Study of the effect of natural polyphenols on the prostate cancer Du145 cells’ cycle

Relevance: Although natural polyphenols are known as useful nutrients, their use is quite occasional. This is probably due to a lack of complete information on their biochemical structure and mechanism of action. Scientific literature offers many studies on polyphenols’ benefits and metabolic effects on the body. Polyphenols are known to improve body function and increase physiological activity. Polyphenols are used in the body’s normal physiological function and metabolic disorders, and their therapeutic effect can be controlled in complex pathological conditions.

Therefore, in our study, we used curcumin and carnosic acid to control the natural polyphenols’ metabolic effects’ structure and function in prostate cancer. Metabolic disorders in mitochondria, one of the organelles responsible for cell energy metabolism, have an irreversible effect on the cell, organ, and the system as a whole. This urges the study of cellular action on individual organelles, including the biochemical and physiological aspects.

The study aimed to determine the effect of natural polyphenols – curcumin and carnosic acid – on the prostate cancer cell cycle.

Results: Combination of curcumin and carnosic acid caused a time-dependent exposure suppression of Du 145 cancer cells growth at G0/G1 and S phases.

Conclusions: The effect of natural polyphenols – curcumin and carnosic acid – on the Du 145 prostate cancer cell cycle was determined. Curcumin 7μM and carnosic acid 10μM in combination stopped phases G0/G1 and S’ prostate cancer cells division.

Keywords: prostate cancer, polyphenol, curcumin, carnosic acid, drug combination.

Introduction: Polyphenols are natural compounds primarily contained in fruits, vegetables, grains, and beverages. Polyphenols are the secondary metabolites of plants and are commonly involved in protection from ultraviolet rays or pathogen aggression. The polyphenols in food impart a distinctive color and bitter taste.

The studies in humans and animals have shown that polyphenols have anti-inflammatory and antioxidant effects in cardiovascular disease, neurodegenerative disorders, cancer, and obesity. In recent years, a comprehensive study of natural polyphenols is carried out at the cellular level in connection with their biological action, low toxicity, as well as their beneficial effect on human health [1, 2].

Another benefit of polyphenols is due to their bioavailability. These nutrients are easily digested and possess normal metabolic properties.

Bioavailability is the main indicator for polyphenols’ intake. However, this does not refer to all polyphenols since their metabolic properties are different and mostly understudied at the cellular level. During the study of these substances, the attention is often devoted to the rate of absorption and circulation of metabolites in plasma, rather than their chemical structure or concentration [3, 4]. Another specific feature is the variety of polyphenols due to their biological properties. An antioxidant capacity increase after consuming polyphenol-rich food indicates that rapid intestinal absorption also depends on the absorption properties [5].

The epidemiological scientific studies on polyphenols have shown that these natural products have anti-inflammatory and antioxidant effects in preventing many diseases. For example, in experiments performed on mice with colorectal cancer, it was observed that when curcumin was injected into the abdominal cavity, the tumor growth has stopped at certain levels [6]. It was also studied the direct effect of curcumin on mitochondrial RNA. Considering that mitochondria are one of the main cell organelles, we know that their structure and functions are poorly studied. Among other relevant topics, we aimed to study the influence of polyphenols such as carnosic acid and curcumin on the processes at the cellular and mitochondrial levels.

Anatomically, the prostate is a glandular organ located at the top of the urethra. At 11-12 weeks of embryonic development, urine turns into the mesenchymal urethral caruncles. The external structure is composed of thin connective tissue, while the parenchyma is composed of mucous glands. Depending on the hormone testosterone, this gland also plays its role in the sperm diffusion.

The number and pathogenesis of diseases caused by inflammation of this gland with complex anatomical structure and function are also complexed. The cancer of interest occurs in every 10th man [7, 8].

It is one of the dynamically developing diseases based on the rapid development of scientific and technological progress, automation of all installations and devices, ecological situation, stress, genetic factors, etc. Etiologically, it is characterized by rapid gland growth vs. the normal status. According to the incidence and age characteristics statistical data, it can be seen that the incidence of prostate cancer or prostate tumor is higher in men over 50 years old [9, 10]. Early prostate cancer has no symptoms. Only the pain in the urethra or clear signs of difficulty urinating can
urge a biopsy examination and a comprehensive analysis to diagnose prostate cancer.

We studied the Du145 prostate cancer cell, one of the most morphologically normal, fast-growing, easy-to-grow cancer cells, resistant in experiments, that quickly metastasize to certain parts of the human body. The cell line was taken from a special cell pool (Manassas, USA) [11].

Materials and Methods: We studied the Du145 prostate cancer cell that began to metastasize to the brain and bone tissue. The cancer cells were grown and prepared in 10% inactivated calf serum (RAA, Pasching, Austria), 1% glutamine solution, 1% sodium pyruvate solution (100 mM), and a special admixture RPMI 1640 (Lonza) in the special incubator at 37°C with 5% CO2. During the experiment, relevant antibiotics were applied to the cells to prevent contamination.

Results: Our study examined the change after 48 hours to track the effect of the dual action of curcumin and carnosic acid on the cell cycle. The dual impact of curcumin and carnosic acid on cell cycle changes was controlled by flow cytometry.

Figure 1 shows our study results: the one prostate cancer cell line obtained as a control did not contain polyphenols Du145. In the second line, the cell received a mixture of curcumin 7 μM and carnosic acid 10 μM. In general, each cell has its own cycle. During the cell growth phase (inter-phase), that is, during the synthesis of DNA and proteins, the process of preparation for cell division is taking place. From the comparison point of view, that process is short for cancer cells.

Figure 1A demonstrates the cell cycle image of Du145 cancer cell in the clean version taken as an observation. Figure 1B shows the image in the flow cytometry structure treated with 7 μM curcumin and 10 μM carnosic acid. Figure 1 shows that while control cells show the cell cycle movement following the mitosis laws (Figure 1A), polyphenol mixture inhibits cell division (Figure 1B).

Polyphenol-containing cells had a greater decelerating effect on the S-cycle compared to the control cells. We observed that the cycle continued till the G2/M phase of Du145 cancer cells grown for 48 hours without adding polyphenols.

50% of undifferentiated cells in the control group were enough to interrupt in the G0/G1 phase the main cell population growth exposed to 7 μM curcumin and 10 μM carnosic acid. The growth stopped at the G0/G1 phase despite the cells' transition to the next cell cycle phase when exposed to the polyphenols mixture.

Many plant-based polyphenols can inhibit the growth of cancer cell cycles. However, this does not work the same way in all cancer cells. For instance, some studies have shown that resveratrol inhibits the growth cycle of U937 lymphoma and HL60 leukemic promyelocytes in the S-phase. Also, there are some data that the grape seed extract has stopped the rapid growth of prostate cancer in mice. Furthermore, the quercetin had a similar effect on prostate cancer cells PC3 as curcumin and carnosic acid on the Du145 cancer cells — polyphenols exposure stopped the cancer cells' growth cycle in the G0/S phase.

Conclusion: In our experiments, the growth of one of the most complex cancer cell lines, prostate cancer Du145 cells, was interrupted in G0/G1 and S phases over time when exposed to a polyphenols mixture, including curcumin and carnosic acid. These findings demonstrate the first experience of studying a polyphenols mixture's inhibitory effect, which gives new scientific prospects.

References: