Therapeutic implications of quinazoline-derived α-1 adrenoreceptor inhibitors in BPH and Prostate Cancer

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KEY WORDS

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ABSTRACT

Quinazoline-derived selective α-1 adrenoreceptor antagonists: doxazosin and terazosin are clinically effective drugs for relieving the symptoms associated with a dynamic component of benign prostatic hyperplasia (BPH). In the last years, the additional mechanisms responsible for long-term clinical activity of both drugs were revealed. Doxazosin and terazosin have been demonstrated to induce apoptosis in benign and malignant prostate cells via an α-1 adrenoreceptor independent mechanism. Our aim is to present the recent data on those supplementary curative properties of certain α-1 blockers.

The presented data come from the recent experimental and retrospective studies concerning the apoptotic activity of quinazoline-derived α-1 blockers.

The evidence, mostly from *in vitro* experiments, supports the theory that the apoptotic activity of quinazoline – based α-1 blockers against prostate cells is related to their interactions with cell signaling pathways and multiple cellular processes including apoptosis, angiogenesis, and adhesion. Moreover, it has been postulated that in clinic quinazoline-derivatives may be an effective therapeutic strategy for advanced prostate cancer, especially while applied in combination with currently used chemotherapeutic agents.

The present review reveals a new therapeutic value of quinazoline-based α-1 adrenoreceptor antagonists. In the future, drugs containing quinazoline compound might become of clinical significance in prostate cancer management.

INTRODUCTION

Benign prostatic hyperplasia

BPH etiology

Benign prostatic hyperplasia (BPH) is one of the most common age-related urological diseases. It affects about 70% of men over the age of 70 [1]. Although BPH is not a life-threatening disorder, its symptoms, in particular urinary obstruction, frequency and nocturia, have a severe negative impact on the quality of life [2]. The etiology of BPH remains not fully understood and it appears not to be associated with other chronic conditions such as diabetes or hypertension [3, 4]. It is believed that age-related changes in hormonal status constitute the predominant factor in the development and progression of the disease. In a healthy prostate, tissue homeostasis is maintained due to the physiological level of androgens. Androgens by the regulation of growth factors *e.g.*: epithelial growth factor (EGF), keratinocyte growth factor (KGF), insulin-like growth factor (IGF) and tumor growth factor (TGF) influence the interactions between stromal (smooth muscle) and epithelial components of the prostate [5]. Because of the balance between cell proliferation and apoptosis, the size of the prostate gland remains constant. Destabilization of androgen-dependent molecular mechanisms responsible for growth and proliferation is considered to be the major cause of glandular enlargement (static component of BPH). The increase of prostate size is due to the significant increase of stromal components. In healthy gland stroma: epithelium ratio is 2 : 1 against 5 : 1 in BPH [6]. Dynamic component of BPH is related to the increase of the prostatic smooth muscle tone that is regulated by the activation of α-1 adrenoreceptors. Both augmentation of prostate volume and increased α-1- adrenoreceptor-mediated smooth muscle tone are responsible for bladder outlet obstruction and the lower urinary tract symptoms (LUTS) including: urinary frequency, urgency, weak stream, and nocturia.

α-1 adrenoreceptor antagonists for BPH treatment

Due to their well established relieving action, with a rapid onset on smooth muscles tone, α-1 adrenoreceptor antagonists have been widely and safely used in the management of BPH and hypertension. At present, they are the treatment of choice for BPH. They significantly (by an average 20-30%) increase the maximal urinary flow rate and improve the symptoms of LUTS [7]. However, this quick α-1 adrenoreceptor-mediated action on the dynamic component of BPH does not fully explain the well documented long-term clinical efficacy of quinazoline-derived α-1 blockers. Activation of other molecular mechanisms, the nature of which is still under investigation, are likely to contribute to the full spectrum of therapeutic effects of these agents.

Prostate cancer

Prostate cancer etiology

The incidence of prostate cancer (PC) is rising steadily and constitutes the most common cancer in men and second leading cause of cancer death in the USA [8]. Radical surgery or radiotherapy constitutes a common treatment regimen for the localized disease. The major therapeutic option for an advanced PC (growth of PC cells is initially hormone-dependent) is androgen ablation with luteinizing hormone-releasing hormone (LHRH) analogs or surgical castration. Nevertheless, after an average 18 months of treatment PC progresses inevitably from hormone-responsive to castration-resistant lethal form, where this therapy is no longer efficient. At present, there is no treatment for hormone refractory PC that would significantly increase the survival rate [9].

Cell lines described in the review

Most of the evidence comes from *in vitro* studies using benign: normal human prostate epithelial cells (PrEC), benign prostatic hy-

perplasia (BPH-1), smooth muscle prostatic cells (SMC-1) and malignant prostatic cell lines *i.e.*: androgen-sensitive metastatic lymph node PC (LNCaP), androgen-insensitive: metastatic brain PC (DU-145), and metastatic bone PC (PC-3) [10].

Quinazoline–derived α–1 adrenoreceptor antagonists–structure and specific activity

Two of the α-1 adrenoreceptor antagonists, doxazosin and terazosin, exhibit a common feature of their chemical configuration; a quinazoline ring, of a similar structure to that of the purine ring forms the center of the compound and is believed to be responsible for specific and analogous activities of both drugs. Due to their particular chemical structure, in addition to their effects on smooth muscle cell tone, doxazosin and terazosin, have been recently demonstrated to possess the ability to induce apoptosis in benign (SMC-1) and malignant prostate cells (PC-3 and DU-145) in a dose-dependent manner without affecting cell proliferation [11, 12]. This mechanism is uninhibited by norepinephrine hence independent of their capacity to antagonize α-1 adrenoreceptor [11, 13], and, more importantly, it seems to be independent of hormone sensitivity status of the cells [13]. Interestingly, Benning and co-workers have demonstrated that *in vitro*, normal prostate epithelial cells (PrEC) were less sensitive to doxa- /terazosin-induced apoptosis than their malignant counterparts (LNCaP and DU-145) [13]. Furthermore, it was suggested that apoptosis of SMC caused by these agents might be one of the most important mechanisms responsible for their long-term therapeutic effects in BPH. The maximal level of SMC apoptosis (15% of cells) was observed in prostate biopsy specimens after 3 months' treatment and remained elevated till one year of continuous application of doxazosin [14]. Subsequent clinical study by the same group confirmed the previous findings in a larger population of 138 patients and showed that terazosin has a similar effect on cell death induction. Moreover, SMC apoptosis in post-treatment prostate biopsy specimens correlated with symptoms score improvement in 34 out of 65 doxazosin and 9 out of 42 terazosin treated BPH patients [15]. These findings explain the therapeutic impact of quinazoline-based α-1 blockers on the static component of BPH and have implied their possible application in the management of hormone–dependent and hormone-naive prostate cancer.

This article provides an up-dated review of currently available findings of *in vitro* and *in vivo* studies on the impact of quinazoline-derived α-1 adrenoreceptor antagonists and, in particular, doxazosin, on cell survival/apoptosis in BPH and prostate cancer.

Mechanisms of quinazoline–induced apoptosis in prostate cells

Accumulated experimental data suggests that the apoptotic activity of quinazoline – based α-1 blockers against prostate cells are due to their interactions and effects on: 1. cell signaling pathways: i. growth factors loops associated with activity of tyrosine kinase (TK), phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase (AKT) ii. growth factors and, in particular, transforming growth factor beta 1 signaling (TGF-ß1) 2. cellular processes: i. cell adhesion to the extracellular matrix; ii. angiogenesis; iii. expression of several pro-apoptotic agents, enzymes and inhibitors of apoptosis (IAP); 3. synergistic activity with radio- and chemotherapy [19-28].

1.i. Growth factors and TK/PI3K/AKT pathway. Significance of PTEN.

Growth factors (GFs) *e.g.* EGF, IGF and TGF activate a TK receptor at a cellular membrane. When two subunits (p85 and p110) of PI3K bind, PI3K is activated and its downstream target is AKT. AKT, while activated (phosphorylated), promotes cell survival by inhibiting apoptosis and increasing proliferation. AKT has been reported to have anti-apoptotic activity and shown to inactivate several pro-apoptotic proteins, including BAD, FKHR, GSK3β, procaspase 9 as well as activate anti-apoptotic agents including mTOR, MDM2 [9, 29]. The PI3K pathway is disrupted in a wide spectrum of human cancers and has been described recently as one of the main growth factor survival pathway in PC [30, 31]. Further, it has been suggested that the activation of this pathway might be critical for the survival of PC cells under the condition of androgen suppression. Therefore, PI3K pathway appears important in PC progression, particularly in the absence of androgens, and in the final development of hormone refractory state [32].

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor gene, which negatively regulates the PI3K pathway, being frequently mutated in the advanced stages of PC (in almost 50% of hormone refractory tumors [33, 34]). Thus, when PTEN is inactive or downregulated, the PI3K pathway is activated leading to PC cells growth and survival [35]. In recent studies both phosphorylation of AKT and/or loss of PTEN expression were correlated with PC aggressiveness [36-38]. A recent *in vitro* study in PC-3 cells shows the ability of doxazosin to induce apoptosis via inhibition of the PI3K pathway. The experimental evidence reveals that the mechanism of doxazosin-induced apoptosis is (α-1 adrenoreceptor independent) and most likely is mediated through the inhibition of phosphorylation of AKT or through dephosphorylation of p-AKT [16]. In this study, Shaw et al. also suggest that structural modifications of doxazosin might have generated a novel group of apoptotic agents with improved efficacy in blocking intracellular AKT activation. Other research supporting this theory reports that quinazoline-based α-1 adrenoreceptors antagonists compete with ATP binding to TK, inhibiting, in this way, various intracellular pathways [39]. This natural capacity of doxazosin and terazosin to interact with kinases appears to be mediated through the specific quinazoline-like structure.

1.ii. Growth factors and TGF ß1

Growth of prostate epithelial cells is stimulated and maintained by a number of GFs such as EGF, TGF, and IGF secreted by stromal cells following androgen stimulation [40]. EGF and TGF seem to play a dominant role in prostatic cell proliferation and survival. On the other hand, TGF ß1 has been shown to possess an apoptotic and anti-proliferative potential in human prostatic epithelial cells. Ilio et al. suggested that apoptotic activity of doxazosin on human prostatic stromal cells is mediated *in vitro* through an autocrine action of TGF ß1 [17]. Glassman and colleagues showed in benign epithelial and stromal prostatic biopsy tissue specimens from men treated for BPH, that doxazosin-mediated up-regulation of TGF ß1 could be the predominant mechanism of apoptosis [18]. Results of *in vivo* study by Yang et al. supports the theory of TGF ß1- mediated pro-apoptotic action of doxazosin. They used an MPR, a mouse prostate reconstitution model mimicking human BPH, in which TGF ß1 was transduced into a single cell population derived from the mouse urogenital sinus and then reimplanted under the renal capsule. In doxazosin treated TGF ß1 transduced MPRs mice, a dose-dependent, α-1 adrenoreceptor-independent increase in epithelial cell apoptosis as well as a 30% reduction in wet weight of prostate gland were observed [19]. Interestingly, Zhao et al. did not observe any change, at the RNA level, in TGF ß1 expression in doxazosin treated normal and BPH stromal cells. Therefore, this study does not support the conjecture that TGF ß1 is involved in apoptotic activity of doxazosin in normal and BPH stromal cells. They reported instead an involvement of TNF α signaling pathway (not TNF α itself) in doxazosin treated cells [41]. However, doxazosin was considered to

mediate PC-3 cells apoptosis by initially inducing the expression of TGF ß1 and subsequently by up-regulating IκBα [20].

2.i. Anoikis

Cell-extracellular matrix attachment (mediated mainly by integrins) and their interactions are necessary for growth and survival of epithelial cells. Anoikis is a specific process leading to the disruption of integrin-mediated epithelial cell contact with extracellular matrix (ECM) [42]. Most cells when they lose contact with ECM undergo detachment-induced apoptosis. Anoikis-resistance (ability to survive when detached from the tumor site) correlates with cell malignant potential and plays a pivotal role in the metastatic process. Doxazosin leads to anoikis by inhibition of cell adhesion to the ECM. *In vitro* study confirms the ability of doxazosin to induce anoikis in benign stromal and epithelial components of the prostate as well as in LNCaP and PC-3 cells. Anoikis was observed both in benign and malignant cell lines, although PC-3 cells were more refractory to the effect of doxazosin. This study also shows an increase in caspase 3 activity and decrease in focal adhesion kinase (FAK) without significant effect on integrin linked kinase (ILK) in doxazosin treated PC-3 cells [21]. Kaledjian et al. further investigated effects of quinazolines on cell-ECM attachment in PC-3 cells and showed doxazosin induced a decrease in PC cells adhesion to gelatin and collagen but not to fibronectin. They observed as well that, the effect could be antagonized by Bcl-2 [22]. A recent study supports the theory that doxazosin has an impact on cell-ECM interactions. Garrison and Kyprianou showed a significant reduction at the mRNA levels of certain integrins and major changes in E-cadherin, ß catenin, laminins and selectins in doxazosin treated PC-3 cells [23].

2.ii. Angiogenesis

Angiogenesis is a complex process enabling cancer cells to spread and grow. Therefore, targeting this process is of great therapeutic value in cancer management. Using an *in vitro* model of PC-3 and PC-3 transfectant clones overexpressing the Bcl-2 (PC-3/Bcl-2), Kaledjian et al. reported that doxazosin treatment results in anti-angiogenic effect by leading to transient, 6 to 12 hours, down regulation (2-fold decrease) of VEGF in PC-3 cells. In transfected PC-3 cells the effect of quinazoline based compounds antagonists on VEGF expression is partially reversed by Bcl-2. However, doxazosin does not lead to any significant change in the expression of hypoxia inducible factor-1α (HIF-1α) neither in PC-3 nor in PC-3/Bcl-2 cells [22]. Furthermore, it has been recently reported that doxazosin suppresses *in vitro* angiogenesis and growth of human umbilical vein endothelial cell (HUVEC) via disruption of VEGF and fibroblast growth factor 2 (FGF-2)–mediated interactions. Doxazosin antagonizes the VEGF-mediated angiogenic response of HUVEC cells by restraining cell adhesion to fibronectin and collagen-coated surfaces and inhibiting cell migration [24]. Although clinical data concerning quinazoline-based α-1 adrenoreceptor antagonist induced apoptosis still remain very scant, there is some evidence to suggest that terazosin decreases prostate tumor vascularity, induces apoptosis in prostate cancer cells and reduces tissue PSA expression [25]. The ability to inhibit cell migration and prevent angiogenesis, cell adhesion, and invasion makes quinazoline-derivatives a very promising tool in the management of tumor associated angiogenesis in advanced PC [24].

2. iii. Pro-apoptotic agents and inhibitors of apoptosis. Significance of Bcl-2.

The Bcl-2 family of proteins comprises the Bcl-2 group (i.e. Bcl-xL and Bcl-w) which inhibits apoptosis and the Bax (i.e. Bak and Bok) and Bim (i.e. Bik, Bad and Bid) groups which promote cell death. The apoptotic effectors consist of proteolytic enzymes – proteases, termed caspases, which trigger cell death. Caspases are activated by promoters of apoptosis and down regulated by inhibitors. These enzymes play a pivotal role in the final stages of the apoptotic process. Bcl-2 is believed to suppress the release of caspase-3 and is supposed to be a predictor of aggressive tumor behavior. Bcl-2 up-regulation seems to be one of the predominant mechanisms of PC progression [43]. It was reported that high resistance to apoptosis (poor therapeutic response) is more likely to occur if the expression of Bcl-2 is high and Bax low [43]. In addition, Bcl-2 is reported to have a high potential of inhibiting apoptosis by influencing angiogenesis. Bcl-2 overexpression in PC-3 cells (PC-3/Bcl-2) results in a partial reversion of doxazosin-mediated decrease of VEGF and therefore, inhibition of angiogenesis.

Fas/CD95 and TRAIL/Apo3 are the receptors at the cell membrane, which upon ligand binding activate a death receptor-mediated pathway. Recently, Garrison and Kyprianou reported doxazosin-induced temporary changes in the expression of several regulators of apoptosis, including up-regulation of Bax and Fas/CD95, and down-regulation of Bcl-xL and TRAIL/Apo3. Doxazosin induced apoptosis in benign (BPH-1) and malignant (PC-3) prostate cells was mediated by the activation of caspase-8 and caspase-3 that occurred within the first 6 to 12 hours of treatment. The apoptotic effect was then reversed by specific caspase-8 inhibitor, confirming that in both cell lines, cell death was mediated by caspase-8 [23]. Partin et al. further showed a strong activation of caspase-3 in doxazosin treated PC-3 cells [20]. Chiang and colleagues demonstrated increased expression of Bax and activated caspase-3 in doxazosin treated mice [26]. They suggested that doxazosin, given chronically at a very high dose, might have been useful for preventing prostate tumor formation, decreasing prostate tumor weight as well as limiting or even suppressing metastasis. Yono et al. reported that long-term α-1 adrenoreceptor blockade with high dose of doxazosin changes the expression of several hundred genes in the rat prostate (39 of them take part in cell death, proliferation, growth) and up-regulates the expression of anti-apoptotic mediator – clusterin [27].

3. Doxazosin with radio- and chemotherapy

Neither radio- nor chemotherapy is considered to be much effective in hormone refractory prostate cancer, although there are some experiments trying to make PC cells more sensitive to the effects of the systemic therapy. There are data suggesting that doxazosin has a radiosensitizing effect. PC-3 cells were treated with doxazosin prior to and after the exposure to ionizing radiation. Synergistic apoptotic effect on cell cultures was observed suggesting that doxazosin could be used in combination with radiotherapy for treatment of castration resistant tumors. Radiosensitizing effect was independent of α-1 adrenoreceptor blockade and did not seem to be correlated neither with caspase-3 nor with Bax protein expression. The mechanism underlying this activity currently remains unknown [28]. Study by Cal et al., has demonstrated doxazosin-mediated cytotoxity in DU-145 and PC-3 cells. Furthermore, the study shows that the combination of doxazosin with some chemotherapeutics *i.e.* adriamycin or etoposide has synergistic cytotoxic activity in these PC cells. Therefore, the authors have postulated that doxazosin could be a new cytotoxic drug either used alone or combined with the above agents in the treatment of hormone refractory disease [44].

CONCLUSIONS

The understanding of molecular pathways of the cell cycle are critical in cancer–research studies. In particular, revealing and

clarifying molecular mechanisms involved in the pro-apoptotic action of quinazoline derivatives *i.e.* doxazosin and terazosin, clinically established and accepted agents, and their interactions with the recognized molecular pathways could provide a biological basis for the design of advanced cancer therapies. New and safe quinazoline-based compounds could be of paramount significance in the management of static component of BPH and in at present untreatable hormone refractory prostate cancer. Further, subsequent modulation of these molecular mechanisms might open new possibilities of effective therapeutic strategies to revert the malignant process.

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