The Relationship Between Testosterone, Estradiol, and Prostate Cancer

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Abstract: Recent experiments have shown that estradiol is the primary cause of prostate cancer. Since estradiol in men is produced by the action of aromatase on testosterone, testosterone is only the secondary cause of prostate cancer. In normal prostate epithelial cells estradiol causes mitosis, whereas testosterone prevents mitosis. Estradiol is mutagenic through the formation of depurinating estrogen–DNA adducts 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua. For decades, doctors have been taught that testosterone is the primary cause of prostate cancer. This misunderstanding has not only been believed by the medical profession, but by the general public as well. As a result of this, too few researchers are exploring the therapeutic uses of testosterone in treating prostate cancer, funding requests are being denied due to the ignorance of funding reviewers, and volunteers for being treated with testosterone are hard to find. The solution to all of these problems is to educate the medical profession and the general public about the true relationship between testosterone, estradiol, and prostate cancer.

Introduction

For decades the medical profession has believed that testosterone (T) is the primary cause of prostate cancer (PCa). They also have believed that T promotes the growth of PCa and that T is an essential survival factor for PCa. These beliefs have permeated the consciousness of the general public. There are a number of important studies that have led to these beliefs.

In 1941, Charles Huggins showed that removing T killed most of the PCa in his patients [1]. This was an example of PCa needing T to survive. In 1977, Robert Noble showed that he could cause PCa in almost 20% of the strain of rats he was using by exposing them to prolonged high doses of T [2]. This was the first example that showed that T could cause PCa. In 1981, Fowler and Whitmore showed that when high dose T was administered to men with advanced PCa, 73% of them demonstrated a noticeable worsening of their symptoms within 30 days [3]. This was consistent with T promoting the growth of PCa. In addition, using bicalutamide to block the intracellular androgen receptor (iAR) in the PCa cell line LNCaP resulted in mitosis being halted between the G1 and the S phase [4]. This suggests that androgen binding to iAR is essential for PCa growth to occur.

Estradiol and Prostate Cancer Carcinogenesis

One thing that all of the above *in vivo* experiments have in common is that none of them used aromatase inhibitors (AIs) to prevent the conversion of T to estradiol (E2). In part, this was due to the fact that AIs were not discovered until the early 1970s [5]. However, to date nobody has tried to add an AI when reproducing any of the above experiments.

There have been experiments which strongly suggest that E2 plays a role in the carcinogenesis of PCa. Adding E2 to T resulted in 100% of the animals developing PCa in rats [6] and in mice [7]. Keeping the level of T constant, but increasing the amount of E2 present resulted in 100% of rat prostate epithelial tissue cultures developing PCa [8].

If in fact E2 is the cause of PCa carcinogenesis, it would have to cause both mitosis and mutations of the prostate epithelial cells. E2 stimulated the growth of EPN cells, a cell line derived from normal human epithelial cells [9]. Both estrone and E2 were found to be mildly mutagenic by forming the estrogen-DNA adducts $4OHE_1(E_2)$-1-N3Ade and $4\text{-}OHE_1(E_2)$-1-N7Gua [10]. Significantly higher ratios of these adducts to other estrogen metabolites have been found in men with prostate cancer as compared to healthy men. Sufficiently high levels of E2 should increase the amount of these mildly mutagenic adducts thus increasing the likelihood that the mutations needed to cause PCa will occur.

Another question to ask is why doesn't E2 alone cause PCa without needing T? When the level of T drops to castrate level or less most PCa cells undergo programmed cell death, called apoptosis [11], as do normal prostate epithelial cells [12] due to calcium ion influx. This explains why it is necessary to have some T present when inducing PCa with E2 in tissue cultures [8]. When a sufficiently large amount of E2 is used *in vivo*, it reduces T to castrate levels [13]. This means that normal prostate epithelial cells will either undergo apoptosis before E2 has time enough to induce the needed mutations or there won't be sufficient E2 present to induce PCa. E2 also has an effect on estrogen receptors (ERs), with E2 binding to ER-α which upregulates the antiapoptotic protein bcl-2 and E2 binding to ER-β which downregulates bcl-2 [14]. Since normal prostate epithelial cells have almost no ER-α [15], the effect of E2 would be to decrease bcl-2 and make these cells more prone to undergo apoptosis. This is one of the reasons that E2 has been used to treat PCa. However, besides the side effects known to occur with high levels of E2 in men, this treatment will result in a huge selective growth advantage to those PCa cells which mutate to increase their ER-α levels. Since 94% of castrate resistant prostate cancer (CRPC) cells have significant ER-α activity [16], the increase in ER-α levels is something that ordinarily happens during the evolution of PCa and accelerating this process through the use of E2 is not likely to increase survival rates. In theory, if there were no CRPC cells, then lowering T to castrate levels would be curative. In practice, this is not the case, and the way that PCa kills men is through the growth of CRPC cells.

How does E2 cause PCa when serum levels high enough to cause mitosis and mutations of prostate epithelial cells will also result in castrate levels of T? The obvious answer is that physiological serum levels of E2 cannot be carcinogenic, and it must be high local levels of E2 which are carcinogenic. Although normal prostate epithelial cells do not express aromatase, it is present in malignant epithelial cells and in PCa cell lines [17]. In addition, in ArKO mice which lack the aromatase enzyme, the effectiveness of T plus E2 to cause PCa was significantly less than in wild type mice [7]. This makes sense because adding the same amounts of exogenous T plus E2 to both types of mice should result in higher serum levels of T plus lower levels of E2 in the ArKO mice because no T will be converted to E2. This should result in higher intraprostatic levels of T and lower levels of E2 in the ArKO mice. Lower levels of E2 will decrease the chance of developing PCa. In addition, higher intraprostatic levels of T will also decrease the chance of developing PCa, since T acts on iAR to prevent mitosis [18]. It is likely that this is because iAR upregulates the androgen shutoff 3(AS3) protein [19].

While all of the previous arguments are strongly suggestive that E2 is responsible for PCa carcinogenesis, they still are not conclusive proof. However, treating ER-α knockout mice with T plus E2 resulted in no cases of PCa as opposed to the 100% rate in wild type mice [7]. This proves that ER-α is essential for PCa carcinogenesis. Since T does not bind to ER-α, but E2 does, this proves that E2 is a primary cause of PCa and T is only the secondary cause because T is converted to E2 in men due to the action of aromatase.

Castrate Resistant Prostate Cancer

Much research has been done to try to determine how PCa changes from being androgen dependent to becoming androgen independent. The assumption is made that T is a survival factor for PCa and that some very specific mutations must occur in order for PCa to be able to grow in the absence of T. However, little attention is paid to the possibility that T is not a survival factor and that androgen deprivation simply increases the rate of apoptosis. If this is in fact the case, then all that is needed for some PCa cells to turn into CRPC is for their rate of cell growth to be greater than their rate of cell death when the PCa is subjected to castrate levels of T.

If the absence of T simply increases the rate of apoptosis, then most CRPC cells should have mutations that affect apoptosis, either by interfering with the effectiveness of apoptotic processes such as proapoptotic proteins or by increasing the amount of antiapoptotic proteins present. Mutations in the proapoptotic protein p53 occur as high as 94% in advanced CRPC [20]. "Bcl-2 overexpression, which is observed in a high percentage of patients with CRPC, has a critical role in the transition from androgen-dependent to androgen-independent tumor growth" [21]. Evidence for this is the fact that using antisense bcl-2 oligodeoxynucleotides delay the development of tumors from becoming CRPC in mice [22]. However, while these experiments show that CRPC may be less susceptible to apoptosis than androgen dependent PCa, they do not prove that resistance to apoptosis is what changed androgen dependent PCa to become androgen independent PCa, since such mutations give a selective growth advantage to all types of PCa.

What seems to be a strong argument in favor of T being a survival factor is the fact that bicalutamide binding to iAR halted LNCaP mitosis [4]. However, adding physiological levels of E2 in addition to bicalutamide allowed mitosis to proceed normally [23], a fact that negates the significance of iAR alone in mitosis. If in fact androgen binding to iAR was needed for mitosis to occur and even for PCa cell survival, then adding E2 should not have had such an effect, since E2 does not bind to iAR in LNCaP cells. Even more convincing is the fact that adding a vector of cDNA for bcl-2 to LNCaP cells is all that is needed to change those cells from being androgen dependent to becoming androgen independent [24]. If CRPC only needs sufficient resistance to apoptosis, then the change from androgen dependent to androgen independent can readily be explained by androgen deprivation selecting those PCa cells which already have increased resistance to apoptosis. This is a straightforward Darwinian explanation and is much easier to conceptualize rather than the Lamarckian postulation that PCa cells somehow undergo fundamental changes to become androgen independent.

Even if T is not ordinarily a survival factor for PCa, it is possible that it may become so following the right mutations. For example, the gene fusion *TMPRSS2:ERG* occurs in roughly half of PCa patients. This is the fusion of *TMPRSS2*, a gene that is controlled by androgens, with *ERG*, a gene involved with oncogenic pathways. The result of this fusion is to put the ERG gene

under control of androgens. Patients with this gene fusion respond better to androgen deprivation to those who do not have it [25]. If T were in fact a survival factor for PCa, then the effect of androgen deprivation should be the same whether or not a gene fusion is present. However, if increasing the rate of cell growth and decreasing the rate of cell death is how PCa develops androgen independence, then the more androgen dependent genes which are required for mitosis or for protecting against apoptosis, the more effective androgen deprivation should be.

There are practical considerations to take into account when deciding which approach to take in treating CRPC. If treatments are used based on the assumption that T is a survival factor, then those treatments will fail if in fact T is not a survival factor and CRPC occurs because mutations occur that affect apoptosis or cell growth. However, if treatments are used to increase the rate of apoptosis or decrease the rate of cell growth, then such treatments will benefit the patient whether or not T is a survival factor for PCa.

Adverse Side Effects of Testosterone

There are two indisputable side effects of T, namely it can increase hematocrit and E2 levels. High hematocrit levels can be lowered by bloodletting and high E2 levels can be lowered by using AIs. Unfortunately, although it is possible to monitor and control these side effects, no studies that report adverse side effects when administering T have done so. Also, some of the studies with exogenous T show correlations that are exactly the opposite of the results found for endogenous levels of T. These reversals make it more likely that the observed adverse side effects are due to the increase in hematocrit or E2 levels and not due to the increase in T levels.

A study which administered exogenous T to 67 men and a placebo to another 67 men found that T worsened sleep-disordered breathing [26]. There was no monitoring of hematocrit or E2 levels in this study. A different study examined the endogenous levels of T for 1312 men and found that low T correlated with more severe sleep-disordered breathing [27]. This is an example of opposite results for exogenous and endogenous T.

There are some conflicting reports with regards to T and liver function. One oral form of T is known to increase the risk of liver toxicity [28]. This may be because oral T passes through the liver, whereas T produced endogenously does not. However, when T is given in the form of parenteral T undecanoate, liver enzyme measurements improved as did all of the factors known to be associated with the risk of metabolic syndrome [29].

While gynecomastia or enlarged breasts have been reported to be a side effect of administering exogenous T to men, it is believed that this is because of the conversion of T to E2. Not surprisingly, administration of an AI along with T prevented gynecomastia [30]. This is another example of why it is necessary to monitor and control E2 levels whenever administering exogenous T.

High hematocrit levels are significantly correlated with cardiovascular disease in men. This includes chronic heart disease, myocardial infarctions, angina pectoris, stroke, and intermittent claudication [31]. Also, high endogenous levels of E2, but not of T, are associated with coronary heart disease [32]. However, low levels of endogenous T are correlated with cardiovascular disease in men [33].

Recently, the mainstream media has been publicizing the results of articles that indicated that exogenous T resulted in adverse cardiovascular events [34-36]. None of these studies attempted to monitor and control the hematocrit or the E2 levels for the men treated. In addition, there has been some severe criticism of the methodology used by these studies [37]. The authors of this criticism also made the astute point "Public health may be harmed not only by inadequate appreciation of an actual risk but also by the failure to offer beneficial treatment for a medical condition because of false claims of risk concerns. On the basis of the current state of evidence, placing restrictions on the appropriate use of T therapy for T-deficient men is likely to result in compromise of public health and a substantially increased future financial burden on the US health care system".

Using Testosterone to Treat Prostate Cancer

While it would have been unthinkable to give T to men with PCa not too many years ago, this is part of the cutting edge research currently in progress. An *in* vitro study showed that supraphysiological androgen levels induce cellular senescence in human prostate cancer cells [38]. The researchers used R1881, an androgen which cannot be converted to estrogen. This demonstrated that the senescence was caused by the androgen receptors and not estrogen receptors. Phase 1 trials were done in which T was administered to men with early stage CRPC. In one study of 15 men, one patient showed symptomatic progression and three patients experienced drops in their PSA [39]. The study concluded that high dose exogenous T could be safely administered. Another similar study came to the same conclusion, with 7 out of 12 patients observing some drop in PSA, although many of the patients were treated with different doses of T and for different durations [40]. A different study analyzed the effect of T on men with low T and early stage PCa that had no previous treatments performed. If T fueled PCa, then some of the patients should have experienced rapidly growing PCa, but none did. In fact, the 13 men who were treated for an average of 3.1 years had an average decrease in their PSA by over 33% [41].

These experiments demonstrated that the old beliefs about T were wrong, but they did not point the way to how T might be used as a treatment option. The most important thing to consider when using T therapeutically is that there must be a relative balance between the iAR and the membrane androgen receptor (mAR) in order for PCa to grow [42]. This is because mAR downregulates AS3, upregulates bcl-2 but upregulates proaptotic proteins even more, with T and

DHT binding equally well to mAR. Apoptosis occurs in the presence of mAR agonism with no iAR agonism [42]. On the other hand, iAR upregulates AS3 and downregulates bcl-2 as well as the proapoptotic proteins upregulated by mAR [14]. This means that if there is iAR agonism with no mAR agonism, then, in theory, the PCa should be unable to proliferate. In practice, when the LNCaP cell line was modified to have the ability to grow in very low levels of androgen, it resulted in amplification of iAR by at least 10-fold. This new cell line, named LNCaP 104-R2, could not grow in the presence of T, but could grow in the presence of T plus finasteride [43]. Finasteride is a drug that inhibits 5α-reductase (5AR) type 2, which is an enzyme that converts T to 5α-dihydrotestosterone (DHT). In the prostate cell, T is almost always converted to DHT by 5AR type 2 and DHT binds to iAR approximately 5 times more strongly than T does [14]. Therefore, even though there is some agonism of T to mAR, with T and DHT having equal agonism to mAR [42], there is much more agonism to iAR because of its amplified number. This imbalance results in mitosis being prevented, presumably because sufficient AS3 is being upregulated. When finasteride is added to prevent the conversion of T to DHT, this reduces the agonism to iAR 5-fold. Although there is still more agonism than normal of iAR as compared to mAR, this is not enough to prevent mitosis.

A clinical study was designed to take advantage of the vulnerability of PCa when there is a large amount of iAR present. Fourteen men with CRPC were treated with high dose T alternating with low dose T, but higher than castrate levels. Every 28 days the men received an injection of testosterone cypionate. The average serum level of T was greater than 1500 ng/dl two days after injection, dropping to around 150 ng/dl on day 28. No man had their T drop to castrate level. Seven of the men experienced a drop in PSA with four of them experiencing a drop of over 50%. However, PSA levels eventually rose in these seven men, with the time from the start of enrollment until PSA progression ranging from 95 to 454 days [44]. When there is reduced agonism to iAR, the amount of iAR tends to increase. Even just using finasteride to block the conversion of T to DHT is sufficient to amplify the amount of iAR in LNCaP [45]. The interesting question is not why this treatment worked, but why it eventually stopped working. It is possible that there was an increase in mAR or a decrease in iAR so that there was no longer a sufficient imbalance between the androgen receptors. Another possibility is that because E2 was not monitored or controlled, the increase in E2 which results when T is administered may have led to an increase in the amount of ER-α present, which would mean more upregulation of bcl-2 [14]. Increased levels of bcl-2 will decrease the rate of cell death and make it more likely that the rate of cell growth will exceed the rate of cell death. More research is needed to determine why this treatment eventually fails and if anything can be done to forestall that.

There typically is a large increase in the amount of mAR when normal prostate epithelial cells become cancerous [46]. One treatment that took advantage of this used a form of testosterone replacement therapy (TRT) that used high dose T along with 5AR inhibitors, typically finasteride, dutasteride (which inhibits both type 1 and type 2 5AR), or both, to reduce

the production of DHT. Because DHT binds to iAR five times more strongly than T does, removing DHT creates an imbalance in which agonism to mAR predominates. While this does not produce as great an imbalance as using T-BSA does, it still had some positive effect. Another possible benefit to this treatment is that the use of 5AR inhibitors helps to prevent the conversion of progesterone to 5-α pregnanes. 5-α pregnanes promote cell growth and metastasis in breast cancer [47] and are likely to do the same in prostate cancer. The charts of 96 men who underwent this protocol during the period of 2000-2007 were reviewed. "In reviewing our experience with high-dose TRT in hypogonadal survivors of prostate cancer it is evident that for ~40% of patients TRT is not associated with increasing PSA levels, even over a long period" [48]. It is not clear exactly what time frame "a long period" represents. Again, the interesting question becomes what is the difference between the mAR, iAR, and ER-α present in those men for whom this treatment was not successful versus in those for whom it was successful.

Discussion

There is still much to be learned about the relationship of T and PCa, especially regarding the optimum use of T in treating PCa. Researchers who have used T both with and without 5AR inhibitors have demonstrated that, under the right conditions, T is capable of killing PCa and reducing PSA. However, the success rate of this treatment is still less than 50%, so more research is needed in order to determine how best to use T to treat PCa patients. It is possible that while T alone is sufficient when there is a high iAR to mAR ratio, when the receptors switch to a high mAR to iAR ratio, then T plus a 5AR inhibitor must be used. Also, it may be possible to improve both of these treatments by also targeting other hormone receptors, using antagonists to block those receptors that promote PCa growth and using agonists to stimulate those receptors that promote PCa death [14]. Ideally, the goal should be for men to die with PCa and not because of PCa.

One obstacle to doing further research into the use of T to treat PCa is the difficulty in getting human volunteers. This is both because the general public believes that T is the primary cause of PCa and because the mainstream media has been sensationalizing recent studies that purport to show that T has serious adverse side effects, especially with regards to adverse cardiovascular events. While it is possible that if T is not used responsibly there will be an increase in adverse side effects, to date no study has shown that any adverse side effects ensue if E2 and hematocrit levels are controlled. It might be useful if the FDA were to put a warning label on T stating that E2 and hematocrit levels should be monitored and controlled whenever T is administered to men. For men with chronic heart disease, the mortality rate was lowest when the serum estradiol level was between 21.80 and 30.11 pg/mL. The highest mortality rate was for men whose serum E2 levels were less than 12.90 pg/mL, with the next highest mortality rate being for men whose levels were more than 37.39 pg/mL [49]. Although these studies were on men who already had cardiovascular problems, it is likely that the safest levels for them would also be the safest levels for men with no cardiovascular problems. Because the highest risk

occurs when there is too little E2, it is not sufficient to administer AIs when giving T to men without also monitoring E2 levels. The highest risk of developing adverse cardiovascular events for men was with hematocrit values of 49% to 70% [31]. Endocrine Society guidelines are to not allow hematocrit levels to exceed 54% [50].

The problem in educating the general public that E2 and not T is the primary cause of PCa is more insidious. Most people, including medical professionals, have been taught that T is the primary cause of PCa all of their lives. In order for the facts to filter their way to the general public, first they must be accepted by the general medical profession which historically is very slow to change. A good start would be for all medical schools around the world to teach the fact that E2 is the primary cause of PCa and hopefully, papers such as this one will help convince educators to do so.

The problem with members of the medical profession not understanding that E2 is the primary cause of PCa is that the advancement of science in determining the ways that T can be used to treat PCa is occurring at too slow a pace. There is a problem with funding being denied and articles being rejected simply because doctors have been taught incorrect facts in medical school and hold on to these beliefs in spite of new studies that contradict them. This represents an impediment to the advancement of science.

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