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Estradiol prodrugs (EP) for efficient *oral* estrogen treatment and abolished effects on estrogen modulated liver functions

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ABSTRACT

Oral compared to parenteral estrogen administration is characterized by reduced systemic but prominent hepatic estrogenic effects on lipids, hemostatic factors, GH-/IGF I axis, angiotensinogen. In order to avoid such adverse metabolic effects of oral treatment, estradiol (E2) prodrugs (EP) were designed which bypass the liver tissue as inactive molecules. Carbone17-OH sulfonamide [-O_2–NH_2] substituted esters of E2 (EC508, others) were synthesized and tested for carbonic anhydrase II (CA-II) binding. CA II in erythrocytes is thought to oppose extraction of EP from portal vein blood during liver passage. Ovariectomized (OVX, day minus 14) rats were orally treated once daily from day 1–3. Sacrifice day 4. Uteri were dissected and weighed. Cholesterol fractions and angiotensinogen were determined in plasma. Oral E2 and ethinyl estradiol (EE) generated dose related uterine growth and important hepatic estrogenic effects. EP induced uterine growth at about hundred-fold lower doses. This was possible with almost absent effects on plasma cholesterol or angiotensinogen. Preliminary pharmacokinetic studies with EC508 used intravenous and oral administration in male rats. Resulting blood levels revealed complete oral bioavailability. Further high blood- but low plasma concentrations indicated erythrocyte binding of EC508 *in vivo* as potential mechanism of low extraction at liver passage. Very high systemic estrogenicity combined with markedly lower or absent adverse hepatic estrogenic effects is evidence for a systemic release of E2 from sulfonamide EP. In conclusion, tested oral EP bypass the liver in erythrocytes furnishing systemic estradiol at hydrolysis. This mechanism avoids the hepatic estrogenic impact of conventional oral estrogen therapy.

1. Introduction

1.1. Ethinyl estradiol

The introduction of a 17α-ethinyl group into testosterone or estradiol (E2) by Hohlweg and Inhoffen resulted in two *orally active* compounds of ethinyl testosterone (ET) and ethinyl estradiol (EE), respectively [1]. Just like its parent molecule, EE was estrogenic, and ET – surprisingly – was also found to have progestational properties. These discoveries technically paved the way for orally active progestins (e.g. norethisterone) and estrogens such as EE, both of which form the basis of oral birth control methods. Not only the chemical, but also the pharmacological diversity among the progestins is pharmacologically important [2].

Abbreviations: AUC, area under the curve; BW, body weight; C_0, extrapolated concentration time zero (i. v.); C_{max}, maximum concentration; CA-II, carbonic anhydrase II; CBG, cortisol binding globulin; COC, combined oral contraceptive; EDTA, ethylene diamine tetra acetic acid; EE, ethinyl estradiol; EMATE, estrone sulfamate; EP, estradiol prodrug; ER, estrogen receptor; ERT, estrogen replacement therapy; ES, estradiol sulfamate; E2, estradiol; FSH, follicle stimulating hormone; GH-/IGF1, growth hormone / insulin-like growth factor 1; hCAII, human carboanhydrase II; i. v., intravenous; OVX, ovariectomized; PMSF, phenylmethyl-sulfonylfluorid; p. o., per osil; rpm, round per minute; s. c., subcutaneous; SHBG, sex hormone binding globulin; STS, steroid sulfatase.

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In view of the range of important adverse metabolic effects of EE, including the lipid metabolism and in particular the effect on blood clotting and blood pressure [3], it is remarkable that EE is still the leading estrogen for combined oral contraceptive (COC) technology. Attempts were made to modulate metabolic effects of EE by counteracting properties of the progestin, which remains an unsolved issue.

It appears that estrogens and androgens interfere with the hemostatic system and various other metabolic functions of the liver in humans [4]. In the case of non-androgenic progestins intending to avoid adverse androgenic effects, an unopposed action of EE in the liver leads to a functional analogy of the factor V Leiden mutation, a resistance to an endogenous anticoagulation mechanism with an increased risk of deep vein thrombosis. Under COC, other factors of the clotting system are apparently determined by a similar interaction [4].

Another attempt to modulate EE-related cardiovascular risks of COC *via* the progestin component in COC is drospirenone, chemically a derivative of spironolactone and testosterone. Unlike other progestins, this compound has potent aldosterone antagonistic effects at therapeutic doses [5]. This property was shown to oppose the unfavorable effects of elevated angiotensinogen and its implications for the electrolyte metabolism and blood pressure regulation. However, drospirenone is still suspected of having a somewhat higher risk of deep vein thrombosis than (androgenic) levonorgestrel-based products [2].

The core issue associated with EE is excessive hepatic estrogenic effects. With oral treatment, EE has a roughly 100-fold higher FSH-lowering activity than micronized estradiol in humans. Concerning the CBG- and SHBG-elevating potential, EE exceeds estradiol by a factor of 1000 and 614, respectively [6,7]. Mandel et al. [7] investigated EE in postmenopausal women measuring parameters of estrogen-modulated liver functions, including SHBG (sex hormone-binding globulin). All tested oral doses ranging from 5 to 50 μg EE/day triggered massive increases in SHBG. This constellation discourages the hope of finding a dose of EE which could reconcile good cycle control, control of ovarian function and tolerable metabolic side effects.

In terms of pharmacokinetics, EE is also far from ideal. Its mean oral bioavailability is only about 40% and exhibits great individual variability [8]. A significant individual variation of pharmacokinetic parameters was confirmed in another study [9].

1.2. Mechanisms and role of hepatic estrogenic effects

As the target organ of estrogens, the liver differs from the genital tract and other target tissues. Hepatic ER synthesis is not controlled by estradiol itself, but by joint effects of growth hormone as well as glucocorticoids and thyroxin [10,11]. Estrogenic effects in the liver [12,13] are the result of complex interactions with these and other hormones. An array of important functions, such as growth, body composition (GH-/IGF1 axis [14]), lipid and electrolyte metabolism [3], hemostasis [4], are apparently estrogen-modulated at this level and are adversely affected by oral estrogens. The assessment of these metabolic effects is attempted using the OVX rat model with measurements of plasma angiotensinogen and cholesterol as representative parameters of hepatic estrogenicity.

1.3. Prodrug research, estradiol sulfamate

The current search for estradiol prodrugs had precursors: N-alkylated 3-sulfamates of EE synthesized by Schwarz et al. [15] as long-acting estrogens. Unidentified metabolites of these EE derivatives were found at a high concentration in erythrocytes [16].

Independent further research revealed that N-dealkylated metabolites of EE 3-sulfamates account for this high affinity to erythrocytes which was later explained by carbonic anhydrase-II binding. It was then discovered that erythrocyte binding has a strong impact on estrogen pharmacology beyond EE: the estradiol sulfamate (E2-SO_2-(NH_2) (J995/ES) was found as the first potent orally active estradiol prodrug [17,18].

One problem with ES as estrogen prodrug appears its metabolism to EMATE. This is the dominant fraction of the carbonic anhydrase bound depot in erythrocytes. Its hydrolysis leads to estrone, a much less potent estrogen than E2. In spite of high levels of ES and EMATE in the circulation, only insignificant E2 levels and *no* estrogenic effects were generated in humans [19]. We expect E2 as product of hydrolysis in case of tested sulfonamide EP. Further, EMATE is a potent inhibitor of the STS [20]. It is obvious from estrogenicity studies that this property impairs the release of estrone and E2 in a species varied manner. STS inhibition in the human was probably the mechanism for very long lasting high ES- and EMATE concentrations in erythrocytes compared to shorter initial peak values of E1 and E2 in the plasma.

Species differences existed concerning plasma concentrations of E1 and E2, corresponding to differences of estrogenic effects (rat > cynomolgus monkey > human). Nevertheless, a common key role of STS as hydrolyzing enzyme appears likely. Chander et al. [21] combined EMATE with another potent STS inhibitor in OVX rats. This reduced the uterotropic effects of EMATE.

These authors concluded that STS is the sole hydrolytic enzyme in case of EMATE. The speed of hydrolysis of ES or EMATE is seen by us to determine the released amount of E1 and E2 per time unit in given species, and thus the strength of estrogen effects of ES or rather EMATE.

A new approach was therefore to separate the binding to carbonic anhydrase II and a sulfatase-independent moiety that could be hydrolyzed *in vivo* and release the parent molecule estradiol (see Fig. 1). Several authors compared sulfonic acid derivatives as well as sulfamate and its derivatives with respect to STS inhibition and found much lower or absent STS inhibition of sulfonamides [22]. This applies to a group of prototype 17OH sulfonamide estrogens which were evaluated to check the plausibility of a 17-sulfonamide approach. As basis of STS inhibitory properties in case of sulfamates appears the linking oxygen (C-O-SO_2-NH_2) [23].

We trust that 17-sulfonamide esters of E2 with reduced STS inhibitory effects and carbonic anhydrase binding properties are possible. Rather than by STS their hydrolysis is expected according to those mechanisms which liberate therapeutically used 17OH-esters of testosterone and estradiol (as testosterone-17undecanoate, estradiol-17 benzoate or estradiol-17 valerate). The plasma contains a large number of species varied esterases [24] Regarding the apparent complexity we doubt that an animal model exists which covers all aspects of biotransformation of this type of EP in humans. Uncertainties remain at this stage of the project. Preliminary incubation studies with estrogen 17-sulfonamides with plasma including the human blood showed hydrolysis in plasma, not in buffer [19].

The cleavage of the ester function is of critical importance for prolonged estrogenic activity and high oral bioavailability of released E2. We therefore chose amino acids as a class of compounds that offer a wide variety of steric and electronic variance, potentially leading to different release rates *in vivo*.

Steroid esters of amino acids have been described in the patent literature before claiming activity as antitumor and cytotoxic agents [25,26]. However, to the best of our knowledge, the combination of an amino acid ester with sulfonamide function to generate EPs is new.