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ORIGINAL PAPER

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Lycopene activity on lung and kidney cancer cells by T_2 relaxation time ^{1}H Magnetic Resonance Imaging *in vitro*

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ABSTRACT

Introduction. The paper presents the results of a study of cell cultures of lung cancer and kidney cancer using lycopene performed using clinical magnetic resonance imaging.

Aim. The aim of the study was to evaluate lycopene activity on tumor cell cultures.

Material and methods. For this purpose, MR tests were performed using the technique of determining transverse relaxation.

Results. Described here studies demonstrated that lycopene may inhibit the growth of A549 and ACHN cell lines.

Conclusion. We determine changes in spin lattice relaxavity T_2 to monitor treatment of lung cancer cell line A549 and kidney cancer cell line ACHN cells treatment with lycopene.

Keywords. cell cultures, lung cancer, lycopene, kidney cancer, magnetic resonance imaging, relaxation times

Introduction

Lycopene is an antioxidant from the carotene group, containing unsaturated hydrocarbon. It has many health-promoting properties, primarily it helps in inhibiting the development of tumors, reduces the risk of heart attack and improves resistance to infection.[1] It is a carotenoid (a natural dye) in blood serum. We find it in fruits and vegetables, the most popular source are tomatoes and preserves. Studies show that the highest content is found in red extracts, but we can also find it in green tomato extracts.[2] The so-called oxidative stress in the body leads to the development of various types of diseases, including cancer, hence the need to use substances with a strong antioxidant effect. Such health-promoting properties result from the use of lycopene. It has the ability to neutralize free radicals, and also has the ability to regenerate antioxidants such as, for example, lutein. In addition, it removes free radicals from the body responsible for cancer development and reduces the risk of cancer.[3-6] Numerous scientific

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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studies present the use of lycopene in scientific studies using methods to determine relaxation times. The transverse relaxation time T2 is the time after which they reach a certain defined value. In the case of T2 it will be about 37% of the total magnetization value. There are many reports about the beneficial effects of lycopene, one of them is the work of Arab et al. on the content of antioxidants in the lining of the lung epithelium, which may have a protective effect.[7] Lian et al. in 2007, he presented the effects of apo-10'-lycopenoic acid in inhibiting the growth of bronchial cancer cells and A549 non-small cell lung cancer cells in both in vivo and in vitro studies.[8,9] Muzandu et al. presented the effect of lycopene and beta-carotene on cellular modifications that may contribute to the capture of reactive oxygen (ROS).[10] In addition, lycopene has the ability to inhibit the expression and growth of prostate, colon and lung cancer cells.11 In addition, lycopene may reduce the incidence of lung cancer.[12-13] In his clinical trials, Talwar et al. in 1997 they presented the effect of inflammation on the content of antioxidants, among others, lycopene in patients with non-small cell lung cancer (NSCLC), concentrations were much lower than in the control group.[14-16]Aizawa et al. in 2016 conducted research on the effects of smoking and alleviation of these effects with the help of lycopene supplementation, ferrets in which lung cancer was induced, among others, were examined.[17] In addition, Satia et al. in their work from 2009 they describe the long-term use of supplements, including lycopene and the assessment of lung cancer risk.[18] Huang et al. and Chow in their studies have shown that lycopene can contribute to the inhibition of tumor metastasis in in vitro studies.[19-20] While Shareck et al. showed an inverse relationship between the intake of selected carotenoids and vitamins and the risk of lung cancer.[21]

Aim

The aim of the study was to evaluate lycopene activity on tumor cell cultures.

Material and mehods

Cell culture

In the experiment we used A549 lung cancer cell line (American Type Culture Collection, VA, USA) and ACHN renal cancer cell line (American Type Culture Collection, VA, USA). Cells were cultured in standard conditions: temperature 37°C, 5% CO2 and 95% humidity. Culture medium consisted of Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, MO, USA), Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham (Sigma-Aldrich, MO, USA), Fetal Bovine Serum (Biochrom, Germany) and Penicillin-Streptomycin-Neomycin Solution Stabilized (Sigma-Aldrich, MO, USA). The culture of lung cancer cells was passaged in 3rd day with the use of Accutase Cell Detachment Solution (Corning, NY, USA) to five 70 ml Tissue Culture Flasks (ThermoFisher Scientific, MA, USA). In 7th day different doses of Lycopene ≥ 90% from tomato (Sigma-Aldrich, MO, USA). Cells were treated with lycopene (2.5 µL/mL, 5.0 µL/mL and 25 µL/mL). Two another cell culture were negative controls – one with addition of 5 µl Dimethyl sulfoxide (VWR, PA, USA) per 1 ml of culture medium and the second one without any supplements. 24 hours after supplementation with Lycopene (Sigma-Aldrich, MO, USA) cells were treated with Accutase Cell Detachment Solution (Corning, NY, USA) and washed in Phosphate Buffered Saline Dulbecco without Mg^{2+} and Ca^{2+} (Biochrom, Germany). Cells were counted with the use of Muse Cell Analyzer (Merck Millipore, MA, USA). In the next step samples were centrifuged (5 min., 250xg in room temperature) in 1,5 ml Eppendorf tubes (Eppendorf, Germany) and supernatant from samples was discarded.

ACHN renal cancer cell line was cultured also in five 70 ml Tissue Culture Flasks (ThermoFisher Scientific, MA, USA). Cells were treated with lycopene (2.5 µL/mL ,5.0 µL/mL, and 25 µL/mL). After 24 hours incubation with Lycopene (Sigma-Aldrich, MO, USA) cultures were also treated with Accutase Cell Detachment Solution (Corning, NY, USA) and cells were counted with the use of Muse Cell Analyzer (Merck Millipore, MA, USA).

MRI quantitative technique

All MR scans were performed with Optima MR360 magnetic resonance from General Electric Healthcare (Milwaukee, Wisconsin, USA). The camera was supported in the SV23 software version. The prepared samples were placed in an MR tunnel and then a series of measurements was made to determine the T2 relaxation time. The lung cancer cells in the vials were placed on the FLEX Small transceiver coil. To perform the measurements, the Fast Spin Echo (FSE) sequence was used with the following parameters: FOV field of view=10x10 [cm]; Matrix=320x224; NEX=2.0; Slice Thickness=1.0 [mm]; Spacing=0.5). TE time varied in the range of 1÷170 [ms] (1, 5, 10, 15, 20, 30, 40, 50, 70, 100, 120, 150, 170ms). TE time was 15,000 m.

Results

Measurements were made to determine the T2 relaxation time in tomatoes (*Lycopersicon esculentum L.*). Figure 1 below shows sample DICOM images.

The next stage of the study were as follow:

- measurements of T2 relaxation time in lung cancer cells A549
- measurements of T2 relaxation time in lung cancer cells A549 in DMSO

Fig. 1. Sample images of T2 FSE DICOM for TR = 12000 and TE 10, 500, 1000, 15000, 2000 ms, respectively

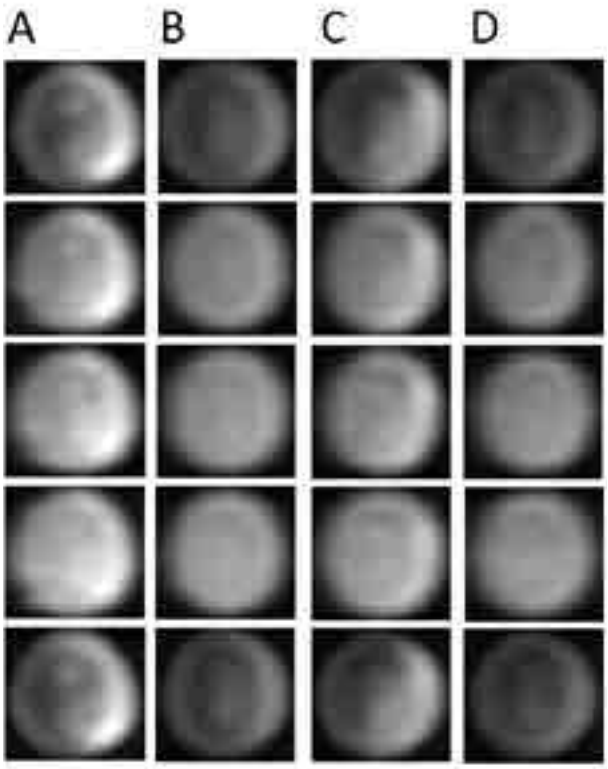

Fig. 2. Sample images from the determination of T2 relaxation times (A) control sample, lung cancer cells, (B) lung cancer cells+2.5μL Lycopene, (C) lung cancer cells+5μL Lycopene, (D) lung cancer cells+20μL Lycopene

– measurements of T2 relaxation time in lung cancer cells A549 treated with lycopene in different concentrations

Columns from AD in Fig. 2 show examples of DICOM images for various samples: (A) control sample, lung cancer cells, (B) lung cancer cells+2.5μL Lycopene, (C) lung cancer cells+5μL Lycopene, (D) lung cancer cells+20μL Lycopene. The viability of cells treated with Lycopene was 96.2%, 94%, 92 and 87%, after 24 h treatment with 0 μl, 2.5 μl, 5 μl and 20 μl of Lycopene respectively. Images were taken for the following echo and repetition times: TE = 5; 50, 100, 150, 200 ms for constant TR = 12000 ms. T2 relaxation curves for lung cancer cells and the same cells in DMSO and with lycopene at various concentrations are shown in the figure below.

Table 1. Relaxation times of lung cancer cells with the addition of lycopene and DMSO

Sample	A549	A549+2.5 μl lycopene	A549+5 μl lycopene	A549+25 μl lycopene	A549+DMSO
T_2 [ms]	77	77	76	57	142

The columns from AD in Fig. 3 show examples of DICOM images for various samples: (A) control sample, kidney cancer cells, (B) kidney cancer cells+2.5 μL Lycopene, (C) kidney cancer cells+5 μL Lycopene, (D) kidney cancer cells+20 μL Lycopene, the viability was 98, 94, 92 and 87% respectively. Images were taken for the following echo and repetition times: TE=5; 50, 100, 150, 200 ms for constant TR = 12000 ms.

Analyzing the results obtained, it can be seen that in the case of the A549 lung cancer cell line, based on the

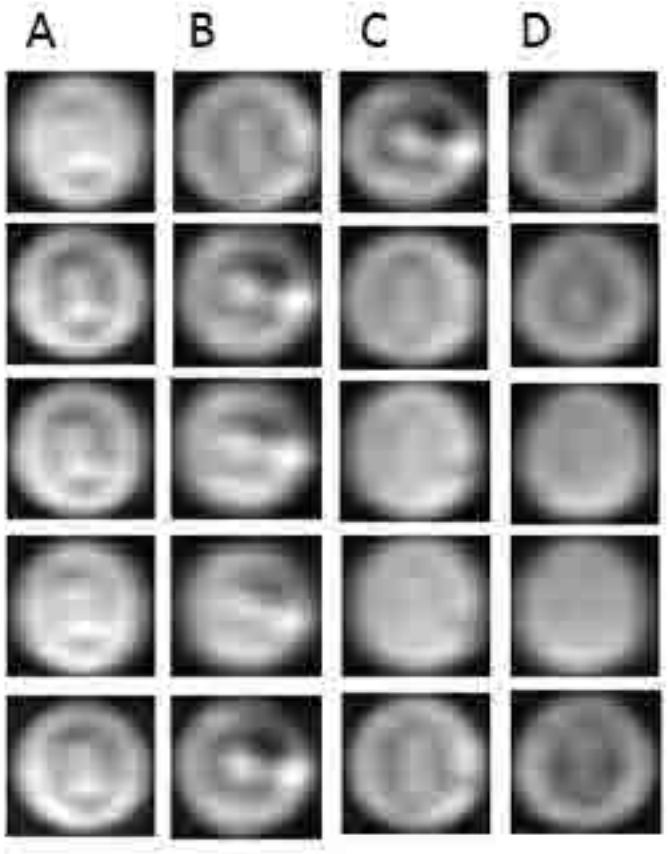

Fig. 3. Sample images from the determination of T2 relaxation times, (A) control sample, kidney cancer cells, (B) kidney cancer cells+2.5μL Lycopene, (C) kidney cancer cells+5μL Lycopene, (D) kidney cancer cells + 20μL Lycopene

results from the T2 relaxation time, we can see a downward trend as the concentration of lycopene increases. The difference between the T2 relaxation time of the control sample with pure A549 cells and the sample with 25 μl of lycopene is 20 ms.

Table 2. Relaxation times for cell cultures of kidney cancer, kidney cancer with addition of lycopene and DMSO

Sample	ACHN	ACHN+2.5 μl lycopene	ACHN+5 μl lycopene	ACHN+20 μl lycopene	ACHN+DMSO
T_2 [ms]	92	83	77	76	68

However, in the case of the ACHN kidney cancer cell line, it can be seen that the difference between the T2 relaxation time of the clean cell control sample and the sample with 20μl of lycopene is 16 ms.

Discussion

Lycopene is found mainly in tomatoes, and in smaller amounts in guava, pink grapefruit, watermelon and papaya. In tomatoes with intense red color, the average lycopene content is 5.6 mg/100g and in lighter tomatoes, available in the spring and autumn only 2.6 mg/100g. Lycopene does not dissolve in water, but in fat, which is why fats are necessary for its absorption by the body. Its bioavailability is also increased by heat treatment.

In work presented here we used lycopene (2,5 μl, 5 μl and 25 μl) to treat lung and kidney cancer cell cultures. With increasing concentrations of lycopene we observed lower values of T2 due to cell killing process. T2 values decreased much faster in lung cancer cells than kidney cancer cells. The health-promoting effect of lycopene is mainly due to its strong antioxidant properties. Lycopene is the most active antioxidant in the carotenoids group and significantly exceeds beta-carotene and alpha-tocopherol in this respect.

Conclusion

The trend in T2 relaxation time may indicate damage to cancer cell nuclei.

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