α-blockade is not effective in decreasing tissue bulk in patients suffering from BPH, an *in vitro* study

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KEY WORDS

prostate ◗ stem cells ◗ alpha-blockade ◗ cell viability

ABSTRACT

Introduction. Stem cells play an important role in the etiology and development of prostate diseases. CD133 allows discrimination between stem and differentiated cells of the prostatic epithelium. Doxazosin (Dox) induces apoptosis in both the prostatic epithelial and prostatic stromal cells. The aim of study was to assess the influence of doxazosin on cell viability within progenitors and differentiated cells cultured *in vitro* from prostate epithelium.

Material and methods. Prostatic epithelial stem and differentiated cell cultures were acquired from 10 patients suffering from benign prostatic hyperplasia (BPH). Cells were magnetically sorted to obtain pure populations of stem and differentiated cells. Primary co-cultures of stem with differentiated cells and pure CD133 cell cultures were obtained as well. After 14 days, primary cultures were incubated for 12 hours with increasing concentrations of doxazosin, 20, 50 and 80 μM, respectively. Cell viability was estimated using trypan blue exclusion test.

Results. Ninety co-cultures contained both stem cells (CD133+) and differentiated prostatic epithelial cells (CD133-). Forty-one cultures contained only sorted epithelial stem cells (CD133+). Doxazosin significantly decreased cell viability in co-cultures of CD133+ and CD133- cells after 12 hours of incubation when compared to control. There were no significant changes in living cell population within CD133+ cultures after 12 hours of incubation with doxazosin when compared with the control.

Conclusions. Doxazosin decreased cell number within co-cultures of stem and differentiated prostatic epithelial cells. Stem/progenitor (CD133+) cells were not sensitive to doxazosin treatment. There is a suspicion that differential influence of doxazosin on progenitor and differentiated cells can be partially responsible for lack of prostate volume decrease after α1-antagonist treatment.

INTRODUCTION

Stem cells play an important role in the etiology and development of prostate diseases [1, 2]. Stem cells are defined as clonogenic, pluripotent, and self-renewing progenitors that can generate one or more specialized cell types [3]. They are generally quiescent and reside in a specialized cellular location known as a niche, which provides a microenvironment that maintains the balance between quiescence and self-renewal of the stem cell population. In prostate the niches are located in the proximal region of prostatic ducts within the basal layer of epithelium [4].

There are few cell surface markers that can be useful for identification of the prostatic stem cell-like population. Collins et al. demonstrated that $\alpha_2\beta_1$ integrin expression can be used to isolate stem cells directly from prostate tissue using differential adhesion to type I collagen [5]. Richardson et al. demonstrated that 25% of cells within the $\alpha_2\beta_1^{hi}$ population expressed another surface cell marker, CD133, while CD133 expression was only observed within the $\alpha_2\beta_1^{hi}$ population. High surface expression of the $\alpha_2\beta_1$ integrin and CD133 ($\alpha_2\beta_1^{hi}$/CD133$^+$) correlates with the potential to regenerate a fully differentiated prostate epithelium *in vivo*. In contrast, a cell population with high expression of the $\alpha_2\beta_1$ integrin and without CD133 expression ($\alpha_2\beta_1^{hi}$/CD133$^-$) was incapable of forming prostate epithelium *in vivo* [6]. Another prostatic stem cell surface marker known as stem cell antigen (Sca-1) was proposed by Burger et al. The Sca-1^{hi} cells that co-express α_6 integrin (CD47f) and anti-apoptotic factor Bcl-2 are almost exclusively confined to the proximal region of the prostatic ducts and they have high proliferative potential *in vivo* [7]. Stem cells are characterized by low sensitivity to action of pro-apoptotic agents which is correlated with high expression of anti-apoptotic proteins, high ability to repair damaged DNA and high expression of ATP-binding cassette drug transporters [8].

It was proven that the α_1-adrenoreceptor antagonists, terazosin and doxazosin, induce prostate programmed cell death (apoptosis) without affecting cell proliferation *in vivo* or *in vitro* via α_1-adrenoreceptor-independent actions [9, 10, 11].

The aim of this study was to compare doxazosin's influence on, both, populations of stem and adult cells of the prostate epithelium.

MATERIAL AND METHODS

Tissue specimens were obtained from 10 patients suffering from BPH treated by an open prostatectomy. The specimens were taken from the transitional zone. They were chopped into small pieces and digested for 6 hours with 0.5% collagenase (Sigma, Germany) [11]. Released epithelial cells were labeled with CD133 MicroBeads

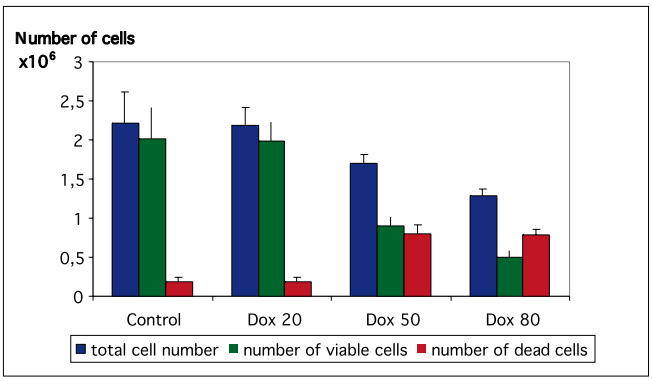

Fig.1. CD133(+)/CD133(-) cells viability estimated with trypan blue test.

and sorted using the SuperMACS II device. Co-cultures of $CD133^+/CD133^-$ cells and $CD133^+$ cells were established separately [12,13]. After 14 days, both types of primary cultures were incubated for 12 hours with 20, 50, and 80 μM concentrations of doxazosin. Cell viability was estimated using the trypan blue exclusion test. Doxazosin was supplied by Pfizer UK. Results were presented as means with standard deviations. Means were compared with t-Student test. $P < 0.05$ was considered important. Correlations were calculated.

RESULTS

Ninety co-cultures of $CD133^+/CD133^-$ cells and 41 primary cultures containing $CD133^+$ cells were established. Twelve-hour incubation of $CD133^+/CD133^-$ co-cultures with doxazosin in concentrations 20 μM/l (Dox 20), 50 μM/l (Dox 50), and 80 μM/l (Dox 80) resulted in a decrease in total cell number estimated with trypan blue test by about 1.3% ($p = 0.8$), 23.1% ($p < 0.001$), and 42.1% ($p < 0.001$), respectively, when compared to $CD133^+/CD133^-$ co-cultures incubated without doxazosin. Twelve-hour incubation of $CD133^+/CD133^-$ co-cultures with doxazosin in concentrations 20 μM/l (Dox 20), 50 μM/l (Dox 50), and 80 μM/l (Dox 80) resulted in the decrease in the number of viable cells estimated with trypan blue test by about 1.31% ($p = 0.8$), 55% ($p < 0.001$), and 75.23% ($p < 0.001$), respectively, when compared to $CD133^+/CD133^-$ co-cultures incubated without doxazosin (Table 1, Fig. 1). A high correlation ($R = -0.98$) between total cell number and doxazosin concentration was noticed in $CD133^+/CD133^-$ co-cultures group. Also, a high correlation ($R = -0.96$) between the number of viable cells and doxazosin concentration was noticed in $CD133^+/CD133^-$ co-cultures group. There were no significant changes in total and living cell number in $CD133^+$ primary cultures after 12-hours of incubation with doxazosin (Table 2, Fig. 2).

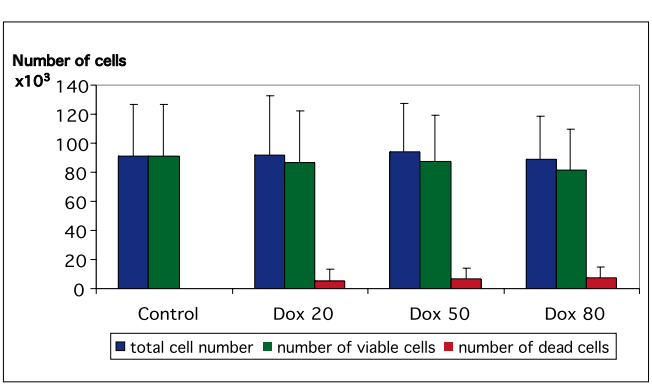

Fig. 2. CD133(+) cells viability estimated with trypan blue test.

Table 1. CD133(+)/CD133(-) cells viability estimated with trypan blue test.

	Total cell number ($x10^6$)	Number of viable cells ($x10^6$)	Number of dead cells ($x10^6$)
Control	2.21 ±0.41	2.02 ±0.39	0.19 ±0.06
Dox 20	2.18 ±0.23	1.99 ±0.24	0.19 ±0.06
Dox 50	1.70 ±0.12	0.90 ±0.12	0.80 ±0.12
Dox 80	1.28 ±0.09	0.50 ±0.08	0.78 ±0.07

DISCUSSION

Doxazosin, a quinazoline derivative and postsynaptic α_1-adrenoreceptor antagonist, is used widely in BPH treatment. The mechanism of action of doxazosin is known and well documented. By selectively inhibiting α_1-adrenoreceptors in the prostate, the urethra, and bladder neck, doxazosin reduces the tone of prostatic smooth muscle and improves urinary flow rate as well as the obstructive and irritative symptoms characteristic for BPH [14]. It is also proven that doxazosin induces apoptosis among prostate stroma smooth muscle and epithelial cells *in vivo* and *in vitro* via α_1-adrenoreceptor-independent actions [9, 10]. Some authors linked increased apoptotic index of both stromal and epithelial cells with improved BPH symptoms in treated patients [15]. Thus, does doxazosin improve BPH symptoms in two different ways? Furthermore, why doesn't usage of doxazosin result in a decrease in prostate volume even though doxazosin increases dead cell number by the induction of apoptosis? Chon et al. demonstrated that doxazosin doesn't affect cell proliferation [9]. We think quinazoline derivatives cannot induce apoptosis in cells responsible for the regeneration of prostate stem cells and that's why there is no decrease in prostate volume in patients treated for benign prostatic hyperplasia. The results of our study seem to confirm this observation. In co-cultures of $CD133^+/CD133^-$ cells, we observed a significant decrease in total and viable cell number after incubation with doxazosin when compared to co-cultures incubated without doxazosin. The same concentrations of doxazosin used during the incubation of $CD133^+$ (stem cells) cultures caused no significant changes in total and viable cell number when compared to $CD133^+$ cultures incubated without doxazosin.

Stem cells play an important role in the etiology and development of prostate diseases – benign prostatic hyperplasia and prostate cancer [1, 2]. Therefore, induction of programmed cell death seems to be a very promising method for treatment of different diseases, especially cancers, with the condition that the stem cells' "security system" against apoptosis can be broken. The mechanism by which doxazosin induces programmed cell death is not exactly known; hence further investigations could be helpful.

CONCLUSIONS

Doxazosin induced apoptosis and decreased cell number in co-cultures of progenitor and differentiated prostatic epithelial cells.

Table 2. CD133(+) cells viability estimated with trypan blue test.

	Total cell number ($x10^3$)	Number of viable cells ($x10^3$)	Number of dead cells ($x10^3$)
Control	90.98 ±35.90	90.98 ±35.90	0
Dox 20	91.82 ±40.70	86.36 ±35.85	5.45 ±8.20
Dox 50	94.44 ±32.83	87.78 ±31.53	6.67 ±7.07
Dox 80	89.09 ±29.14	81.82 ±27.86	7.27 ±7.86

However, progenitor cells were not susceptible to apoptosis after doxazosin treatment. There is a suspicion that the differing influence of doxazosin on progenitor and differentiated cells can be partially responsible for the lack of prostate volume decrease after α1-antagonist treatment.

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