

EFFECT OF PC-3 PROSTATE CANCER CELL LINE SUPERNATANT ON APOPTOSIS IN MACROPHAGES

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Abstract: Particular types of cell death, for example apoptosis, play important role in metastatic processes. Apoptosis is programmed cell death, which is characterised by specific morphological changes. In this study we aimed on topic of affecting of supernatant from prostate cancer cell line PC-3 on a healthy cells of immune system, macrophages concretely. Peripheral blood monocytes were cultivated for 7 days to macrophages. Macrophages were stimulated for 24 hours by lipopolysaccharide (LPS) and cultivated with supernatant for another 24 hours. Preparations were prepared and analysed by light microscopy. Macrophages with normal morphology show the largest rate in our experiment. In samples macrophages + supernatant there was an increase mainly in late stage of apoptosis. A lot of observed cells showed feature of rupture and spillage of cell contents into the microenvironment. In samples macrophages + supernatant + LPS there is a noticeable decline in the incidence of normal morphology compared to the control. This means that effect of cancer cells and their supernatants on macrophages can induce apoptosis on macrophages and thus prevent proper course of immune responses.

Key Words: prostate, cancer, supernatant, macrophage, apoptosis

INTRODUCTION

Prostate gland is often affected by cancer. Carcinoma of this gland is the most common oncological disease in developed countries. The incidence of this disease has increased by 300% compared to 1995. According to information from National oncological register, on 100.000 healthy men belong 131 cases of carcinoma of prostate gland. This is an intensive increase because of ageing of population and preventive medical examination (Zvolský 2014). In the most cases, they are punished men older than 65 years (Král 2014).

Prostate cancer is a very major sociable problem. Nowadays, many experiments are aimed on research and development of a new diagnostical approaches. Particular types of cell death, for example apoptosis, play important role, because of understanding of treatment impact on cancer cells. Apoptosis is a word of a Greek origin meaning dropping off and refers to the leaves falling from the trees in autumn. Apoptosis was first described by Kerr et al. in 1970 (Wong 2011). It is an intrinsic cell-suicide programme which guarantee tissue homeostasis, elimination of unnecessary or unwanted cells and cells which may represent some form of danger for the organism as a cancer cell (Cairrão, Domingos 2010). Cells which succumb to apoptosis exhibit specific morphological and biochemical changes. Cell shrinkage, nuclear condensation and fragmentation calls karyopyknosis, dynamic membrane blebbing -zeiosis- and loss of adhesion to neighbours or to extracellular matrix (Ouyang et al. 2012). Finally, cell is divided into apoptotic bodies (cell lysis), which are phagocytosed by neighbouring cells (Cairrão, Domingos 2010).

Many researches are focused on studying of influence between cancer cell and some treatment, for example cytostatics, chemotherapy, drugs. Whereas only a few articles published study about interference cancer treatment – healthy cells of immune system. In this study we aimed on a topic how supernatant from prostate cancer cell line effects healthy cells of immune system, macrophages concretely. It is also very notable to know about behaviour of crucial part of human body involved in a successful treatment.

MATERIAL AND METHODS

Human monocytes, as part of PBMC (Peripheral Blood Mononuclear Cell) were isolated from 40 ml men blood by Histopaque 1077 (Sigma-Aldrich) protocole. Blood was diluted in a ratio 1:1 by PBS (Dulbecco's Phosphate Buffered Saline, without Ca and Mg, Lonza Verviers SPRL), centrifugated (speed 2000 rpm, time 40 min, brake 0, temp. 24 – 40°C). Then PBMC were obtained by aspiration from the respective ring of density gradient from Histopaque 1077, centrifugated with PBS (speed 1500 rpm, time 10 min, brake 9, temp. 21 – 29°C). Cells in pellet were suspended in RPMI and seeded into Multi well Culture Plate in concentration 10⁶/well. Cultivation was carried out for 7 days with GM-CSF (Recombinant Human Granulocyte Macrophage Colony-Stimulating Factor, Animal Origin Free, Gibco by Life Technologies) in concentration 5 µl GM-CSF/1 ml cell culture medium RPMI 1640 with L-glutamin (Lonza Verviers SPRL) in 5% CO₂ at 37°C. On day 7, macrophages were stimulated by LPS (from E. Coli, Sigma-Aldrich) in concentration 20 µl LPS/1 µl cell culture medium and with PC-3 prostate cancer cell line supernatant. Supernatant was obtained 5 days after starting of cultivation of cancer cells. Macrophages cultured without supernatant were used as a control. After 24 hours macrophages were harvested mechanically by repeated washes in medium RPMI 1640.

Preparations on glass slides were prepared by smearing of harvested macrophages in medium on the slide and coloured by the hematoxylin-eosin method. Capture of occurrence of characteristic features of cell death was provided by light microscope Olympus BH2. Photographs were taken by camera Canon 1100 d. Immersion oil was used for observation, magnification 1000x. In every samples representative selective set of cells was analysed. For each measurement one hundred of cells were collected and divided into particular categories. From each category percentage of occurrence was analysed. Study Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics by Kerr, Wyllie and Currie 1972 was default for sorting of cells into particular categories (Kerr et al. 1972). Statistics and *p* values were computed by software Statistica in *t* test. It was carried out comparasion of percentage occurrence of particular type of cell death between kind of treatment. Statistical significance was declared when *p* value was equal to or less than 0.05.

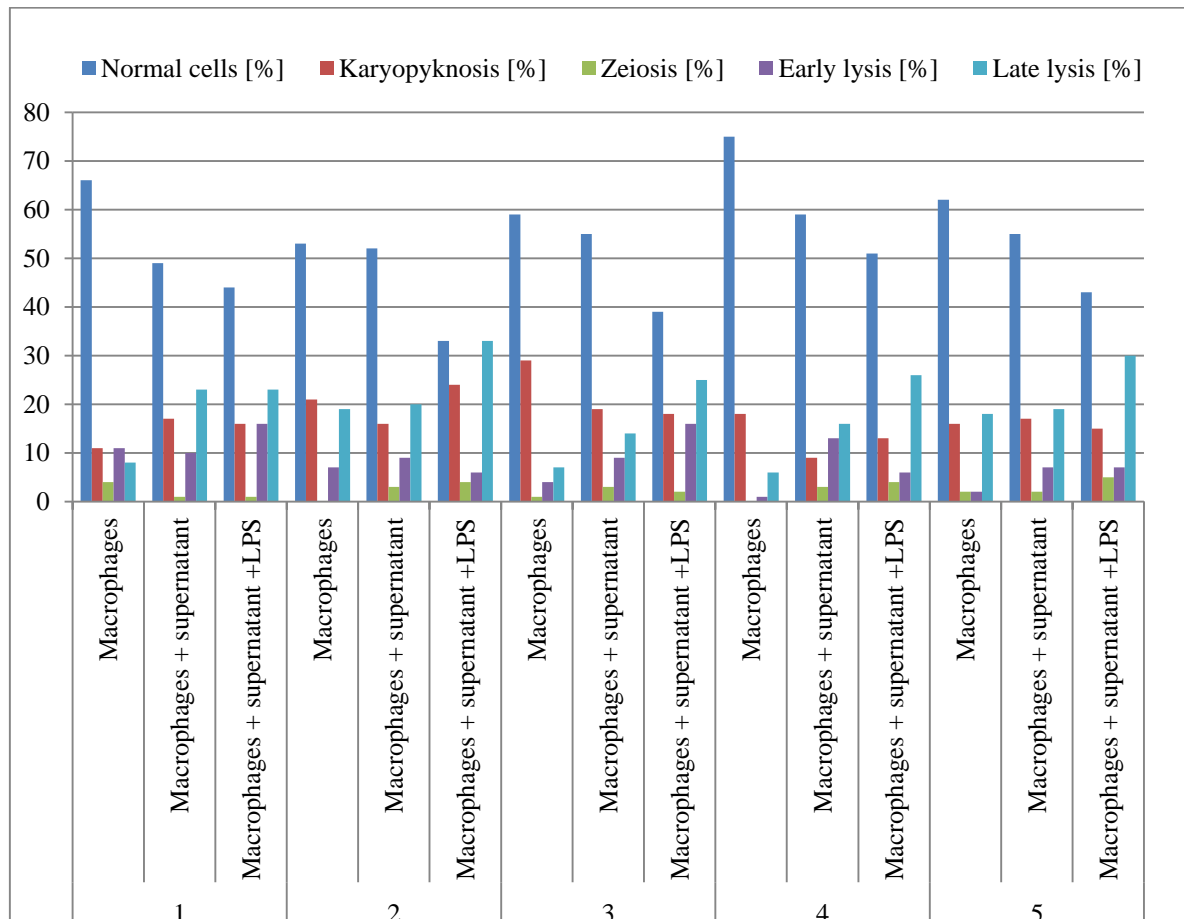
RESULTS AND DISCUSSION

We chose the 24 hours experiment because of sufficient time for incubation of macrophages with the supernatant and for complete appearance of morphological changes associated with apoptosis (Su et al. 2015). Process from the initiation of cell death to the final cellular fragmentation usually takes several hours (Wong 2011). Our samples were analysed by light microscopy. We elected light microscopy although more sophisticated methods are available. This approach is available and thus widely used, which is documented by other authors. Light microscopy can identify the various morphological changes that occur during apoptosis (Elmore 2007). Light microscopy still represents an appropriate approach for identifying apoptotic cells (Bottone et al. 2013). This is a preliminary study. Therefore, it was used 5 samples for experiment.

As it is obvious from results in Figure 1, macrophages with normal morphology show the largest rate in our experiment. However, representation in particular types of treatment is different. In control, where were only macrophages without supernatant and also without LPS, more than 50% of normal macrophages were observed. This occurrence was expected. There is not reason for excessive apoptosis, because of good live conditions for macrophages grow. It is very positive fact because of impact on inner immunity system and we checked that our methodology is right. In other samples, appearance of normal morphology was lower. On the other side, incidence of particular types of apoptosis rises. In these cases influence of treatment was manifested. In samples macrophages + supernatant there was an increase mainly in late stage of apoptosis. A lot of observed cells showed feature of rupture and extrusion of cell contents into the microenvironment. This is due to the action of the supernatant. Cancer cells and their metabolites affect the behaviour of macrophages. For example, Sánchez-Reyes et al. confirmed in publication changing of immunophenotype of macrophages from M1 to M2 only by action of soluble factors secreted by cells of cervical cancer (Sánchez-Reyes et al. 2014). The second published article presents similar results. Caras et al. used supernatant from colorectal and laryngeal cancer samples and realised similar experiment. Their results support the hypothesis that supernatant from cancer cells can modulate functional polarization of macrophages (Caras et al. 2010). For our experiment

we chose cancer cell line PC-3. This cell line is derived from the 4st degree of adenocarcinoma of prostate cancer in human. PC-3 has a great potential to form metastases in the body and we can observe the strongest affecting of healthy cells. Data of occurrence of particular types of apoptosis in macrophages after treatment of supernatant from cell line PC-3 with or without LPS stimulation are not at disposal. This study is the first, which published results of this cell type in similar design of experiment. It is possible that soluble factors from cancer cells cause or encourage the development of apoptosis in macrophages. LPS influences proinflammatory gene expression in macrophages (Aung et al. 2006).

Figure 1 Occurrence of particular types of apoptosis.



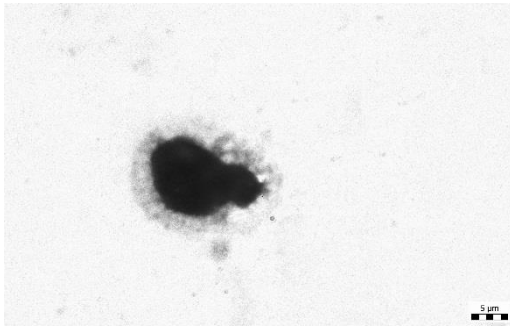
Macrophages have an ability to recognize pathogens from the outside environment via pathogen-associated molecular patterns, such as LPS. LPS represents an important endotoxin to which the body responds to strong immune responses (Blagih, Jones 2012). In our experiment, LPS in combination with the supernatant still increases the incidence of apoptosis in macrophages. This phenomenon can be explained by the action of several stress conditions to macrophages. LPS was added to macrophages 24 hours before the addition of supernatant to activate proinflammatory profile M1 macrophages. Therefore it was expected to increase the proportion of normal morphology. But the results show the opposite trend. In samples macrophages + supernatant + LPS there is a noticeable decline in the incidence of normal morphology compared to the control. This means that effect of cancer cells and their supernatants on macrophages plays really important role. Analysis of the expression profile and secretion of anti or inflammatory cytokines macrophages after contact with the cancer supernatant and after activation by LPS is the subject of our further research. This will bring better understanding of this topic at the molecular level. Statistical significance differences were observed in stage of cell death late lysis between treatment macrophages × macrophages + supernatant + LPS (p value = 0.0015), macrophages + supernatant × macrophages + supernatant + LPS (p value = 0.0055).

Figure 2 represents particular type of cell morphology during apoptotic changes. Karyopyknosis is characterised by shrinking of apoptotic cell. Zeiosis is characterised by blebbing of cytoplasm. During

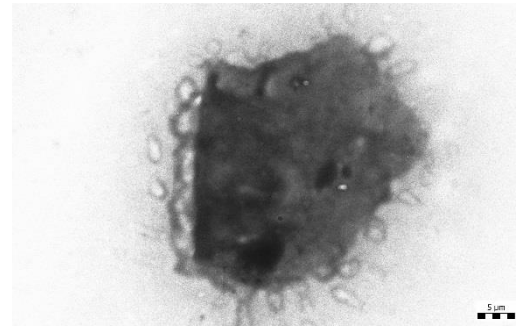
early lysis cell structure is distinguishable, biomembrane is ruptured, there is a tendency to spillage of cell content. Typical features for late lysis are remains of the nucleus and cytoplasm is completely spilling. Figures obtained in our experiment are similar to those in the publication Elmore (2007).

Figure 2 Particular types of changes in cell morphology during apoptosis.

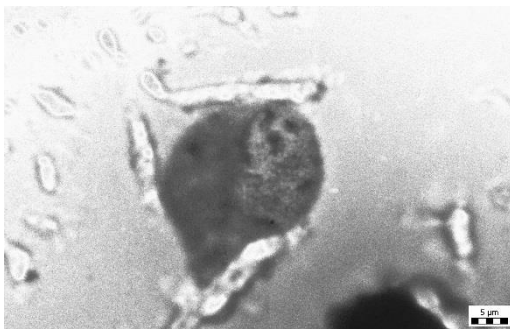
A) Karyopyknosis



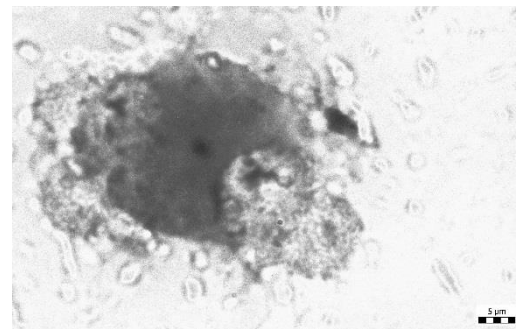
B) Zeiosis



C) Early lysis



D) Late lysis



CONCLUSION

Cultivation of macrophages with supernatant from prostate cancer cell line PC-3 indicated that macrophages succumb to apoptosis in greater extent. Supernatant in association with treatment by LPS induces increase of the lysis of macrophages. It can be said that macrophages undergo to apoptosis when they are exposed to supernatant and also activation of macrophages by LPS does not descent occurrence of apoptosis. Macrophages than can not engulf the apoptotic, unnecessary or unwanted cells, especially cancer cells, and it can lead to outbreak of cancer proliferation and formation of metastasis.

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