Prostate epithelial stem cells are resistant to apoptosis after α1-antagonist treatment. The impact for BPH patients

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KEY WORDS

doxazosin ◗ apoptosis ◗ epithelial stem cells ◗ prostate

ABSTRACT

Introduction. Induction of apoptosis in prostatic epithelial cells by doxazosin, terazosin and prazosin has been well documented. However, the biochemical pathways of doxazosin action is still unclear. Aforementioned drugs should lead to decrease of prostate volume, although this effect was never observed in patients suffering from BPH after treatment with α1-antagonists. Probably, it is connected with cancer stem cells' resistance on chemotherapeutic agents. The aim of this study was to compare incidence of apoptosis induced by doxazosin in progenitor and differentiated cells isolated from human prostate epithelium.

Material and methods. For this purpose tissue specimens were obtained from 10 patients suffering from BPH, the primary cultures of prostate epithelium were established and CD133 MicroBeads sorting was prepared. Both, CD133(+)/CD133(-) co-cultures and CD133(+) cells were incubated with different concentration of doxazosin for 12 h. Cell viability and apoptosis was estimated with Annexin V-FITC.

Results. 12 h incubation of CD133(+)/CD133(-) co-cultures with doxazosin resulted in increase of apoptotic cells, while in CD133(+) cultures no changes were observed. Correlation between apoptotic cell number and doxazosin concentration in CD133(+)/CD133(-) co-cultures group was high (R = 0.99).

Conclusion. Doxazosin induced apoptosis in co-cultures of progenitor and differentiated epithelial cells. However, progenitor cells were not susceptible to apoptosis, what can be a reason of treatment failure in BPH patients.

INTRODUCTION

Doxazosin, terazosin and prazosin induce apoptosis in prostatic epithelial and stromal cells *in vitro*. Doxazosin induce apoptosis in prostate cancer cell lines [1]. It was even stated that terazosin and doxazosin induce apoptosis within prostates of patients with benign prostatic hyperplasia, but the biochemical pathways of doxazosin action are still not defined [2, 3]. These drugs should lead to decrease of prostate volume. This effect was never observed despite long-term treatment with α1-antagonists in a huge number of patients suffering from BPH all over the world. Few years ago it was suggested that each tissue including a malignant one has a progenitor layer or niche. Progenitor cells, which residue within such a niche have a different properties from their proliferating and differentiating counterparts. Resistance to many drugs is a characteristic feature of progenitors and stem cells. Drewa et al and Miki et al proposed CD133 as a marker of prostate epithelial progenitors [4, 5, 6]. Toward to resolving the question, why α1-antagonists treatment does not decrease prostate volume in BPH patients we compared incidence of apoptosis induced by doxazosin in progenitor and differentiated cells isolated from human prostate epithelium.

MATERIAL AND METHODS

Tissue specimens were obtained from 10 patients suffering from BPH and undergoing adenomectomy. Local Ethical Committee agreement was obtained. Establishment of human prostate epithelium primary cultures and cell incubation with doxazosin was previously described [7]. Released epithelial cells were labeled with CD133 MicroBeads and sorted using SuperMACS II device (Miltenyi Biotec). Co-cultures of CD133(+)/CD133(-) cells and CD133(+) cells were established separately. After 14 days both types of primary cultures were incubated for 12 h with 20, 50 and 80 μM concentrations of doxazosin as previously described [7]. To detect apoptotic cells Annexin V conjugated with fluorescein izothiocyanate (Annexin V-FITC, Immunotech, USA) and propidium iodide (PI), (Immunotech, USA) were used. Doxazosin, supplied by Pfizer Ltd. UK, was added to each culture after 24 h preincubation. Apoptotic cells were counted after 12 h incubation. Cells incubated with PBS were used as control samples. Analysis was performed using flow cytometry EPICS XL (Coulter) with System 2 Software Version 1.0.

Results were presented as means with standard deviations. Means were compared using t-Student test. Correlations were calculated. $P < 0.05$ was considered important.

RESULTS

90 primary co-cultures of CD133(+)/CD133(-) cells and 41 primary cultures containing CD133(+) cells were established. 12 h incubation of CD133(+)/CD133(-) co-cultures with doxazosin resulted in decreasing number of viable cells and significant increase of apoptotic cell number (Fig.1). High correlation (R=0.98) between cell number and doxazosin concentration was noticed in CD133(+)/CD133(-) co-cultures group. Correlation between apoptotic cell number and doxazosin concentration in CD133(+)/CD133(-) co-cultures group was high (R = 0.99). There was no

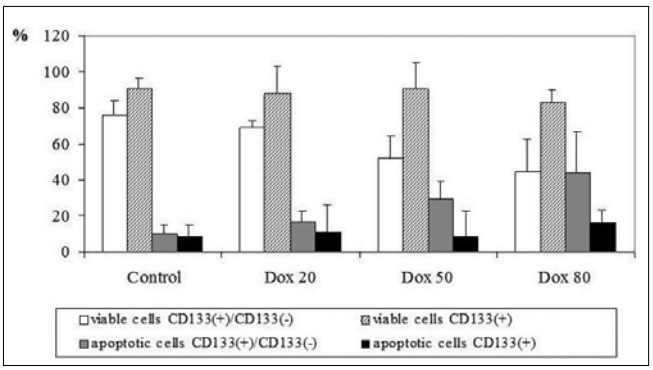

Fig. 1. Apoptotic and viable cells after incubation with increasing concentrations of doxazosin (20, 50 and 80 μM) measured using flow cytometry (Annexin V-FITC and iodide propidium – IP labeling).

significant changes in living and apoptotic cell number in CD133(+) primary cultures after 12 h incubation with doxazosin (Fig. 1).

DISCUSSION

The cancer stem cell hypothesis suggests that these cells are a minority population that drives tumor growth and possess resistance mechanisms against widely used drugs [8]. Cytotoxic chemotherapy eliminates most cells in a tumor, but cancer stem cells probably survive. Currently, there is a huge pressure on inquiring mediators of apoptotic signal, which is very important in prostate cancer management [9-11].

Doxazosin is commonly used in BPH treatment and induces apoptosis among prostate stroma smooth muscle and epithelial cells [12]. In our previous study we observed decreasing number of viable cells in co-cultures of CD133+/CD133- cells after doxazosin application [7]. Why therefore treatment with doxazosin does not result in a decrease in prostate volume? In presented study we showed that doxazosin induces apoptosis in co-cultures of progenitor and differentiated prostatic epithelial cells. Moreover, we observed different effect on progenitor cells, which were not susceptible to apoptosis after doxazosin treatment. Similar effect is noticed after treatment of acute myeloid leukemia (AML), which often failed, because administered drugs do not target leukemic stem cells (LCSs) [13, 14]. Although, drug resistance may differ among various cancer stem cells, 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. In spite of small number of cancer stem cells, their properties are probably sufficient for allowing tumor recurrence. The mechanism responsible for the resistance of CD133+ cells to conventional therapies remains to be elucidated. One of the explanation could be increased expression of BCRP1, a putative drug resistance protein, which was observed in CD133+ hepatocellular carcinoma and glioblastoma cell lines [15, 16]. The phosphorylation of Akt and accumulation of anti-apoptotic signals in Akt/PKB survival pathways have also been suggested to contribute the chemoresistance of CD133+ tumor cells [17]. The survival of cancer cells could be also promoted by Oct-4, the homeobox protein [18].

CONCLUSION

Doxazosin induced apoptosis in co-cultures of progenitor and differentiated epithelial cells. However, progenitor cells were not susceptible to apoptosis, what can be a reason of treatment failure in BPH patients.

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