UDC: 616-006-65-008.9+577.1 DOI: 10.52532/2663-4864-2020-4-58-44-46

***S.O. OSSIKBAYEVA*[1], *B.G. YESZHAN*[2]**

[1]JSC «Kazakh Research Institute of Oncology and Radiology», Almaty, the Republic of Kazakhstan;
[2]Kazakh Academy of Sports and Tourism, Almaty, the Republic of Kazakhstan

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 Du145 cancer cells growth at $G_{0/1}$ and S phases.*

Conclusions: *The effect of natural polyphenols – curcumin and carnosic acid – on the Du145 prostate cancer cell cycle was determined. Curcumin 7μM and carnosic acid 10μM in combination stopped phases $G_{0/1}$ 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 colon 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 diffluence.

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.

Our study revealed that double polyphenolic compounds interrupt the cell cycle. The interaction of 7 μM curcumin and 10 μM carnosic acid inhibited a certain cell cycle phase.

The overall cell cycle is known to include four specific phases or stages, such as the G_1-growth period, DNA S-doubling, G_2-growth, and M-mitotic division. One of the main cell cycle phases of the G_1-phase is the predominant stage of synthesis of other cellular structures and mRNA. The differentiated cells stop their mitosis and are at a quiescent state, i.e., in the G_0-phase. The G_2-stage is the final stage of preparation of the cells for mitosis.

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 (interphase), 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.

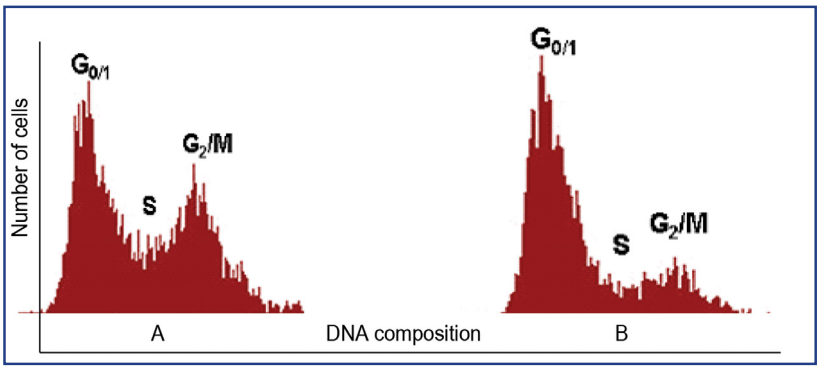

Figure 1 - Comparative analysis after the DU145 cell exposure to polyphenol

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 G_2/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 $G_{0/1}$ phase the main cell population growth exposed to 7 μM of curcumin and 10 μM of carnazolic acid. The growth stopped at the $G_{0/1}$ 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 G_1/S phase.

Conclusion: In our experiments, the growth of one of the most complex cancer cell lines, prostate cancer Du145 cells, was interrupted in $G_{0/1}$ 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:

1. Aruoma O.I., Halliwell B., Aeschbach R., Löligers J. Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid // Xenobiotica. *– 1992. – №22. – P. 257–268;*

2. Yesil-Celiktas O., Sevimli C., Bedir E., Vardar-Sukan F. Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines // Plant Foods Hum. Nutr. – 2010. – №65(2). – P. 158-63;

3. Zinov'yeva V. N., Spasov A. Mekhanizmy protivorakovogo deystviya rastitel'nykh polifenolov I. Blokada initsiatsii kantserogeneza [Blockade of initiation of carcinogenesis] // Biomed. Chem. – 2012. – №58 (2). – P. 160-175 (in Russian);

4. Scalbert A., Manach C., Morand C., Remesy C. Dietary polyphenols and the prevention of diseases // Crit. Rev. Food Sci. Nutr. – 2005. – №45. – P. 287-306;

5. Spencer J.P., Abd E.I., Mohsen M.M., Minihane A.M., Mathers J.C. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research // Br. J. Nutr. – 2008. – №99. – P. 12-22;

6. Siegel R.L., Miller K.D., Jemal A. Cancer statistics // CA Cancer J. Clin. – 2018. – №68. – P. 7-30;

7. Khan N., Syed D.N., Ahmad N., Mukhtar H. Fisetin: A dietary antioxidant for health promotion // Antioxid. Redox Signal. – 2013. – №19. – P. 151-162;

8. Manach C., Scalbert A., Morand C., Remesy C., Jimenez L. Polyphenols: Food sources and bioavailability // Am. J. Clin. Nutr. – 2004. – №79. – P. 727-747;

9. Fraga C.G., Galleano M., Verstraeten S.V., Oteiza P.I. Basic biochemical mechanisms behind the health benefits of polyphenols // Mol. Asp. Med. – 2010. – №31. – P. 435-445;

10. Pandey K.B., Rizvi S.I. Plant polyphenols as dietary antioxidants in human health and disease // Oxid. Med. Cell. Longev. – 2009. – №2. – P. 270-278;

11. Hu M. Commentary: Bioavailability of flavonoids and polyphenols: Call to arms // Mol. Pharm. – 2007. – №4. – P. 803-806.

ТҰЖЫРЫМ

С.О. Осикбаева[1], Б.Ғ. Есжан[2]

[1]«Қазақ онкология және радиология ғылыми-зерттеу институты» АҚ, Алматы, Қазақстан Республикасы;
[2]Қазақ спорт және туризм академиясы, Алматы, Қазақстан Республикасы

Қуық асты безі ісігінің Du145 клетка циклына табиғи полифенолдардың әсерін зерттеу

Өзектілігі: *Табиғи полифенолдардың тағамдық зат ретінде пайдалы екенін білсек те қолдануға көп жағдайда шешім қабылдай бермейміз. Себебі полифенолдардың толыққанды биохимиялық құрылымы мен әсер ету механизмі туралы ақпараттың аздығынан да болса керек. Дегенмен де ғылыми әдебиеттерде полифенолдардың пайдасы мен ағзадағы метаболизмдік әсері жайында жазылып жатқан және жүргізіліп жатқан зерттеулерде көп. Сол зерттеулердің нәтижелерінен көріп, бақылағанымыздай полифенолдардың әсерінің ағза жұмысын жақсартып, физиологиялық тұрғыдан белсенділігін арттыратыны туралы да ақпараттар да бар. Полифенолдардың ағзаның қалыпты физиологиялық қызметіне ғана емес, зат алмасу үдерістерінің бұзылысы кезінде қолданылатынын ескере отырып күрделі патологиялық жағдайларда емдік әсерін бақылауға болады. Сондықтан да, ғылыми-тәжірибелік зерттеулерімізде қуық асты безінің қатерлі ісігінде табиғи полифенолдардың құрылымы мен қызметі жағынан метаболизмдік әсерін бақылау мақсатында куркумин және карназол қышқылын алып қолданып отырмыз. Жасушаның энергетикалық алмасуына жауапты органоидттардың бірі митохондриядағы алмасу процестерінің бұзылысы жасушаның, мүшенің және жалпы жүйенің патологиялық қайтымсыз үдерісіне алып келеді. Сондықтан зерттеулерді жасушалық, оның ішінде жекелей органоидтардағы биохимиялық, физиологиялық тұрғыдан зерттеу маңызды болмақ.*

Зерттеудің мақсаты: *Табиғи полифенолдардың, оның ішінде куркумин және карназол қышқылының қуық асты без ісігі жасушаларының циклына әсерін айқындап, көрсету болып отыр.*

Нәтижелері: *Куркумин және карназол қышқылының қоспа (комбинация) түріндегі мөлшері уақыт аралығына сәйкес Du145 қуық асты без ісігі жасушасының арнайы бөліну фазаларына сәйкес жасуша өсуін $G_{0/1}$ және S тежеді.*

Қорытынды: *Қуық асты без ісігі Du145 жасушасының циклына табиғи полифенолдардың (куркумин және карназол қышқылы) әсері айқындалды. Куркумин 7мкМ, карназол қышқылының 10мкМ қоспа түрінде қуық асты без ісігі жасушасының бөлінуін $G_{0/1}$ және S фазаларына сәйкес тоқтатқанын ағынды цитометрия құрылғысы негізінде анықталып зерттелді.*

Түйінді сөздер: *Қуық асты без ісігі, полифенол, куркумин, карназол қышқылы, комбинация.*

АННОТАЦИЯ

С.О. Осикбаева[1], Б.Г. Есжан[2]

[1]АО «Казахский научно-исследовательский институт онкологии и радиологии», Алматы, Республика Казахстан;
[2]Казахская академия спорта и туризма, Алматы, Республика Казахстан

Исследование влияния природных полифенолов на клеточный цикл рака простаты Du145

Актуальность: *Зная, что природные полифенолы полезны как питательные вещества, мы часто не решаем их использовать. Вероятно, это связано с отсутствием информации о полной биохимической структуре и механизме действия полифенолов. Однако в научной литературе есть много исследований о пользе полифенолов и их метаболическом влиянии на организм. Как известно из результатов исследований, действие полифенолов улучшает функции организма и увеличивает его физиологическую активность. Учитывая, что полифенолы используются не только для нормального физиологического функционирования организма, но и при нарушениях обменных процессов, терапевтический эффект можно контролировать в сложных патологических состояниях.*

Поэтому в своем исследовании мы используем куркумин и карназоловую кислоту для контроля метаболических эффектов природных полифенолов с точки зрения структуры и функции при раке простаты. Нарушение обменных процессов в митохондриях, одной из органелл, отвечающих за энергетический обмен клеток, приводит к патологическим необратимым процессам в клетке, органе и системе в целом. Поэтому важно изучать клеточные механизмы воздействия на отдельные органеллы, в том числе с биохимической и физиологической точек зрения.

Цель исследования: *определить влияние природных полифенолов куркумина и карназоловой кислоты на клеточный цикл клеток рака простаты.*

Результаты: *Куркумин и карназоловая кислота в комбинации подавляют рост раковых клеток Du145 на фазе $G_{0/1}$ и S в зависимости от времени воздействия.*

Выводы: *Определено влияние природных полифенолов (куркумина и карназоловой кислоты) на цикл клеток рака простаты Du145. Куркумина в концентрации 7мкМ и карназоловая кислота 10 мкМ в комбинации останавливали деление клеток рака простаты в соответствии с фазами $G_{0/1}$ и S.*

Ключевые слова: *рак предстательной железы, полифенол, куркумин, карназоловая кислота, комбинация препаратов.*