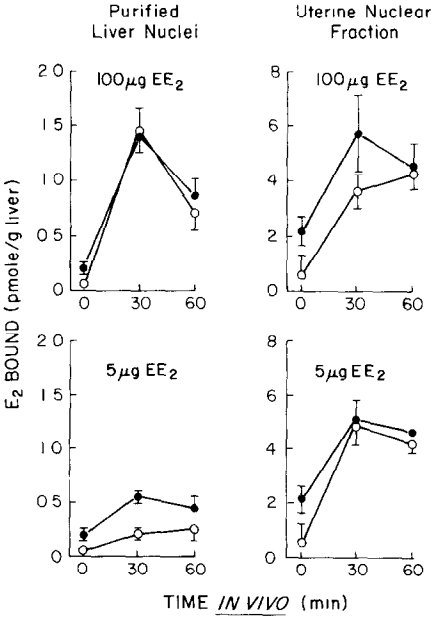

Fig. 2. Estrogen receptor levels in liver and uterine nuclear fractions after administration of 5 or 100 μg of ethinyl estradiol *in vivo*. Ethinyl estradiol was injected subcutaneously in propylene glycol. At 30 or 60 min after administration, purified nuclei from liver and the nuclear fraction from uteri were isolated. The levels of estrogen receptors were determined by exchange assays with radioactive estradiol. To determine specific binding, replicate samples were incubated in the absence and presence of an excess of non-radioactive DES. The exchange reactions were conducted both at 30°C and 0°C in order to estimate the levels of total receptor (●) and of occupied receptor (○). The bars depict the SEM. The cytosol level in the vehicle injected controls was 2.0 pmol/g of liver and 7.2 pmol/g of uterus. (Adapted from *Endocrinology* [17] with the permission of the Endocrine Society.)

Estradiol (E2) is weaker than ethinyl estradiol (EE2) for promoting nuclear estrogen receptor retention in liver

We have compared liver nuclear receptor retention of E2 with that of EE2, the biologically active estrogen from almost all combined oral contraceptives. E2 is much weaker than EE2 for increasing retention of the estrogen receptor in nuclei of isolated hepatocytes. About 100-fold higher concentration of E2, 10^{-5} M, is required to promote nuclear levels of estrogen receptor comparable to those with 10^{-7} M EE2 (Fig. 2) [19]. *In vivo*, E2 is also much weaker than EE2 for promoting nuclear retention of the liver estrogen receptor. The dose of EE2 to half-maximally increase nuclear estrogen receptor levels in liver is about 15 μg, while more than 100 μg of E2 is required (Fig. 3) [20]. Another laboratory using a different strain of rat finds that liver nuclear estrogen receptor levels determined at 1 h are maximal following 10 μg of EE2 or 50 μg of E2 [21]. The high doses required in our studies for liver nuclear binding contrast with the low doses of only a few micrograms of either E2 or EE2 required for maximal uterine binding [20, 22].

These nuclear receptor results provide an explanation for the observations that higher doses of estrogen are required to produce a liver response compared to a uterine response and that EE2 is substantially more potent than E2 for increasing plasma renin substrate while the difference in their potency for uterine weight gain is much smaller [23].

Metabolism of estrogen to inactive metabolites diminishes the potency of estrogens for nuclear receptor complex formation in liver

Differences among estrogen in their liver metabolism is the cause of the difference in potency of estrogen for promoting nuclear estrogen receptor complexes in liver. In isolated rat hepatocytes we find that estrogens are rapidly metabolized. For example, by 2 min after addition of 10^{-7} M E2 or EE2 at 37°C, only 3% of the E2 is not metabolized and only 18% of the EE2 is not metabolized [19]. Adding an inhibitor of the microsomal drug metabolizing system, SKF 525A, maintains the level of unchanged EE2 for a longer time period and enhances the potency for promoting nuclear complexes (Fig. 3). With E2, addition of SKF together with testosterone, which transiently prevents the oxidation of E2 to estrone, temporarily maintains the level of E2 at a higher concentration and increases the potency for nuclear retention of the estrogen receptor (Fig. 3). The major difference in metabolism between E2 and EE2 is that E2 is rapidly and extensively metabolized to estrone while EE2, because of the 17 alpha ethinyl group, cannot be oxidized by estradiol dehydrogenase.

Metabolism also influences the identity of the estrogen which is attached to the receptor in the nucleus [24]. After incubations with EE2, the estrogens attached to the nuclear receptor are unchanged EE2 and its catechol metabolite. After incubations of E2 with female rat liver cells, the estrogen attached to the nuclear receptor is entirely unchanged E2, even though most of the non-polar radioactivity during the incubation is the metabolite estrone.

The inhibitors of estrogen metabolism also increase the potency of estrogens for nuclear estrogen receptor retention in the adult female rat liver *in vivo* [20]. SKF increases the potency of EE2 approximately 10-fold. SKF plus testosterone increases the potency of E2 about 10-fold (Fig. 4). Thus, the *in vitro* and the *in vivo* results are consistent with

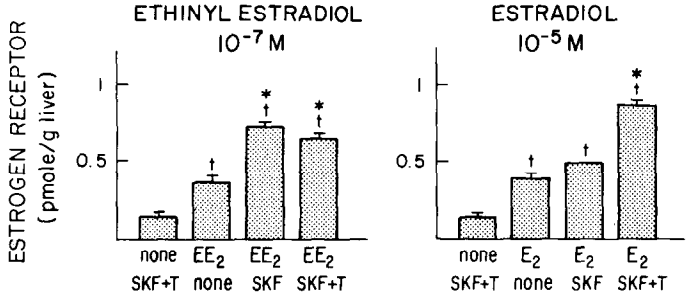

Fig. 3. Effects of ethinyl estradiol, estradiol and inhibitors of estrogen metabolism on estrogen receptor levels detected in nuclei of isolated rat hepatocytes. Isolated hepatocytes were prepared for adult female rats and incubated with 10^{-7} M ethinyl estradiol (EE2) or 10^{-5} M estradiol (E2) at 37°C. Replicate flasks also contained 2×10^{-3} M SKF 525 (an inhibitor of the drug microsomal metabolizing system) with or without 2×10^{-5} M testosterone (T) (which functions in this system as a transient inhibitor of the oxidation of estradiol to estrone). The control flasks contained SKF and testosterone without an estrogen. After incubation for 15 min the cells were washed, homogenized and the purified nuclear fraction obtained by sedimentation through dense sucrose. The estrogen receptor levels in the nuclei were determined by exchange assays. The bars depict the total receptor level +/− SEM. Statistical significance was determined by analysis of variance separately for the ethinyl estradiol groups in the left panel and for the estradiol groups in the right panel. The dagger in the figures indicate a higher level ($P < 0.05$) in a estrogen-treated group compared to the control group. The asterisk indicates higher levels ($P < 0.05$) than in the other groups in the panel. The cytosol level in the control group was 1.0 pmol/g liver. (Adapted from *Endocrinology* [19] with the permission of the Endocrine Society.)

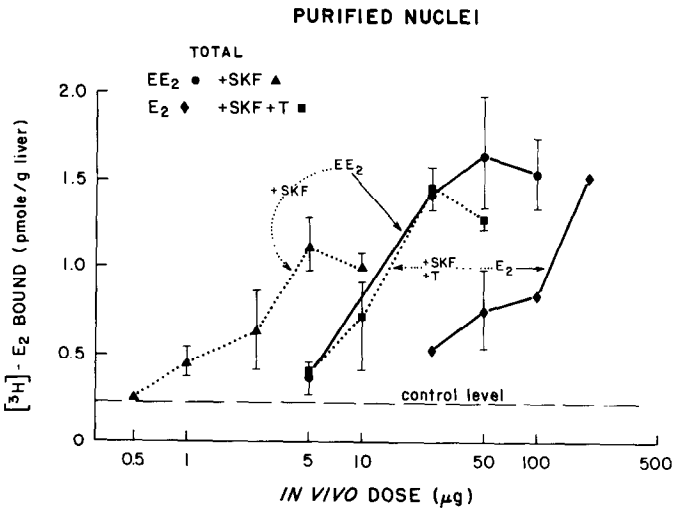

Fig. 4. Effect of dose of ethinyl estradiol or estradiol administered without and with inhibitors of estrogen metabolism on the estrogen receptor levels in purified nuclei from adult female rat liver. Adult female rats were injected with different doses of ethinyl estradiol (EE2) or estradiol (E2). Some of the groups also received the metabolic inhibitors SKF 525 and testosterone (T) (which in isolated hepatocytes functions as a transient inhibitor of the oxidation of estradiol to estrone). Thirty min later, the levels of total (occupied and unoccupied) estrogen receptor in highly purified nuclei were determined by exchange assays. The figure indicates that ethinyl estradiol is more potent than estradiol. The metabolic inhibitors enhance the potencies of the estrogens for liver nuclear complex retention. (Adapted from *Endocrinology* [20] with the permission of the Endocrine Society.)

estrogen metabolism in liver diminishing the potency of estrogens for promoting a nuclear estrogen receptor complex in liver.

Androgen receptor in liver

In our early studies of adult male rat liver we found, using radioactive dihydrotestosterone, very high levels of a binding protein [25]. This binding protein, which is androgen induced, was originally thought by another laboratory to be the androgen receptor [26]. However, we find that this protein has a relatively poor affinity for both androgens and estrogens and that its specificity is not consistent with that of an androgen receptor [25].

In recent studies we have shown that rabbit liver contains an androgen receptor [27]. The receptor has a high affinity, K_d of 0.9 nM, for [^{3}H]1881 (methyltrienolone), a capacity of 79 fmol/mg cytosol protein, and a specificity appropriate for an androgen receptor. Following administration of 1881 *in vivo*, the receptor is detected predominantly in highly purified nuclei (Fig. 5). All these properties of the androgen binding system in rabbit liver are consistent with this protein being an androgen receptor. The concentration of the receptor in liver is in the same range as in the male reproductive tract.

Recently, an androgen receptor has also been described in the liver of male rats [28]. It binds

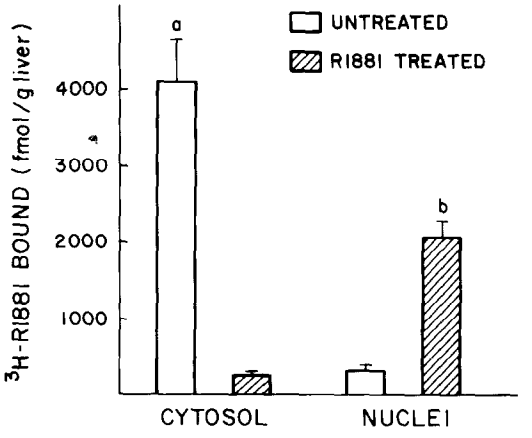

Fig. 5. The effect of methyltrienolone administration *in vivo* on the androgen receptor levels in cytosol and nuclei of liver from rabbits. R-1881, methyltrienolone, 100 μg, was administered subcutaneously to adult female rabbits 1 h before sacrifice. The controls received the vehicle alone. The 35% ammonium sulfate fraction of liver cytosol (which contains the receptor in the untreated group) was incubated with 5 nM R-1881 for 16 h in the absence and presence of 250 nM non-radioactive R-1881. (All incubations also included non-radioactive triamcinolone acetonide and cortisol.) Liver nuclei were purified by sedimentation through dense sucrose, and the nuclear receptor levels were determined by an exchange assay. (a) Significantly higher than all other groups ($P < 0.05$); (b) significantly higher ($P < 0.05$) than untreated group, by analysis of variance. (Adapted from *Endocrinology* [27] with the permission of the Endocrine Society.)

R-1881 with a K_d of 2 nM, a capacity of 19 fmol/mg cytosol protein, and a specificity appropriate for the androgen receptor. Apparent translocation from cytosol to the nucleus following *in vivo* R-1881 has been shown. Although there is specific androgen binding in sucrose gradients using cytosol from male rats there is no specific binding in the gradients using cytosol from female or testicular feminized male rat liver.

Sex steroid receptors in the human liver

Our laboratory has found, in ongoing studies, that human liver cytosol binds estrogens with high affinity and specificity [29]. However, the levels of binding in human liver are much lower than those we found in liver of rat, mouse, rabbit and green monkey. Our results confirm the results of other laboratories indicating that human liver cytosol contains low levels of binding consistent with estrogen receptor [30, 31].

Human liver cytosol binds androgens at much higher levels than it binds estrogens [29]. The properties of the androgen binding are consistent with androgen receptor. It is notable that the levels of liver cytosol androgen receptor are in the same range as the levels detected in classic androgen target organs of the male reproductive tract. Our finding of androgen receptor in human liver cytosol agrees with observations in some recent reports [32, 33].

A progesterone receptor has not been detected in human liver. Furthermore, a progesterone receptor has not been found in the liver of any mammal we have studied. Although we have not detected a progesterone receptor in the rat liver following estrogen treatment, the possibility that estrogens induce progesterone receptor in mammalian liver requires further investigation. Since the human liver may not have a progesterone receptor, it is possible that progestins influence liver function by attaching to the high concentrations of androgen receptor. This is supported by our observation that some progestins, either 19 nor-testosterone derivatives or progesterone derivatives, are capable of attaching to the androgen receptor in human liver cytosol [29]. This observation is concordant with observations that progestins can bind to androgen receptors of other target organs and can produce some androgenic responses [34].

Sex steroid receptors in human liver tumors

Recent information suggests that some human liver tumors have sex steroid receptors.

We have preliminary results concerning the receptor levels in a liver adenoma which most likely was oral contraceptive induced. The adenoma was excised from a young woman who had used oral contraceptives for 10 yr. The levels of sex steroid receptors in this benign adenoma are similar to the levels in normal human liver. Benign liver adenoma is an extremely rare tumor, which is detected only in adult women and is markedly increased in women who use oral contraceptives [35]. The increase is 60-fold for long-term oral contraceptive users.

Focal nodular hyperplasia, another type of benign liver tumor, has low levels of cytosol estrogen receptor [36].

Low concentrations of putative estrogen receptors are found in the cytosol from some cases of primary hepatocellular carcinoma [37, 38]. Androgen binding consistent with receptor is also found in cytosol from most cases of primary hepatocellular carcinoma [33, 39]. Primary hepatocellular carcinoma is one of the most common types of cancer in the world with a particularly high incidence in Africa and Asia. There is a strong sex prediliction with 3–9 males affected for every female [40].

In addition to studies of excised human liver tumors, a cell line derived from a human liver carcinoma has been found to contain an estrogen receptor and to exhibit some responses to estrogen [41, 42]. The number of nuclear high affinity binding sites for estrogen increases from 300 to 3000 sites per nucleus after 48 h incubation with estradiol. Estradiol increases the secretion into the medium of two apolipoproteins, apo-C-II and apo-A-I 2.5- and 2-fold respectively. The cellular level of mRNA for apo-C-II doubles by 48 h after the addition of an estrogen to the medium.

At present, the properties of the sex steroid binding in human liver cytosol are consistent with the presence of estrogen receptors and of androgen receptors. Further studies are required to demonstrate that nuclear retention of the receptor steroid complexes occurs in the normal liver parenchymal and tumor cells and that there are direct sex steroid responses.

DISCUSSION AND CONCLUSION

Sex steroid receptor systems in mammalian liver

Our studies show that the mammalian liver contains estrogen receptors and androgen receptors. These receptors provide mechanisms for sex steroids to directly modulate liver function. Our laboratory has found that the liver receptor systems have some unusual aspects that may permit preferentially diminishing the undesired liver side-effects of oral contraceptives and other sex steroid preparations.

Involvement of sex steroid effects on liver in major side-effects of the oral contraceptives

We first became interested in the mechanism whereby sex steroids modulate liver function when we recognized that many of the major side-effects of the oral contraceptives might be consequences of sex steroid acting directly on the human liver. These liver effects include increasing or decreasing the synthesis of certain plasma proteins involved in cardiovascular disease and influencing bile synthesis and liver tumorigenesis [3].

Almost all combined oral contraceptives contain ethinyl estradiol, EE2, or mestranol as the estrogen. Mestranol is inactive *per se* but is metabolized to ethinyl estradiol for biologic activity. The progestin used in the combined oral contraceptive is usually a 19 nortestosterone derivative. Combined oral contraceptive use is associated with an increased risk of cardiovascular disease [43–45]. There are several EE2 effects on certain plasma protein levels, most likely by changing their synthesis in liver, which may contribute to the cardiovascular disease. EE2 decreases the plasma levels of a key clotting inhibitor anti-thrombin III. The strongest relationship between the EE2 dose and cardiovascular disease is with venous thrombosis [44]. EE2 may contribute to hypertension by increasing the synthesis of renin substrate [46]. There are two EE2 effects on lipoproteins which may influence atherosclerosis—EE2 increases the levels of LDL, which may be a detrimental change, but increases HDL which may be a beneficial change [47].

An increased risk of arterial disease (including heart attacks and stroke) in combined oral contraceptive users has been epidemiologically linked, in recent studies, to the dose of progestin [48, 49]. One suggested mechanism for this effect is a progestin dose related decrease in serum HDL, most likely by decreasing its synthesis in liver [49]. A decrease in the serum level of HDL, which transports cholesterol from peripheral tissues including arteries to the liver for destruction, is a known risk factor for heart attacks [50]. The progestin effect of decreasing the plasma level of HDL resembles the effect of androgen administration [51].

Oral contraceptive users have an increase in gall bladder disease [45]. Estrogens enhance cholesterol gall-stones by decreasing liver synthesis of bile acids which maintains cholesterol in the bile in solution [52].

The only tumors which are markedly increased in oral contraceptive users are liver adenomas [5, 35]. Although these tumors are histologically benign, they can be fatal due to massive hemmorhage. These tumors remain rare even though their frequency is increased more than 60-fold by long-term oral contraceptive use. The relative contribution of the estrogen and the progestin to the development of hepatomas remains uncertain.

Involvement of liver in androgen side-effects

Anabolic androgen use also has major liver-associated side-effects [4, 5]. The most serious complication of anabolic androgen use is liver tumors, in particular, primary hepatocellular carcinoma [4, 5]. As previously mentioned, hepatocellular cancer is one of the most common forms of cancer worldwide and there are 3–9 males for every female with this cancer [40]. It is possible that this male predominance might be due to an androgen effect on liver.

Accordingly, diminishing liver effects of sex steroids might decrease a substantial contribution to some major contraceptive and sex steroid side-effects.

Properties of the liver sex steroid receptor system which may permit preferential diminution in liver side-effects

Our laboratory has found that the receptor mechanisms in liver have some unusual aspects that may permit preferentially diminishing undesired sex steroid effects on the liver. We have shown with estrogens that the liver requires higher doses for receptor nuclear complexes than does a target organ such as the uterus. Extensive metabolism of the estrogen in the hepatocyte seems to be the predominant explanation for the high doses required for liver nuclear receptor occupation (Fig. 6). Our studies of estrogens indicate that liver estrogen metabolism reduces liver receptor binding. Similarly, metabolism of progestins (and androgens) in liver is likely to influence their binding to liver sex steroid receptors. This provides an explanation for why reduction of the dose of sex steroids in the oral contraceptives could maintain desired effects in other target organs and yet could diminish liver-mediated side-effects. We have observed that liver has a high concentration of an androgen receptor and may not have a progesterone receptor. Thus, it is also possible that the desired progestin effects in hypothalamic–pituitary axis and uterus may be mediated by progesterone receptors while the deleterious liver effects may be mediated by a weak interaction of progestins with the liver androgen receptor.

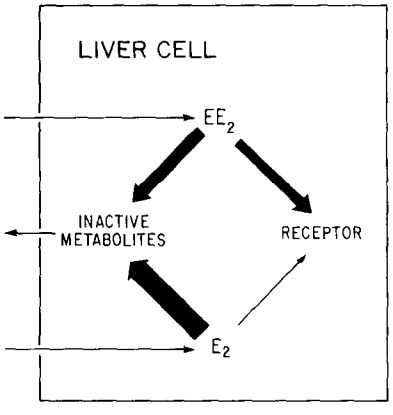

Fig. 6. Modulation of estrogen receptor binding in liver parenchymal cells by conversion of estrogens to inactive metabolites. This schematic diagram contrasts ethinyl estradiol (EE2) and estradiol (E2) for their relative attachment to liver estrogen receptor in the nucleus vs their conversion to metabolites which do not attach to the receptor. The inactive metabolites exit from the liver parenchymal cell into plasma or bile. The thickness of the arrows connecting the estrogens to receptor or inactivation depicts the relative magnitude. As indicated with the thicker arrow, ethinyl estradiol is more potent than estradiol for liver nuclear receptor binding.

Modification of contraceptives

Oral contraceptives—lower doses in the newer combinations. The safety of the combined oral contraceptives has already been improved by lowering the estrogen and progestin doses [49, 44, 45]. The newer oral contraceptives remain effective for birth control and have additional non-contraceptive benefits which may outweigh the serious side-effects [45].

However, it seems likely that some increased risk of serious side-effect remains at least for certain groups of women. This is of particular concern for women above the age of 35 who also smoke cigarettes since these women have the highest risk of arterial disease with the oral contraceptives [43–45]. In addition, the current oral contraceptives are relatively counterindicated for women with certain medical conditions including hypertension [53].

The sex steroid related effects on liver function as indicated by changes in the levels of certain plasma proteins have been partially decreased by reducing the doses of ethinyl estradiol and the 19 nor-testosterone type of progestin in the newer oral contraceptives. With 30 μg of ethinyl estradiol and relatively low doses of progestin, plasma levels of anti-thrombin III, clotting factors and HDL are very little different from levels in non-users [54]. However, plasma renin substrate and corticosteroid binding globulin levels are still 3-fold above control levels indicating that some estrogen-induced liver changes are still occurring [55].

Oral contraceptives—changing the estrogen or progestin. Our results also provide a scientific basis for further minimizing the liver effects of sex steroids. Liver effects could be minimized by careful selection of which estrogen or progestin is administered. Thus, a sex steroid could be selected which is preferentially metabolized upon entering the liver cell rather than attaching to the receptor. An example is observed in our studies of estrogens and the rat liver. There is a marked contrast in liver receptor binding of estradiol compared to ethinyl estradiol due to a more marked metabolism of estradiol to inactive derivatives in the hepatocyte (Fig. 6). In addition, if a progesterone receptor in liver is not present and progestins are producing their deleterious effects via the liver androgen receptor then liver effects could be minimized by utilizing a progestin which does not bind to the androgen receptor or is devoid of liver androgenic activity.

An example of selection of an another estrogen is the experimental trials of combined oral contraceptives containing estradiol [56, 57]. Oral estradiol plus estriol has been compared to EE2. Both the natural estrogens and EE2 were used in combination with the same dose of a 19 nortestosterone progestin. The combination of natural estrogens with a progestin is as effective as the EE2 containing combination in preventing pregnancy. The natural estrogen combination has less estrogenic effects on lipoproteins—LDL does not increase and the progestin-induced decrease in HDL, although slight, is greater than with EE2 combination [47]. However, the natural estrogen combination has more menstrual irregularities than the EE2 combinations. In these studies both formulations prevented pregnancy yet the natural estrogen combination exhibited less estrogen effect on the liver and on the endometrium. There are several factors which may contribute to oral estradiol not maintaining the endometrium. Oral estradiol is extensively metabolized in the intestine and liver to estrone and estrone sulfate before entering the systemic circulation [58]. Endometrium, in addition to liver, contains enzymatic activity for converting estradiol to estrone [59]. Progestin administration both increases the level of estradiol dehydrogenase activity in the endometrium and decreases the levels of estrogen receptor retained in endometrial nuclei during estrogen administration [60]. (The endometrial estradiol dehydrogenase activity even with progestin stimulation is still approximately 50 times less than the activity in liver [61].)

Parenteral routes of sex steroid administration. Liver effects could also be diminished by using routes of administration other than oral. First pass effects would be avoided. Since oral steroids are absorbed from the intestine into the hepatic portal vein, high concentrations of the steroids are in contact with the liver parenchymal cells before entering the systemic circulation. Furthermore, if the steroid enters the systemic circulation before entering the liver there would be more choices in selecting the sex steroid that could still be effective in other target organs and yet has the property of being inactivated once it enters the hepatocyte. For example, as mentioned above, estradiol when administered by mouth is extensively inactivated both in the intestine and liver before entering the systemic circulation; but estradiol when administered parenterally is more potent on non-liver target organs.

One parenteral route of administration for estradiol is transdermal. Skin patches, which release a constant amount of estradiol for half a week are being used for estrogen replacement therapy in postmenopausal women. With transdermal estradiol hot flushes and other symptoms associated with the menopause can be controlled without having detectable estrogen effects on liver as estimated by measuring the level of estrogen responsive plasma proteins [62].

Another parenteral route utilizes sex steroid containing plastic rings inserted in the vagina round the cervix. Experimental silastic rings containing estradiol and a progestin, inserted once a month for 3 weeks duration, are effective contraceptives due to systemic absorption of the steroids [63]. Some formulations are able to maintain the endometrium and yet have no detectable estrogen effects on liver as

estimated by measuring the level of estrogen responsive plasma proteins [55, 63].

These vaginal rings containing estradiol and a progestin have recently been used in a small trial for contraception in hypertensive women [64]. Use of the steroid-containing rings does not exacerbate the hypertension. The levels of plasma renin substrate or anti-thrombin III are unchanged with use of the rings, indicating that there is no detectable estrogenic liver effect. However, plasma HDL is substantially decreased indicating a progestin effect on liver without an opposing estrogen effect on these plasma proteins. Since decreased HDL is a cardiovascular risk factor, it would seem preferable if the progestin effect on liver could also be diminished by further modifications.

Another parenteral route of administration is subcutaneous implants. Low doses of the progestin norgestrel in silastic capsules (norplant) are effective for long-term contraception without changes in lipoproteins [65]. However, norplant has a high incidence of endometrial breakthrough bleeding (which is also observed with use of oral low dose progestin contraceptives without estrogens).

Although this discussion has focused predominantly on contraceptives, similar principles seem applicable for diminishing the liver side-effects of androgenic preparations. Thus, androgens should be selected which are likely to be metabolized on entering the hepatocyte and a parenteral route of administration may be preferable. Androgens which are not 17 alkylated might produce fewer liver side-effects than 17 alkylated derivatives judging from their relative effects on plasma protein levels [2].

To summarize, the mammalian liver contains estrogen receptors and androgen receptors. Estrogen metabolism extensively modulates estrogen receptor binding in liver. Our work provides a scientific basis for minimizing liver effects by using low doses or selecting sex steroids which are extensively metabolized in the hepatocyte. Some of these sex steroids may be more effective in other target organs relative to liver by utilizing a parenteral route of administration. With progestins, additional diminution of liver effects might be achieved by the use of progestins which do not attach to the liver androgen receptor or produce liver androgenic effects.

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