Occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin

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Abstract: The aim of this study, which is focused on occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin is get information about distribution of normal, apoptotic and autophagic cells by exposure to plumbagin. Plumbagin is one of the simplest plant secondary metabolite of three major phylogenic families - Plumbaginaceae, Droseraceae and Ebenceae. Plumbagin assigns highly potent biological activities, for example antioxidant, antiinflammatory, anticancer, antibacterial and antifungal activities. Cancer cell line PC-3 is derived from the 4st degree of adenocarcinoma of prostate. The type of cell death is important for developing of new diagnostical approaches.
In this study types of cell death were studied by using experiment which take 48 hours. During this time cells were exposed to plumbagin, which cause one of type of cell death. Action of this metabolite was finished after certain hour depending on the number of sample and after that samples were analysed by light microscope.
Proportion of type of cell death was following. The highest percentage of apoptotic bodies (46%) was observed 6 hours after start of experiment. Apoptotic cells were in the largest frequency 2 hours after start of experiment (10%). Most of residual cells moved to autophagy 48 hours after start of experiment (20%).
To conclusion, treatment of cancer cell line PC-3 by plumbagin showed that this metabolite can cause cell death of cancer cells. This information is important for further development of treatment of cancer diseases.

Key-Words: apoptosis, necrosis, autophagy, cancer, cell, PC-3, plumbagin

Introduction

Carcinoma of prostate gland is the most expanded oncological disease in developed countries. Incidence of this type of cancer is higher about 300% from 1995. On 100 000 men come on 131 case of incidence prostate cancer according to information from National oncological registr. This intensive increase is cause by ageing of population and preventive medical examination, respectively [1]. More than 65% of all causes prostate cancer were determined in patients, who were older than 65 years [2].

Prostate cancer represent very important social problem. This fact is confirmed by experiments, which are targeted on development of new diagnostical approaches. These can help reveal cancer proliferation exactly and efficiently and punctual treatment can be begin. In this time research in cancer diseases is concentrated on process, which are in association with occurrence of some type of cell death, for example apoptosis, necrosis and autophagy.

Apoptosis, also called programmed cell death, is main mechanism, which helps in physiological elimination of cells from organism. This cell death occurs naturally during development and ageing of organism as homeostatic mechanism and for keeping population of cells in tissues. In consequence of damage by diseases or by noxious influences to apoptosis can occur also during imunological reaction as defence mechanism [3]. Apoptotic process is characterised by specific changes in cells structure – shrinking of cell, condensation of cytoplasm and chromatin in nucleus (karyopyknosis),

bubbling cytoplasmatic membrane (zeiosis), division of apototic cell on apoptotic bodies. Apoptotic bodies are recognised and eliminated by phagocytosis. It is Necrosis is passive form of cell death. It is finally resulting of bioenergetic catastrophe, which follows from depletion of ATP on level incompatible with cell survival. Necrotic process occur in typical sequence – fusion of small membrane bubbles into one big bubble, which is separated from rest of cell substrate [5]. Morphologically necrosis is characterised by production of vacuoles in cytoplasm, falling down of plasmatic membrane and initiating of inflamation around dying cell. Cells which are dying by necrosis exhibit changes in morphology of nucleus too, but not fragmentation and condensation as in apoptosis [4].

Autophagy is catabolic process targeted on cell organels and cytoplasmatic components, which are determined for degradation in lysosome. Autophagy proceeds selectively and is concentrated on liquidation of old and useless structures in cell primarily. It provides recyclation of cell components and participates in maintenance of cell homeostasis and cell integrity [6]. Level of autophagic activity is arranged as response on various intracellular and extracellular stimulus, for example ongoing disease, starvation, lack of oxygen and other form of stress [7].

Plumbagin belongs to group of naphthoquinones. It is organic compound, which is extracted as derivate from root of plant *Plumbago zeylanica*. Coloured pigments are the most frequent forms, which are occured in some kind of bacteria, fungus and higher plants. Naphthoquinones have pharmacological effects, high toxicity, antibacterial, antifungal, antiviral and antiparasitic traits. In tissue and cellular cultures anticancer and antiproliferative effects were observed [8].

The aim of this study, which is focused on occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin is get information about distribution of normal, apoptotic and autophagic cells by exposure to plumbagin.

Materials and Methods

This study was treated on cellular cultures of line PC-3. This cancer line is derived from the 4st degree of adenocarcinoma of prostate, androgen independent (HPA Culture Collections, Salisbury, UK).

Cultivation of cell lines

Cultivation proceeded in cultural, sterile bottles at 37 °C and concentration of CO_2 was 5%. Medium for PC-3 is composed from Ham's F12 medium with 7% FBS and antibiotic.

important for absence of inflammation around dying cell [4].

Passage of cells

1. Extraction of old medium from cultural vessel, where are accumulated cells.
2. Washing by EDTA (2 ml).
3. Extraction of EDTA.
4. Washing by trypsin (2 ml, 1x) – time of reaction 30 seconds.
5. Extraction of trypsin.
6. For 3 minutes put cultural vessel with cells into CO_2 thermobox.
7. Washing away deadherent cells into medium.
8. Cell suspension put into centrifugal tube and rotate for 7 min, 2700 revolution, at 4°C.
9. Extraction of supernatant and resuspending of cells in medium.
10. Sowing of cells into new cultural vessel with fresh medium.

Changing of medium:

1. Extraction of old medium from cultural vessel.
2. Washing by rinsing medium (medium + ATB, 2 ml).
3. Extraction of rinsing medium.
4. Putting of fresh medium.

Application and effect of cytostatic in growth medium:

Cytostaticum plumbagin was applicated into growth medium with prostatic cell culture in concentration, which correspondent with IC50 – 2uM plumbagin. Cultivation was interrupted at expiration of time interval and morphological changes were analysed. Duration of action of cytostatic was following: 1, 2, 3, 5, 6, 7, 9, 9.5, 10, 11, 12.5, 13, 27, 48 hours.

Evaluation of results:

Capture of occurence of characteristic features of cell death was provided by inverse light microscope Olympus IX71. Photographs were taken by camera Nikon D80.

In every time interval representative selective set of cells was analysed. For each measurement one hundred of cell were collected. These cells were divided to five categories by their morphology: normal cells, apoptotic bodies, apoptosis, autophagy and necrosis. From each categories percentage of occurrence was analysed. Study Death by design: apoptosis, necrosis and autophagy by Edinger and Thompson, 2004 was default for sorting of cells into particular categories [4].

Results and Discussion

Percentage of occurrence of cell death by dependency on time of plumbagin treatment is shown in Table 1.

Table 1 Occurrence of cell death [%]

Time of treatment [hours]	Normal cell [%]	Apoptosis [%]	Apoptotic bodies [%]	Autophagy [%]	Necrosis [%]
0	100	0	0	0	0
1	68	8	22	2	0
2	58	10	26	6	0
3	68	4	19	9	0
5	75	3	14	8	0
6	41	5	46	7	1
7	76	3	16	5	0
9	68	2	27	2	1
9.5	70	5	18	6	1
10	66	6	25	3	0
11	67	7	24	2	0
12.5	75	4	17	3	1
13	58	7	33	1	1
27	74	2	20	4	0
48	54	3	23	20	0

As it is apparent from these results, cells with normal morphology achieved the greatest occurrence. Treatment of plumbagin wasn't expressed by changes in morphological structure and evidences of upcoming cell death weren't elicited.

Nevertheless, percentage of occurrence of normal cells declined by 30%. One of the lower worth of unchanged cells was registered 48 hours after start of experiment. This effect is caused mainly by increase of autophagy. Cells succumbed to stress (depletion of growth medium, less of space) and process of delaying cell death-autophagy-was choosen.

Desintegration of apoptotic cell into apoptotic bodies was the most numerous morphological changes. The largest occurrence was observed 6 hours after beginning of experiment. It is in association with fact that apoptotic cells were the most plentiful 2 hours after start of testing. Time intervals needful for causing of morphological changes, from shrinking of cytoplasm in apoptotic cell to disruption into apoptotic bodies, were in correlation absolutely. In remaining measurements apoptosis was about 5% and incidence of apoptotic bodies was about 20-25%. In consequence, increased proportion of apoptotic bodies is caused by identification because one apoptotic cell is broken down into several apoptotic bodies. Apoptotic cell is only one, while apoptotic bodies emerge from it much more. Therefore, number of apoptotic bodies is larger proportionally.

Occurrence of autophagy fluctuate during whole experiment. Whereas in initial measurement proportion of autophagy cells was increased, by end proportion was decreased. After 48 hours stressful factors, which affected cells were emerged and autophagy was achieved maximum – 20%. In remaining time interval 5% occurrence of autophagy wasn't overlaped. It was caused mainly by fact, that cell tried to gain delaying from cell death by autophagy. After certain time, depletion of energy supply occurs and necrosis begins.

Traits of necrosis were observed in the making of experiment rarely. This fact can be explained by proportion of autophagy because it was relatively

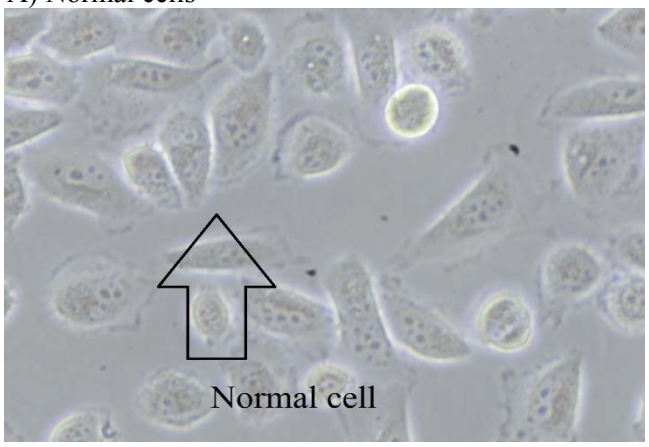
minor and identification of necrotic cell among one hundred other cells is problematic. Moreover, it may be caused by disruption of cell in time between measurements probably and this cell became unrecognizable.

Data of occurrence of cell death in cancer cell line PC-3 after some treatments are not at disposal. This study is the first, which published results of such experiments. But results described above are in correlation with similar experiments with using of different treatment [4].

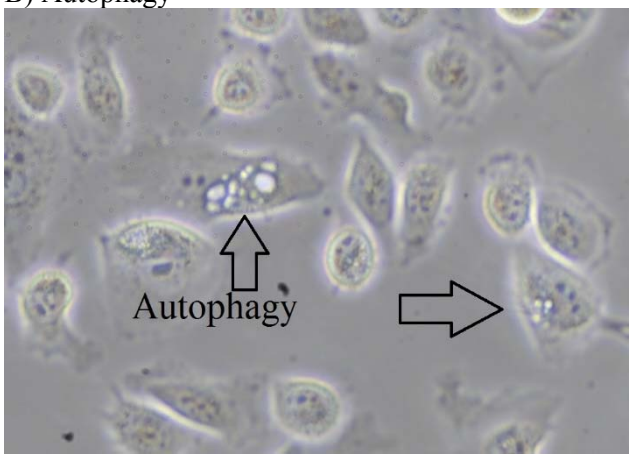
Observed types of cell death are in Figure 1.

Fig. 1 Type of cell morphology associated with cell death

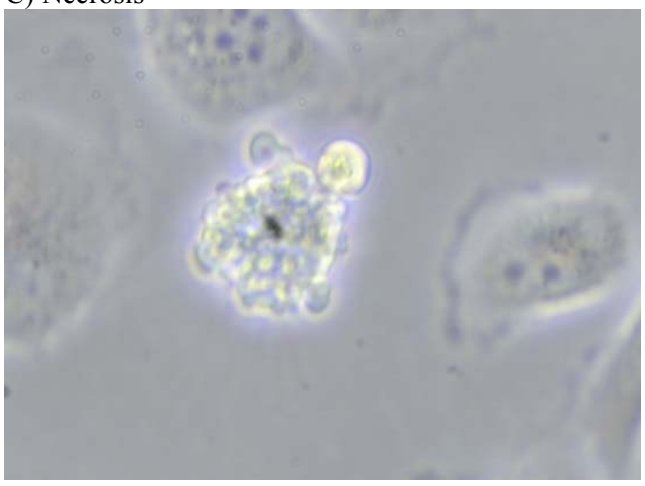
A) Normal cells



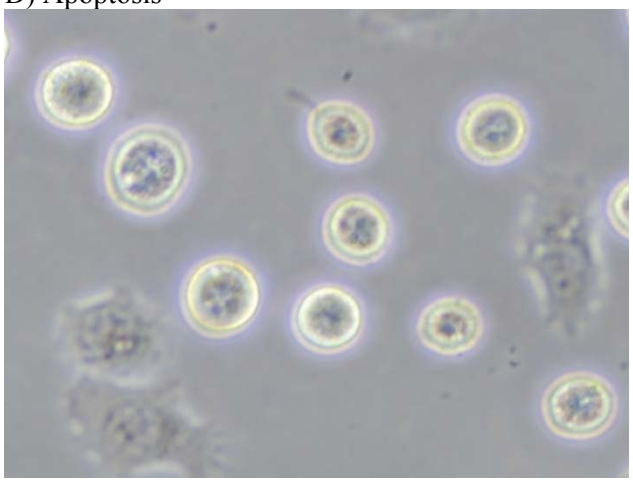
B) Autophagy



C) Necrosis



D) Apoptosis



Conclusion

Treatment of plumbagin on prostate cancer cell line PC-3 indicated that initiating of certain type of cell death was successful. Apoptosis with follow up disruption into apoptotic bodies was mainly observed. This detection confirms importance of using of cytostatics during treatment of cancer diseases.

Current approaches on active service against cancer are concentrated on induction of cell death. By this proliferation and migration of cancer cells would be terminated. It is very important to engage in this question in next study.

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