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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.

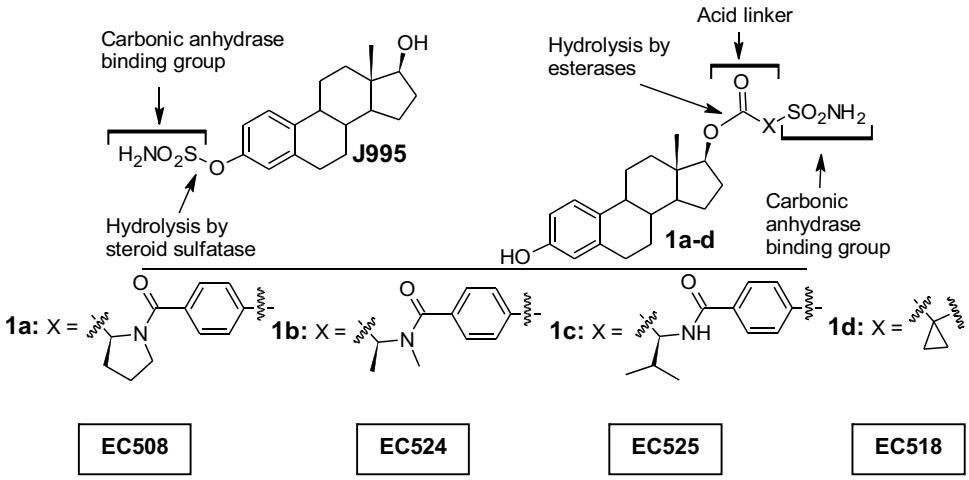

Fig. 1. Estradiol sulfamate, J995 (ES) and novel type of prodrug with different linkages to the CA-II binding group.

2. Material and methods

2.1. Estrogen prodrugs

For the synthesis of the applied compounds see [27].

2.2. Carbonic anhydrase II (CAII) inhibition assay (Iyer)

The catalytic activity of human CA-II was monitored by the hydrolysis of the non-physiological, ester, 4-NPA (Sigma-Aldrich, St. Louis, MO). The 4-NPA esterase activity will be interpreted as ligand interaction with CA-II. The products of the hydrolysis reaction are acetate and nitrophenolate, which ionize to give a bright yellow anion that is detected by measuring its absorbance at 348–400 nm with a SpectraMax Plus 384 ultraviolet-visible spectrophotometer (Molecular Devices, Sunnyvale, CA) using 96-well plates. The inhibition concentration 50 (IC 50) was determined [28].

2.3. Modified uterine growth test

Animals: Sprague-Dawley rats aged 5–6 weeks were purchased from Charles River Germany. The rats were kept in Macrolone® type 4 cages, with 5–6 animals per cage. The laboratory was air-conditioned and artificially illuminated: 12 h of light, 12 h of darkness. Animals had access to pelleted food (SNIFF®) and tap water *ad libitum*. Food was withdrawn on the evening before blood collection. After an adaptation period, animals were ovariectomized (OVX) in anesthesia with a standard combination (Dorbene, midazolam, fentanyl s. c.; reversal of anesthesia with Antisedan, flumazenil, naloxone s. c.).

Treatment: Treatment began 14 days after OVX. The weight of the animals at this time was about 160–180 g. Treatment was performed by oral (gavage) or parenteral (s. c. injection) administration. Compounds or vehicles were administered only once daily in the morning from day 1 to day 3. Animals were sacrificed on the morning of day 4 by neck dislocation in isoflurane anesthesia.

Evaluation: the uterus and vagina were dissected, blotted and weighed. Blood was collected from the orbital vein plexus in anesthesia on day 1 and 4. EDTA plasma was prepared and frozen for the determination of cholesterol fractions and angiotensinogen.

The experiments were approved by the ethics committee for animal experiments at the FSU Jena, registration number 15-001/14 TLLV, 99947 Bad Langensalza, Germany.

The tested doses were equivalent with respect to their E2 content. Substances were suspended in 0.5% methyl cellulose (Sigma-Aldrich, USA)/saline vehicle and orally administered in 0.2 mL. Controls were treated with 0.2 mL vehicle. Parenteral E2: vehicle castor oil/benzyl benzoate (4:1), dose in 0.2 mL by s. c. injection.

Biochemistry: determination of cholesterol fractions and angiotensinogen in plasma: both parameters of hepatic estrogen-modulated functions were determined in an external laboratory (Food GmbH Jena Analytik) using established kits. Cholesterol: total cholesterol test kit no. 981812 and 981813 after hydrolysis of esters with cholinesterase and the generation of a chromophore. Angiotensinogen: determination of angiotensin-I after incubation of the plasma sample at 37 °C for 3 h by direct competitive ELISA (angiotensin-I (PRA) ELISA; ref. no. DB52011 IBL International GmbH, Germany). In order to obtain a complete transformation of angiotensinogen to angiotensin-I, an excess of recombinant human renin (Cat. No. 72041) from MoBiTec, Göttingen, Germany, was used. The determination of angiotensin-I reflects plasma angiotensinogen. Protease inhibitor PMSF is used to prevent the transformation of angiotensin-I to angiotensin-II.

2.4. Parameters and biometrical evaluation

All endpoints as well as doses were investigated after log transformation, as this is convenient for analysis. Results were displayed using the antilog backward transformation. The experimental setup comprised four prodrugs: EC508, EC518, EC524, EC525, plus E2 s. c., E2 p. o., and EE p. o. as comparators. Adjacent doses differed by a factor of 10 for all substances. Up to four dose levels were tested with 5-6 animals per dose group.

As is appropriate for analyzing a screening study, we neither adjusted for multiple testing, nor did we put emphasis on exploring the potentially small differences in outcome between two original series of assays. We did, however, check for outliers, heteroscedasticity, and normality without any signs of relevant violations of assumptions for our analysis.

The observed dose-response relationships exhibit a variety of shapes. Neither common limiting behavior nor common shapes or curves can be assumed. We therefore decided to estimate effective doses for a given target value of uterotropic activity, in this case a uterus weight of 200 mg, corresponding to a two-fold increase, when compared to OVX controls. These 'effective doses' allow for a derivation of relative efficiencies of the different substances with respect to uterotropic activity. Concerning the hepatic estrogenic

activity we compared concentrations of total cholesterol and angiotensinogen in plasma estimated for the corresponding effective doses at uterus weight target. Again, relative efficiencies can be derived for these data. All estimated values were generated using the linear model fit of substance-specific second-degree polynomials in log dose to the endpoints considered. Any simplification of the model resulted in highly significant loss of fit. For the effective doses (e.g. analysis of uterus weight) the delta method was applied in order to obtain approximate standard deviations of the estimates.

In the case of the variables characterizing hepatic estrogenic effects, standard deviations were obtained directly from the linear model summary. The applied model used three parameters per substance, giving the best possible fit in the case of three dose levels (saturated model). The two cases with four dose levels were also characterized well by our approach. We used the open source statistical software products R version 3.0.3 (R Core Team (2014)) and RStudio (RStudio Team (2015)) combined with the R Markup Language and the kit NTR package to generate a statistical analysis with fully reproducible results [29] www.R-project.org/www.rstudio.com.

2.5. Pharmacokinetic investigations in the rat

Pharmacokinetic studies were performed at GVK Biosciences Pvt. Ltd. (Hyderabad, India). Rats received a single oral dose of either EC508 or E2 at a dose of 5 mg/kg body weight (n = 3 per group) by an oral gavage needle in order to compare the presence of both test items in plasma and whole blood. EC508 was also investigated after i. v. administration at a dose of 1 mg/kg body weight. Blood samples were withdrawn after 0.25, 0.5, 1, 2, 4, 8, and 24 h post-dose *via* jugular vein cannulation. 25 μL of sample was taken and precipitated with 0.2 mL of acetonitrile containing internal standard at 200 ng/mL concentration. The sample was than vortexed for 5 min at 850 rpm, centrifuged at 4000 rpm for 5 min at 4 °C, from which 0.1 mL of supernatant was separated and diluted with 0.2 mL of methanol: water (1:1). Analysis was performed using LC–MS/MS (API 4000) under standardized chromatographic conditions. Pharmacokinetic parameters were calculated for individual animals by a non-compartmental model with WinNonlin software version 6.3 (see Fig. 3). A comparison of the i. v./p. o. data are reported in Table 3.

3. Results

3.1. Overview

Parenteral (s. c.) administration of E2 providing constant release from the oily vehicle exhibited very potent uterine growth-inducing effects. Compared to this, oral administration of E2 led to massive loss of uterine efficacy. The EPs were about 100-fold more potent than oral E2 and EE, but less efficient than E2 s. c. The power of the study was not sufficient, however, to demonstrate differences in uterotropic efficiency between the various EPs (see Fig. 2 and Table 1). EPs differ with respect to the maximum inducible uterine growth which is not reached with most known EP or oral E2. Only EP EC524 induces the same maximum level of uterine growth as parenteral E2 (see Fig. 2).

Oral doses of the comparators E2 and EE which were capable of inducing uterotropic effects also exerted strong hepatic estrogenic effects. No such effects were seen with *parenteral* administration of E2 including doses which induced a full uterine response (see Fig. 2 and Table 2).

With regard to hepatic estrogenic activity, most EPs exhibited the same behavior as parenteral E2. There were no significant differences for total plasma cholesterol as endpoint. An exception

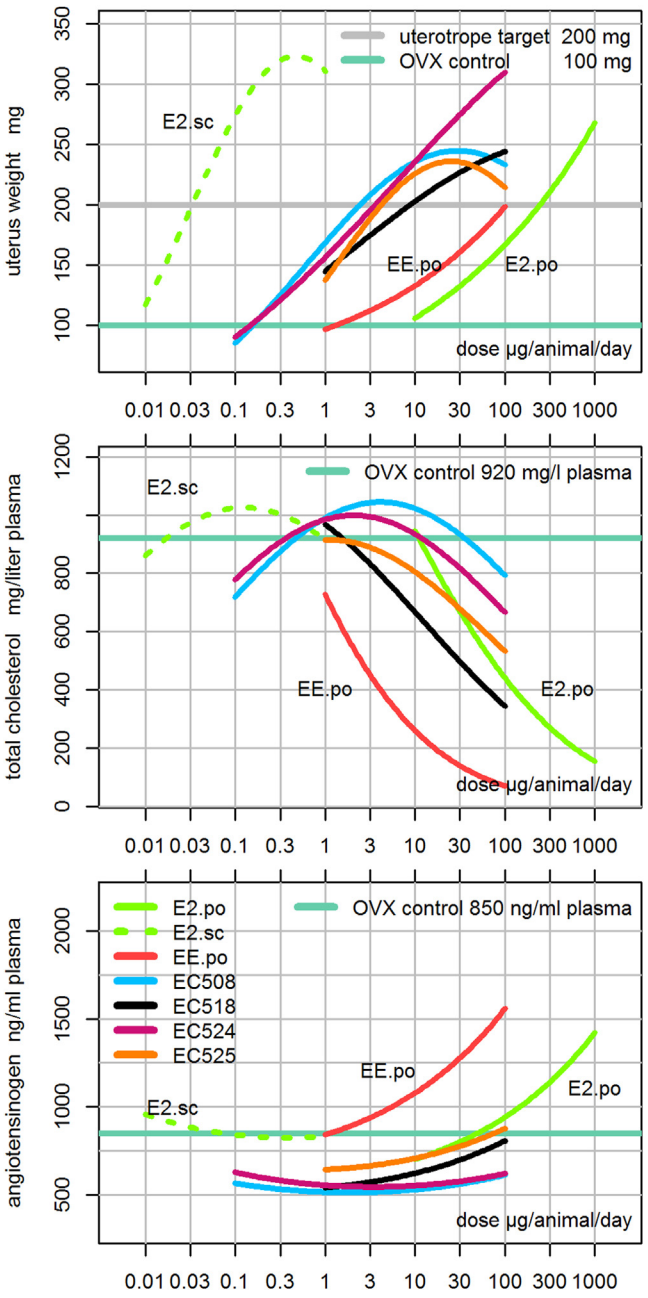

Fig. 2. Dose response for prodrugs and comparators. Relations for uterus weight, total cholesterol, and angiotensinogen. Original fit in log dose and log endpoint value. Endpoints are displayed anti-log transformed, dose axis in log10 style. All plots use the same scale for dosing, so that corresponding values of uterotropic and hepatic activity can be read directly. Hormesis of angiotensinogen concentration occurs significantly for all prodrugs with values below the OVX reference, whereas the total cholesterol concentrations at the dose levels corresponding to the uterotropic target of 200 mg uterus weight show more variation between the prodrugs.

is EC518, which exhibited significantly lower plasma cholesterol values at the 200 mg uterine weight-inducing dose.

Unlike oral E2 or EE, no elevation of plasma angiotensinogen above OVX level was seen with the four EPs (see Fig. 2). This applies to all tested doses. Surprisingly, all EPs showed significantly *lower* plasma angiotensinogen values than with parenteral E2 (see Table 2).

Statistics: when checking assumptions for the model used, a linear model with a second-degree polynomial in log(dose) as predictor of log endpoints (e.g. uterus weight, total cholesterol and

Table 1
Uterotropic activity. Relative efficiencies (ratio of doses, means () 95 % confidence limits, comparator/prodrug orally yielding the target of 200 mg uterus weight. Values greater than 1 mean that a higher dose is needed of the comparator than of the prodrug for achieving the target.

	hCAII inhibition (IC_{50} nmol)	E2 p. o.	E2 s. c.	EE p. o.
EC508	110	101 (45–218)	0.014 (0.006–0.029)	43 (16–117)
EC518	10,000	28 (8–99)	0.004 (0.001–0.013)	12 (3–48)
EC524	203	68 (30–156)	0.009 (0.004–0.020)	29 (10–80)
EC525	202	58 (26–131)	0.008 (0.004–0.017)	25 (9–68)

All differences between prodrugs and comparators are highly significant.

Table 2
Effective doses (μg/day uterus weight 200 mg) mean values and confidence intervals, estimated total cholesterol and angiotensinogen concentrations at effective dose; means () 95 % confidence limits.

	E2 p. o.	E2 s. c.	EE p. o.	EC508	EC518	EC524	EC525
Effective dose uterus (μg/day)	246 (157–385)	0.033 (0.023–0.047)	104 (50–219)	2.4 (1.2–4.8)	8.8 (2.7–28.8)	3.6 (1.8–7.2)	4.2 (2.1–8.3)
Total cholesterol (μg/mL)	304 (273–338)	977 (832–1148)	68 (56–83)	1137 (906–1187)	686 (601–783)	991 (859–1144)	873 (758–1006)
Angiotensinogen at effective dose (ng/mL)	1090 (1019–1166)	883 (798–976)	1569 (1383–1778)	515 (474–561)	618 (569–671)	547 (500–598)	675 (618–737)

angiotensinogen concentration in plasma), no relevant violations of assumptions needed for valid statistical inference were observed. The models explained more than 80 % of the variance (log scale) for all endpoints under consideration.

3.2. Preliminary pharmacokinetic studies with EC508

Studies were performed in male rats fitted with a jugular vein catheter. Bioavailability was assessed comparing blood levels of EC508 after intravenous and oral administration. A comparison of the pharmacokinetic data is reported in Table 3.

Oral bioavailability amounting to 100 % was established. Very high concentrations prevailed in whole blood, whereas the concentration in the plasma was much lower (see Fig. 3). This demonstrates that the majority of EC508 in the circulation is bound in the erythrocyte fraction. No such difference in blood compartments prevails in the case of E2.

4. Discussion

Sulfonamide derivatives of E2 bind to CA-II (EC508, EC524, EC525) but not to the estrogen receptor. This substitution is aimed at achieving erythrocyte binding to avoid extraction from the blood

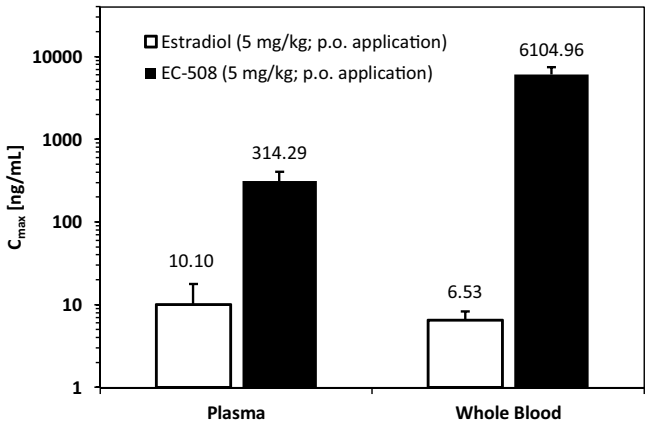

Fig. 3. Peak blood levels of E2 and EC508 in plasma/blood after oral administration. Small difference in concentrations in the blood (plasma + erythrocytes) vs plasma in the case of estradiol; the large difference in the case of EC508 is indicative of the EP depot in erythrocytes.

in the liver. This mechanism was confirmed in the studies with EC508, showing a massive accumulation in the erythrocyte fraction of blood *in vivo*. Extrapolating from its uterotropic activity, the bioavailability of E2 was increased about hundredfold. This was effectively achieved with all tested EPs, including one which does not bind to CA-II (EC518).

Various esters of steroids using the 17ß-OH are known prodrugs of E2, furnishing relevant E2 levels in the circulation after enzymatic ester cleavage. This type of ester is mainly administered by parenteral routes. It does not offer any advantage over estradiol with regard to improved bioavailability or reduced metabolic effects when applied orally [30].

Compared to oral E2, the EPs led to 30–200-fold higher systemic estrogenicity as assessed by the 200 mg uterus weight-inducing dose in OVX rats.

Effects on the entire genital tract constitute evidence of an effective systemic release of E2 from all EPs. A highly reduced or abolished hepatic estrogenicity of EPs indicates a low E2 concentration in the liver tissue compared to systemically equipotent oral doses of E2.

The role of erythrocyte binding was analyzed using J995 (ES) in perfusion studies with isolated rat livers. Whereas J995 was extracted from a control medium, hepatic extraction was strongly reduced by the addition of erythrocytes [18]. With the new EP a mechanistically well-defined, highly effective oral E2-based treatment without the adverse metabolic effects of oral E2 or EE appears to offer a realistic perspective for therapeutic use in humans.

Oral E2 and EE increased angiotensinogen and lowered cholesterol plasma levels at the 200 mg uterus weight-inducing dose. This was not seen under EP. The mechanism of observed paradoxical *inhibitory* effects of very low doses of EP on plasma angiotensinogen levels cannot be explained at this stage of the evaluation.

Table 3
Orientating pharmacokinetic studies with EC508 in male rats using intravenous (jugular vein catheter) and oral administration; Evaluation of blood concentration, including erythrocyte fraction; means: ±standard deviation, C_0 (i. v.).

Administration	n	Dose (mg/ kg body weight)	C_{max}/C_0 (ng/mL)	$AUC_{0\text{-}tlast}$ (ng h/mL) EC508	Relative bioavailability of EC508 (%)
i.v.	3	1.0 mg	4437 ± 382	4459 ± 1280	100 (by definition)
p. o.	3	5.0 mg	6104 ± 1285	26322 ± 9528	122 ± 42

Without a doubt, the active estrogenic principle of all tested EPs is E2. However, the compounds clearly differ in terms of their estrogenic effects. Pharmacokinetic differences may account for this. The speed of hydrolysis may lead to a longer or shorter presence of relevant E2 blood levels between two applications. Uterine growth to the same maximum value as parenteral E2, as induced by EC524, may reflect slow hydrolysis of this EP and more constant E2 levels in the blood.

Once in the systemic circulation, EPs reach all organs and tissues. Regarding the different effects of EP, it appears possible that different distributions of esterases in the blood and tissues modulate the recorded patterns of estrogenic effects of EP.

A problem with J995 was the metabolism to the estrone derivate before hydrolysis and the subsequent release of the less potent estrogen E1 rather than E2. In case of sulfonamide EP oxidation of the 17OH is chemically not possible. However, questions arise concerning the unprotected 3C-OH group of these EPs. Sulfation would reduce their potential estrogenicity. But this may not be an issue. High and similar patterns of non-metabolized EC508 in blood at i. v. and p. o. administration leave little room for the formation of a C3- sulfate analogue at intestinal resorption. Further, an important site of intestinal sulfation in case of estradiol is the (human) liver [31]. Carbonic anhydrase binding in erythrocytes of new EP may be of particular importance preventing hepatic C3-sulfation.

EC518: this substance gradually differs from the other tested EP in terms of its structure and pharmacology. EC518 is an aliphatic sulfonamide ester substituted with a cyclopropyl function in alpha position to the sulfonamide moiety. EC518 exhibits a very low or absent CA-II affinity (see Table 1). However, potent systemic estrogenicity compared to oral E2 is obvious. Absent (angiotensinogen) hepatic effects at the effective 200 mg uterus weight-inducing dose and moderate effects on total cholesterol suggest effective *systemic* generation of E2 by hydrolysis. The question is whether or how this is possible without CA-II and thus probably lacking erythrocyte binding.

COC: estrogen (*EE*)/progestin combinations and *oral* treatment are essential elements for modern-day hormonal birth control. EE is still indispensable as estrogen despite its pharmacokinetic and pharmacodynamics drawbacks. It is obvious that cardiovascular morbidity is the most serious drawback of COC. Hepatic estrogenicity appears to be the key issue in this context. This may be modulated by suitable progestins; however, replacing EE by EP may be more effective in this respect.

The metabolic effect of EE in humans cannot be avoided by parenteral (vaginal) administration [32]. As reflected by its longer half-life in the circulation, EE passes the liver many times prior to its elimination. Therefore we would not suggest a sulfonamide derivative of EE in order to improve its effects on the liver in humans.

5. Conclusion

Sulfonamide esters of estradiol may technically improve oral estrogen therapy. Carbonic anhydrase-II-mediated binding by erythrocytes prevents their extraction during hepatic clearance. Systemic hydrolysis improves the bioavailability of estradiol and prevents a metabolic impact. Estradiol prodrug-based hormone replacement therapy or COC may solve core issues of oral estrogens, such as thromboembolic events.

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