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Evidence of estrone-sulfate uptake modification in young and middle-aged rat prostate

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ABSTRACT

High plasma exposure to estrogens is often associated with prostate cancer. Reducing this phenomenon may present therapeutic benefits. The involvement of estrone sulphate (E1S), the most abundant circulating estrogen in men, has been partially studied in this age-related pathology. To investigate the consequences of plasma E1S overload on blood and prostate sex steroid levels and inflammatory tissue responses, young and middle-aged male rats were treated with E1S with or without steroid sulfatase (STS) inhibitor STX64 for 21 consecutive days. A plasma and prostate tissue steroid profile was determined. STS activity, mRNA expression of *E1S organic anion transporting polypeptides* (*slco1a2, slco2b1, slco4a1*) and *pro-inflammatory cytokines* (*Il1-beta, Il6, TNF-alpha*) were evaluated in prostate tissue according to age and treatment group.

A significant correlation between plasma and prostate steroid levels related to hormone treatment was observed in all rat age groups. However, while the E1S level in prostate tissue increased in middle-aged treated rats ($p < 0.0001$), no significant variation was observed in young treated rats. The protective effect of STX64 during E1S infusion was observed by the maintenance of low free estrogen concentrations in both plasma and tissue. However, this protection was not associated with mRNA expression stability of *pro-inflammatory cytokines* in older rat prostate.

These results suggest that E1S uptake in rat prostate cells increases during aging. Therefore, if a similar phenomenon existed in men, preventively reducing the STS activity could be of interest to limit uptake of estrogens in prostate when high E1S plasma level is assayed.

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1. Introduction

Estrogens were long considered to protect against prostate cancer (PCa) and were used in treating advanced disease [1], even though their protective property was mediated by negative feedback along the hypothalamic-pituitary-gonadal axis, leading to a state of chemical androgen castration. More recently, the possible involvement of estrogens in PCa induction and progression has been suggested in many experimental and epidemiological studies [2–6]. However, it remains difficult to clarify the relationship between PCa and plasma sex steroid concentrations (estrogens, but also androgens). Proposed hypotheses have been supported by evidence that a steady increase in the estrogen/androgen ratio during aging could promote the mitotic and mutagenic properties of estrogen steroids [7]. Testosterone (T) is a major source of estradiol (E2) in men, and a significant positive correlation between plasma T and E2 concentrations has been reported [8]. However, despite an increase in the estrogen/androgen ratio, no significant age-related variation in blood estrogen levels has been demonstrated. Thus, additional mechanisms are required to explain the stable plasma E2 level. To the best of our knowledge, the role of E1S (the most abundant circulating estrogen in men) in the elevation of the estrogen/androgen ratio has never been proposed. To date, E1S has not been the subject of

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many evaluations published in the literature and its role in PCa incidence has been only partially explored [9–11]. There is a lack of data concerning prostate tissue steroid levels during age-related decline in androgens and during the various stages of PCa. Therefore, study of the impact of the progressive modification of circulating steroid levels on steroid uptake by prostate cells during aging is urgently needed.

In adult men, E1S plasma levels vary considerably among individuals, yet its concentration in the same subject appears to remain stable with age [9], unlike the dehydroepiandrosterone-sulfate (DHEAS) plasma concentration, which decreases significantly during aging [12]. Klein et al. (1989) [13] studied the characteristics of sulfatase (STS) in human prostate cells and demonstrated that E1S behaves as a better substrate STS than for DHEAS. In addition, both DHEAS and E1S were shown by the same authors to be competitive inhibitors of STS. Thus, a gradual decrease in the plasma DHEAS level during aging could in parallel increase E1S plasma hydrolysis by reducing STS inhibition, and may release active estrogens into the circulation, especially when E1S plasma concentration is high. In addition, in the human prostate, STS is able to efficiently metabolize E1S to E1. In fact, prostate sulfatase activity is much greater than prostate aromatase activity. Therefore, the sulfatase pathway is the major source of biologically active estrogen derived from E1S in this tissue [6].

Organic anion transporting polypeptides (OATPs, protein expression of *SLCO* transport genes) are required for the transport of plasma steroid sulfates, which cannot freely diffuse across the cell membrane. In men, some OATPs transporting both E1S and DHEAS are expressed in multiple tissues, including healthy and cancerous prostate, and a significant association between OATP expression and prognosis factors in PCa patients has been demonstrated [14–17].

Chronic prostatic inflammation seems to contribute to the development of PCa in men [18–20]. Aging, which is associated with an increase of estrogens/androgens ratio, is characterized by the upregulation of pro-inflammatory cytokines, such as IL1-beta, IL6, and TNF-alpha [21]. In rodents, some reports have revealed a potential link between estrogenic exposures and prostate inflammation [22–24], like in P450 aromatase over-expressing mice (AROM+), and chronic inflammation could be considered one of the histopathological hallmarks of estrogen-treated prostate tissue [25]. The exogenous administration of estrogen or its endogenous overproduction has produced several inflammatory cell infiltrates and upregulation of some cytokines in prostate tissue.

We chose STX64 (Irosustat, an irreversible steroid sulfatase inhibitor) for this animal experimentation, since it displays a similar metabolite profile in rats and humans [26]. In addition, STX64 is an interesting candidate for limiting estrogen uptake of hormone-sensitive tissues [27–28]. In the present work, we wondered whether there was a difference in the hormone profiles of young (low PCa incidence) and middle-aged (increased PCa incidence) rats after E1S induction. For this purpose, we investigated the consequences of plasma E1S overload on blood and prostate steroid concentrations in both age groups. Furthermore, we were interested in studying the inhibitory effect of STX64 on estrogen diffusion, and STS activity in prostate tissue. We also examined the role of STX64 in steroid transport and in the estrogen-related inflammation response in prostate tissue after exposure to E1S.

2. Materials and methods

Water used in this study was purified in-house, using a Milli-Q system (Millipore, MA, USA). Solvents (≥99% pure) were purchased from Carlo Erba (Val-de-Reuil, France) and other chemicals and reagents used were obtained from Sigma–Aldrich.

2.1. Animals and experimental design

Forty-eight intact male Sprague Dawley (Crl:CD[SD]) rats (Charles River Laboratories, L'Arbresle cedex, France) (twenty-four 12 week-old and twenty-four 12 month-old) separated into three different steroid treatment groups (physiological saline = C, E1S = E, and E1S+STX64=E + I; $n = 8$ rats/age and treatment group) were maintained at 22C ± 3 and kept in cages in a 12 h light:12 h darkness cycle. After arrival in our laboratory, rats were housed 4/cage with *ad libitum* food and water. According to treatment, the rats received physiological saline or E1S with a subcutaneous osmotic pump (Alzet(r) 2004, 0.25 μl h^{-1}) (Charles River Laboratories, France). Micronized STX64 (Ipsen, Les Ulis, France)/methylcellulose 1% (M-6385, Sigma–Aldrich)/water suspension was administered daily by gastric gavage (15 mg/rat). All animal surgical procedures and experimentation were approved by the *Direction Départementale des Services Vétérinaires du Val-de-Marne* (Agreement number 94-594).

The experimental protocol was identical for each rat group (age and treatment group). Animals were studied over a period of 21 consecutive days at four principal steps: day 0 (D0, before subcutaneous osmotic pump installation), 7 (D7), 14 (D14), and 21 (D21). Animals were anesthetized with isoflurane/oxygen breathing mixture (Forène(r), Abbott, France) prior to subcutaneous pump implantation (D0) and blood collection. Two ml of venous blood was drawn on D0, D7, D14, and D21. Plasma was separated and kept at −20 C until hormone analysis. Rat weight was also checked during the experiment. Animals were anesthetized and sacrificed by cardiac exsanguination on D21, after which the prostate was extracted, weighed, and immediately frozen at −80 C. The combined all four rat prostate lobes were pulverized with a BioPulverizer (Biospec, Bartlesville, USA) on the day of extraction, and mixed homogeneously at 4 C.

2.2. Preparation of implants

E1S (E-0251) powder was purchased from Sigma–Aldrich. A preliminary test conducted prior to this study enabled us to determine the quantity of steroid to be added to each implant in order to obtain sufficient circulating steroid levels in each rat, according to the osmotic pump's specific features. The E1S implant quantity for a 200 μl saline water/ethanol working volume (V/V) was found to be 1mg (1.25 μg/h/rat).

2.3. Steroid Assay Laboratory procedures

2.3.1. Hormone tissue extraction

Approximately 200 mg of premixed homogeneous prostate tissue was suspended in 1 ml saline water with 50 μl deuterated methanol internal standard working solution. The sample was homogenized with a Ribolyser (Hybaid-RiboLyser FP120-HY-230) and silicone beads for approximately 45 seconds at maximum speed. 3 ml of 1-chlorobutane was added to the homogenized sample and mixed. After centrifugation, the aqueous tissue lower phase (containing the conjugated steroids) was frozen and the upper organic phase (containing the free steroids) collected and subjected to derivatization after preparative silica mini-column chromatography.

2.3.2. Determination of steroid plasma and tissue levels

The procedure of the determination of steroid plasma and tissue levels is roughly similar to that described in our previous papers [9,29]. T, DHT, E1, E1S, E2, and E2S were assayed simultaneously by mass spectrometry coupled with gas chromatography on 1ml of plasma or ±200 mg of homogenous prostate tissue. Briefly, sera and tissues were overloaded with deuterated steroid internal standards and extracted with 1-chlorobutane. The organic extracts were purified on conditioned LC-Si SPE columns

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