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Effect of microwave irradiation on the aminoglycoside antibiotic sensitivity of *Saccharomyces cerevisiae*

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SUMMARY

Nowadays there is an increasing interest concerning the effect of high frequency microwave irradiation on biological targets. The results and interpretations of radiofrequency irradiation effect on living cells are fairly conflicting in the literature. We examined a 2.45 GHz microwave non-thermal effect with constant temperature irradiation protocol on *Saccharomyces cerevisiae* cultures. The irradiation did not alter the viability and the growth characteristics of the yeast strain tested. Aminoglycoside antibiotics per se have no inhibitory effect on eukaryotes, as on *Saccharomyces cerevisiae*. Interestingly, when irradiation was applied to yeast culture containing the antibacterial substances, gentamicin or neomycin a concentration dependent inhibition of yeast multiplication was detected. Conclusively, microwave irradiation induced sensitivity of the yeast strain to these aminoglycoside antibiotics lacking for antifungal activity. This newly observed phenomenon might be the consequence of a transitory, reversible change in plasma membrane permeability upon irradiation and consequently due to the inhibition of protein synthesis in mitochondria of the irradiated yeast cells. Further research is to be done to clarify the exact mechanism and changes in cell.

Keywords: Microwave irradiation, cell membrane permeability, antibiotic sensitivity, *Saccharomyces cerevisiae*.

INTRODUTCION AND LITERATURE REVIEW

The increasing interest in the effect of high frequency microwave irradiation on biological targets *(Banik et al.* 2003, *Beliaev* 2005). Widespread use of devices emitting radiofre-

quency of different wavelengths and output power is becoming more common. Possible health hazard effects are of high importance, and becoming the subject of strict regulation (*ICNIRP* 1998., *EC Directive* 2004). There are conflicting results and interpretations in the literature concerning the biological effects, hazardous character and technological advances of radiofrequency irradiation. Moreover, there is a great variability regarding the level of applied frequency, intensity, duration, modulation of irradiation and the other experimental setups in different publications *(Grundler et a1.* 1977, *Kim et al.* 1985, *Geveke* and *Brunkhorst* 2003). In biological systems the primary targets of thermal and non-thermal effects of microwave irradiation are the water molecules, ionic compounds and the macromolecules of dipole character *(Banik et al.* 2003). It has also been observed that the 2.45 GHz microwave irradiation increased the uptake of p-nitrophenyl-acetate across unilamellar liposomes whose structural integrity was not affected *(Orlando et al.* 1994). Other authors have stated that antibacterial gentamicin and neomycin do not affect the eukaryotic *Saccharomyces cerevisiae* because these antibiotics are unable to penetrate the cell membrane. Noteworthily, these compounds interfere with prokaryotic protein synthesis *(Böttger et al.* 2001). As opposed to prokaryotic cells, the eukaryotic yeast cells contain two types of ribosomes, *j.e.* the eukaryotic ones in the cytoplasm, and another type of them in the mitochondria. Notedly, eucellular mitochondria are originated from endosymbiotic protocellular organisms *(Gabaldón* and *Huynen* 2007) consequently mitochondrial ribosomes are of prokaryotic type, that are sensitive to antibiotics affecting protocellular protein synthesis *(Gilman et al.* 2001, *Zhang et al.* 2005). Antibiotics like gentamicin and neomycin are unable to permate the eukaryotic cell membrane, that is why they cannot express their inhibitory effect on mitochondrial ribosomes of eukaryotic organisms. The objective of present work was to elucidate whether or not the microwave irradiation has an influence on the uptake of antibacterial gentamicin or neomycin by eukaryotic yeast cells.

Materials and Methods

Cell culture: *Saccharomyces cerevisiae* M-26 strain was prepared and maintained on YGC (Biolab CGA20500) agar (4%). The liquid YGC medium contained the following ingredients: glucosemonohydrate (Labomark Ltd., Hungary 0610557) 5 g/L, peptone from casein (Merck 1.11931.1000), yeast extract (Merck 1.03753.0500) 5 g/L, ammonium dihydrogen phosphate (Merck 1.01126.0500), distilled water. Liquid cultures were incubated on a rotary shaker before and after the irradiation, at 37 ^{o}C.

Antibiotics: In some experiments gentamicin-chinoin (Sanofi-aventis Zrt.), neomycin sulfate (Pharmacopea Hungarica XV) antibiotics were applied. They were added at inoculation time to the fluid media culture as filtrated stock solution prepared under sterile conditions. Final antibiotic concentration in the fluid yeast culture media was in case of gentamycin 0,222 mg/mL, in case of neomycin 0,257 mg/mL.

Irradiation: The irradiation was performed with Microwave Accelerated Reaction System, Model MARS™ (CEM Corporation, Matthews, NC, USA). The optimized experimental setup was as follows: 2.45 GHz, 400 W at constant 37 °C temperature, and ambient pressure. Under these conditions the radiation was intermittent with maximum 12 ms sequences. Yeast cultures in early exponential phase, 120 minutes after initiation with standardized inocula were irradiated routinely for 30 minutes. In preliminary experiments, the duration of irradiation varied from 5 to 45 minutes. To monitor cultural growth, samples were collected under sterile conditions at regular time intervals after irradiation. Measurements of optical density referring to yeast cell concentration were carried out by Densichek™ (bioMerieux S.A., Marcy-l'Etoile, France). Mathematical analyses and curve fitting were performed using the SPSS TableCurve 2D Ver. 5.0 program. The fitted curve serial numbers are stated in the captions of the figures.

Results and discussion

Irradiation per se for up to 45 minutes had no considerable effect on the growth characteristics of yeast cultures. The presence of the antibacterial antibiotics, gentamicin or neomycin in non-irradiated cultures did not affect the multiplication of yeast cells either. On the contrary, irradiation for 30 minutes in the presence of gentamicin or neomycin profoundly retarded yeast cultures. The inhibitory effect grew parallel with increasing concentrations of these antibiotics, neomycin in particular *(Figure 1.* and *Figure 2.).*

Figure 1. Concentration-dependent inhibitory effect of gentamicin, in association with 30 min microwave irradiation on 720-minute-old yeast cultures (sigmoid curve fit 8011, $R^2 = 0.9879$)

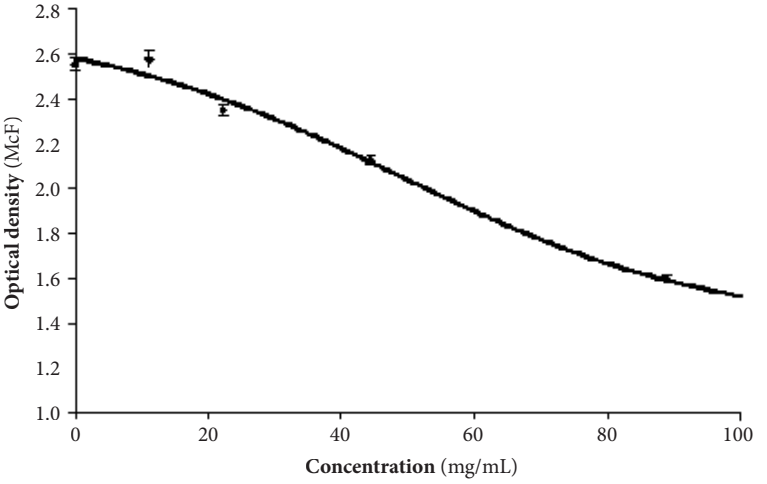
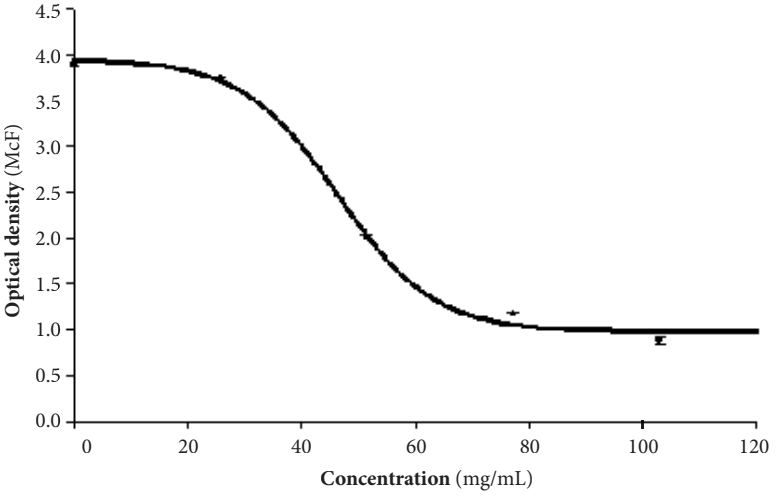


Figure 2. Concentration-dependent inhibitory effect of neomycin, in association with 30 min microwave irradiation on 780-minute-old yeast cultures (sigmoid curve fit 8011, $R^2 = 0.9964$)



There is a wide range of organisms, applied frequency, power and modulation, that have been tested for the effect of radiofrequency irradiation on cells in the literature. Exact and widely accepted explanation does not exist to date, as far as basic mechanisms of the effect of the electromagnetic irradiation on cells and components are concerned. Ions are the first expected target of irradiation. Ionic vibrations were proposed to be responsible for all the observed effects of the oscillating electric or electromagnetic fields (*Panagopoulus et al.* 2000, 2002). If the generated vibrations exceed a certain limit, the molecular structure of the membrane and consequently ion permeability will be modified. The authors state that effects are more pronounced at low, than high frequencies, since the amplitude of movements is inversely proportional with the frequency. *Fröhlich* (1968) proposed that cooperative forces produced at molecular level by alternating electromagnetic fields may cause compression and decompression of membrane structures. In contrast to these, *Adair* (2002) concluded that vibrations in a membrane structure generated by radiofrequency irradiation are too small to affect biological integrity and function.

Conclusions

The observed phenomenon might be the consequence of a reversible change in membrane permeability upon irradiation and due to the inhibition of protein synthesis in mitochondria of the yeast cell by the penetrating antibiotics. The increased permeability for the investigated antibiotics was due to the effect of irradiation applied. The applied irradiation protocol seems to be an effective tool for facilitating the uptake of other compounds by yeast

cells. The phenomenon will be investigated on other cell types as well. Further research is to be done to understand and clarify the exact structural and molecular mechanism of the permeability changes.

A mikrohullámú sugárzás hatása a *Saccharomyces cerevisie* aminoglikozid antibiotikum érzékenységére

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Összefoglalás

Manapság egyre nagyobb érdeklődés kíséri a nagy frekvenciájú mikrohullámú besugárzás biológiai objektumokra gyakorolt hatását. A rádiófrekvenciás sugárzás ilyen irányú hatásairól azonban egymásnak ellentmondó eredmények és értelmezések szerepelnek a szakirodalomban. Egy-egy konkrét hatás pontos megismerése célzott vizsgálatot tesz szükségessé. Ennek érdekében a 2,45 GHz frekvenciájú mikrohullám nem termikus hatását vizsgáltuk *Saccharomyces cerevisiae* tenyészeteken konstans hőmérsékleti-besugárzási protokoll alapján. A besugárzás önmagában nem változtatta meg a vizsgált élesztőtörzs életképességét és szaporodási profilját, mint ahogy önmagában (besugárzás hiányában) az egyébként antibakteriális hatású gentamicin vagy neomicin antibiotikum sem befolyásolta az élesztősejtek szaporodását. Ezzel szemben a besugárzás és az antibiotikum együttes hatására koncentrációfüggő szaporodásgátlás lépett fel. Következésképpen a mikrohullámú sugárzás érzékenységet indukált az élesztőtörzsben a vizsgált aminoglikozid típusú antibiotikumokkal szemben. Az újonnan megismert jelenséget a besugárzás által kiváltott reverzibilis membránpermeabilitás változás, majd ezáltal az élesztősejtek mitokondriumaiba bejutni képes, antibiotikum által előidézett proteinszintézis gátlás okozhatja. További kutatás szükséges a sejtben bekövetkező változások és a mechanizmus megértésének pontos tisztázására.

Kulcsszavak: mikrohullámú besugárzás, sejtmembrán-permeabilitás, antibiotikum-érzékenység, *Saccharomyces cerevisiae.*

Köszönetnyilvánítás

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