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ORIGINAL ARTICLE

Effect of medical castration on CYP3A4 enzyme activity using the erythromycin breath test

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Abstract
Purpose Testosterone administration can lead to increased antipyrine clearance in humans. Medical or surgical castration is a standard treatment of progressive prostate carcinoma, but the effect of the subsequent fall of testosterone concentrations upon drug metabolism has not been reported.
Methods Eleven men with a biopsy-proven diagnosis of progressive prostate cancer were enrolled after providing informed consent. CYP3A4 activity was determined using the erythromycin breath test (EBT) in each patient prior to their beginning with an LHRH-agonist (leuprolide or goserelin). No patients had elected to undergo orchiectomy during the period of subject accrual. Each subject underwent a second EBT 2 months after beginning LHRH suppression. Blood samples were collected at these time points to determine changes in testosterone and leutinizing hormone.
Results All subjects had a predictable drop in serum testosterone concentrations over the 8-week course of the study, but concentrations in three did not fall below castrate levels (<50 ng/dl). There was no statistically significant change in CYP3A4 activity using the EBT method ($p = 0.88$). The extent and direction of changes in CYP3A4 activity was highly variable, with three subjects experiencing an increase in activity, and five demonstrating a decrease in activity.
Conclusion There is no clinically significant change in CYP3A4 activity after medical castration. No changes in the clearance of docetaxel or other CYP3A4 substrates are likely during and after medical castration. Although similar findings are expected after orchiectomy, we were not able to test this presumption because of patient preference for medical castration.

Keywords Erythromycin breath test · Testosterone · CYP3A4 · Castration · Orchiectomy

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Introduction

Elimination or suppression of testosterone production of the testes is standard treatment for advanced or symptomatic prostate cancer [7]. As a substrate of the CYP3A4 drug-metabolizing enzyme, this therapeutic reduction in testosterone concentrations may affect the metabolism of other drugs.

Docetaxel is a taxane that has been shown to provide a survival benefit in men with recurrent and metastatic prostate cancer [9, 12]. Docetaxel is predominantly metabolized by the CYP3A4 isoenzyme, and inhibition of

the enzyme has been shown to significantly raise docetaxel exposure and the risk of toxicity [2, 16]. Dose modifications of docetaxel or other CYP3A4 substrates would be needed if the drop in testosterone concentrations following medical or surgical castration led to substantial changes in enzyme activity.

Rats demonstrate sex-dependent activity of P450 class drug metabolizing enzymes. Kyerematen et al. [5] showed that the higher P450 content and nicotine C- and N-oxidation seen in male rats decreased to female levels after bilateral orchiectomy. Reconstitution of castrated male rats with exogenous testosterone raised enzyme activity and P450 content to pre-castration levels.

Johnson et al. [4] tested the effect of supplementing normal men with 400 mg testosterone for 21 days. The half-life of testosterone decreased from 10.7 ± 2.7 to 7.0 ± 1.2 h, inferring a 53% increase in its metabolism. This suggested a hypothesis that a decrease in circulating testosterone in men with surgical or medical castration will affect drug metabolism. We report here the results of the first clinical trial on men with prostate cancer to identify the effects of castration on CYP3A4 activity.

Methods

Eligible subjects for this trial were men with prostate cancer scheduled to receive a bilateral orchiectomy or testosterone suppression with the LHRH-agonists leuprolide or goserelin. Twelve subjects were sought from each group (medical vs. surgical castration). The protocol was approved by the University of Wisconsin Health Sciences Human Subjects Committee and signed informed consent was obtained before subjects entered the trial.

Determination of CYP3A4 activity utilized the radioactively labeled erythromycin breath test (Metabolic Solutions, Nashua NH) [6, 15]. Patients were therefore ineligible if they had a known allergy to erythromycin, or if they were taking drugs known at the time to be CYP3A4 substrates. Subjects were administered a 3 μCi intravenous dose of ^{14}C-N-methyl-erythromycin in the Nuclear Medicine department prior to undergoing castration. The N-methyl group of the erythromycin is metabolized by CYP3A4 and is exhaled as $^{14}CO_2$. Twenty minutes after the injection, subjects exhaled through a CO_2 trap containing hyamine hydroxide, ethanol, and thymolphthaleine until the pH indicator underwent its color change, indicating entrapment of 2 mmol CO_2 [15]. The radioactivity (DPM) of trapped CO_2 was measured by a calibrated liquid scintillation counter and was corrected for background. The percent ^{14}C-erythromycin metabolized per mmol CO_2 was determined from Eq. 1.

$$\%\ \text{metab per mmol } CO_2 = \left(\frac{(20\,\text{min breath dpm} - \text{background dpm})}{(2.22 \times 10^6\ \text{dpm}/\mu\text{Ci dosed}) - \text{Residual } ^{14}\text{C}}\right) \times \left(\frac{100}{\text{mmol } CO_2 \text{ collected}}\right) \quad (1)$$

The percent ^{14}C-erythromycin metabolized per hour was calculated using the regression equation reported by Wagner ([13], Eq. 2), in which the ^{14}C-erythromycin metabolism (EM) has been adjusted for total CO_2 production estimated as 5 mmol CO_2/m^2/min [1].

$$\%\ ^{14}\text{C erythromycin/hr} = (-65.988 \times \text{EM}^2) + 54.645 \times \text{EM} + 0.0377 \quad (2)$$

Within 2 weeks prior to their scheduled surgical or medical castration, a blood sample was obtained to measure baseline concentrations of free testosterone and leutinizing hormone (LH). Blood hormone levels and erythromycin breath tests were repeated 2 months after the start of LHRH-agonist treatment or orchiectomy. This ended the subjects' participation in this study.

Pre- and post-castration EBT CYP3A4 activity ratios were tested for a change from unity using the Student's *t* test. The intrasubject variability of the EBT assay is <5% [3, 6]. The power to detect a 20% difference in CYP3A4 activity using this assay in 12 subjects was >95%. Hormone concentrations were compared using Student's *t* test for paired observations after logarithmic transformation of the concentrations.

Results

No patients were enrolled in the surgical castration arm, reflecting the preference of men in this community to choose suppression of testosterone using LHRH agonist drugs. Eleven of the intended 12 men were found and consented to the medical castration arm during the funding period of the study. The age of the subjects ranged from 59–78 years (Table 1).

Panels a, b of Fig. 1 show the individual changes in testosterone and leutinizing hormone. Of note, 3 of the 11 subjects did not have castrate levels (<50 ng/dl) of testosterone at the time of re-assessment. Similarly, three subjects had incomplete suppression of LH. Only one subject demonstrated both measurable LH concentrations and incomplete testosterone suppression.

No difference was noted in CYP3A4 activity as measured by the erythromycin breath test. Figure 2 illustrates the changes in CYP3A4 activity noted for individual

Table 1 Subject demographics and treatment effects

Subject	Age	Testosterone (ng/dl)		CYP3A activity (%/hr)			Body weight (kg)		
		Pre GnRH	Post GnRH	Pre GnRH	Post GnRH	% Change	Pre GnRH	Post GnRH	% Change
1	78	235	51	2.62	2.12	–18.9	91	89	–2.2
2	72	365	11	1.95	1.6	–17.6	97	99	2.7
3	63	444	5	3.55	2.89	–18.5	90	89	–1.0
4	76	361	13	2.94	2.66	–9.7	68	70	2.0
5	77	40	17	2.9	3.25	11.8	108	104	–3.4
6	59	265	14	2.49	2.1	–15.7	123	123	0.0
7	77	355	< 4	3.64	3.63	–0.2	79	84	5.7
8	74	380	10	3.38	3.3	–2.3	98	99	0.9
9	60	480	24	3.46	3.43	–1.0	82	80	–3.3
10	63	553	20	2.32	3.53	52.2	119	125	4.6
11	60	166	10	1.72	2.4	39.6	81	85	3.8

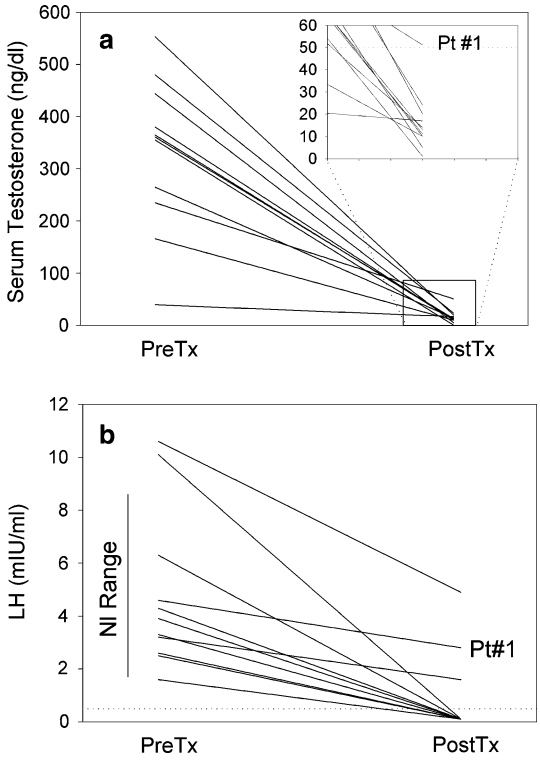

Fig. 1 Pre- and post-castration testosterone and leutinizing hormone concentrations. *Dotted line* at 50 ng/dl shows upper limit of desired "castrate" testosterone concentrations

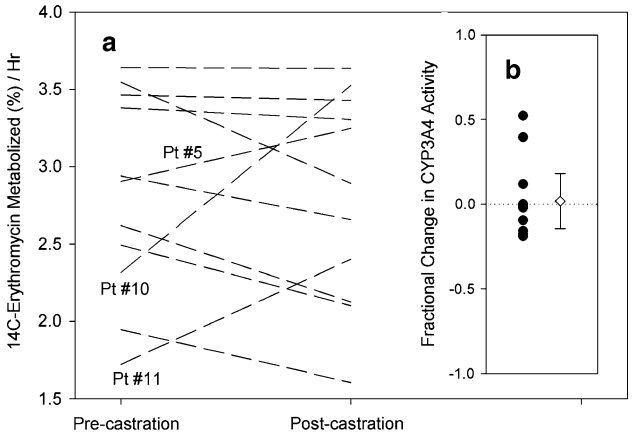

Fig. 2 **a** CYP3A4 activity by erythromycin breath test before and 2 months after medical castration. **b** Fractional change in CYP3A4 activity after medical castration. *Solid circles* are individual subjects. *Diamond* and *error bars* are mean and 95% CI

subjects. It is evident that the extent of changes was highly variable, with three subjects experiencing an increase in CYP3A4 activity, and five demonstrating a decrease. The 95% confidence interval for the ratio of pre- and post-castration CYP3A4 activities was 0.862–1.16. Because this range of pre/post ratios of CYP3A4 activity includes 1, it can be concluded that there is no significant and consistent effect of medical castration upon the activity of this drug-metabolizing enzyme. There were no correlations of fractional change in CYP3A activity with age or baseline CYP3A activity or fractional changes in testosterone (data not shown).

Our finding in men undergoing medical castration with leuprolide or goserelin was that the lowering of circulating testosterone concentrations was not accompanied by a consistent change in CYP3A4 enzyme activity as measured by the labeled erythromycin breath test. The effect of surgical castration on drug metabolism was not addressed because no eligible men chose this route of hormone suppression. Our study was limited in size, but the 11 subjects studied provided a power of >95% to detect a change in CYP3A activity of 20% from baseline.

Discussion

The results of the study reported here suggest that the decrease in testosterone concentrations established by medical castration with LHRH agonists does not lead to a significant change in the activity of the CYP3A4 enzyme. The change over 2 months following the initiation of testosterone suppression was mixed among individuals. The acceptance of the null hypothesis of no difference in EBT results before and after medical castration suggests that any effect of castration on CYP3A4 is not clinically significant.

The effect of reducing testosterone concentrations upon other CYP isotypes, acetyltransferases, glucuronidases or other drug metabolizing enzymes was not evaluated in this study. Testosterone has been shown to be a substrate for CYP3A4 [8, 14] and the supposition was made that changes in testosterone concentrations would be reflected in enzyme activity. Administration of testosterone to normal males leads to a significant increase in the clearance of antipyrine, but other human enzymes in addition to CYP3A4 have been shown to be responsible for metabolizing antipyrine, including CYP 1A2, 2B6, and 2C9 3C4 [4, 11]. Any effect of castration upon these enzymes would not have been apparent in our study, since erythromycin is metabolized by CYP3A4 and 3A5. It can be anticipated that a substantial effect of medical castration on 2C9 is unlikely in the absence of any reports of INR changes after castration in men receiving warfarin.

The potential outcome from a study of patients undergoing orchiectomy rather than GnRH treatment can be considered. The use of LHRH agonists such as leuprolide overwhelms the usual pulsitile LHRH release and leads to LRHR receptor down-regulation and testosterone suppression secondary to the suppression of luteinizing hormone [10]. In contrast, bilateral orchiectomy leads to a stimulation of LH production. Only if there is an unidentified effect of LH upon CYP3A4 activity would a different outcome in surgically castrated patients occur. The lack of a relationship between changes in LH concentrations and EBT activity (Figs. 1b, 2) argues against separate outcomes from orchiectomy versus LHRH-agonist treatments.

One significant difference between surgical and medical castration may lead to a significant, transient change in CYP3A4 activity. Unlike orchiectomy, in which serum testosterone concentrations begin dropping immediately after the resection of the testicles, treatment with LHRH-agonists such as leuprolide and goserelin leads to a temporary increase in LH and testosterone concentrations. Subsequent down-regulation of the LHRH receptor leads to sequential drops in circulating LH and testosterone concentrations. It is possible that the temporary elevation of testosterone concentrations during this transition period may have increased CYP3A4 activity. Obtaining only one follow-up EBT test of CYP3A4 activity 2 months after starting the LHRH-agonist treatment would not have identified this effect.

Conclusion

This investigation demonstrated no significant effect of medical castration upon CYP3A4 activity in men with prostate cancer treated with LHRH agonists. No empiric dose adjustment of drugs such as docetaxel metabolized by CYP3A4 appears to be needed in men undergoing medical castration for prostate carcinoma. Furthermore, although the effect of orchiectomy upon CYP3A4/5 activity was not studied, it appears unlikely that surgical castration would elicit a substantial change in the activity of this enzyme in men.

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