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Original Article

Remote action of clotrimazole on yeast under the synergistic effect of electromagnetic resonance and an electromagnetic amplifier

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Abstract: Material information can remotely affect cells through the mediation of electromagnetic resonance (EMR). We previously reported that EMR-mediated Clotrimazole (CLT) inhibits the growth of yeast *Saccharomyces cerevisiae* (*S. cerevisiae*). In this study, we found that an electromagnetic amplifier (EMA) enhanced the EMR-mediated inhibition of CLT on yeast growth and expanded the transmission distance of CLT information. This is the first report about the synergistic effect of EMR and EMA which amplify material information and transmit it over longer distance. The possible mechanism of remote action and amplification of CLT structure information was discussed, which provides a reference for further research and possible application approaches.

Keywords: Remote action, clotrimazole, yeast, electromagnetic resonance, electromagnetic amplifier, synergistic effect

Introduction

Studies have shown that information of DNA can be transmitted through an electromagnetic field (EMF), which may be related to the quantum effects of the DNA-water system [1]. Two complementary DNA strands form a DNA double helix structure with high charge density through the nucleotide base complementation mechanism, which can generate electromagnetic signal (EMS) of specific frequencies, and create the long-distance transmission of DNA structure information under the action of EMF. Gibberellin has a conjugated electron structure with high charge density. Meyl found that when gibberellin was placed on the transmitting coil of an electromagnetic resonance (EMR) apparatus, the germination and growth of soybeans were remotely affected [2, 3]. They also found that EMR-mediated information can regulate communication between molecules and even between cells [4, 5]. Many subsequent studies have proved that material information can be transmitted and duplicated by electromagnetic waves in a certain space and distance, and corresponding biological effects can be observed [6-9].

In these reports, the molecular structures of DNA, gibberellin and other materials all have a ring structure. When free electrons in the molecular ring structures (such as benzene rings) are stimulated by external signals (frequency, EMF, EMR, etc.), EMSs containing material characteristic information will be generated and emitted, which can affect other materials in a specific field through resonance, conduction and other forms of non-contact actions.

CLT is a broad-spectrum antifungal agent, which has an obvious inhibitory effect on the growth of yeast. We have previously reported that EMR-mediated CLT inhibited the growth of yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) in both solid and liquid media, and its effects were influenced by the surrounding environment and showed seasonal patterns [10, 11]. However, the remote effect of CLT structure information can only be realized when the two coils of the EMR apparatus are resonant, which limits the transmission distance and intensity

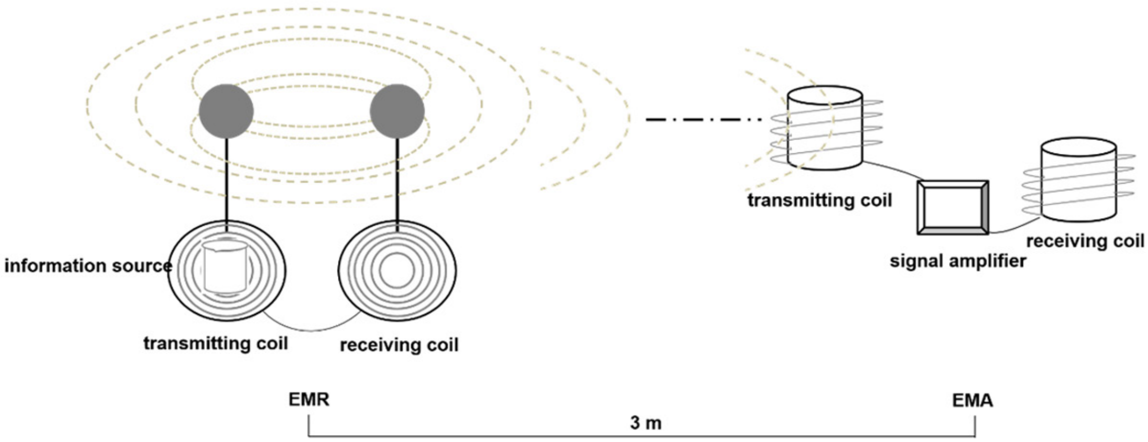

Figure 1. Experimental device schematic diagram. The device was composed of an EMR and EMA apparatus. The transmitting coil of EMA was 3 m away from the receiving coil of EMR.

of CLT structural information. It is known that an electromagnetic amplifier (EMA) formed by electromagnetic coils can excite, collect and transmit structural information of specific substances. In this study, EMA was introduced in the EMR apparatus to investigate whether EMA can enhance the EMR-mediated CLT effect. We found that EMA can enhance the inhibition of EMR-mediated CLT on yeast growth and expanded the transmission distance of CLT information. These results indicate that the structural information of CLT is amplified and transmitted over long distance under the synergistic effect of EMR and EMA. The possible mechanisms of remote action and amplification of CLT structural information were discussed.

Materials and methods

Strain and yeast culture

Industrial instant dry yeast *S. cerevisiae* (5 g* 400/F, ANGEL YEAST CO.LTD., China) was selected as the experimental strain. Yeast cells were cultured in rich medium YPD (1% yeast extract, 2% tryptone, 2% D-(+)-glucose) at 30°C in a shaker with 180 rpm to the logarithmic phase of growth and then used for each experiment.

Experimental device

The experimental device consisted of an EMR and EMA apparatus (**Figure 1**). The EMR apparatus is the same one used in previous studies [10, 11]. The EMR apparatus consists of two standard pancake Tesla coils made on a printed circuit board that functions as a transmitter and a receiver respectively. The secondary coil is printed on the top of the printed circuit board with 16 m total length, 0.5 mm width and 0.5 mm space between two wires. The center of the secondary coil is connected to a stainless steel ball of 120 mm in diameter. A ground wire is used to link the transmitter and receiver coil connecting the outside end of the secondary coil. The primary coil is printed under the printed circuit board with the same width and space, but shorter length and connected directly to the DDS (Direct Digital Synthesizer) generator, whose output impedance is usually under 10 Ohms. When the two coils meet the condition of same frequency, same wave shape and opposite phase shift, self-resonance of the whole system will be generated. In our experiments, the resonant frequency varied between 3.2 and 3.6 MHz due to the field attenuation by the flasks and the glass bottles containing the yeast or drug powder. The EMA apparatus consists of a power supply module, a signal transmitting coil, an amplifier and a receiving coil. The transmitting coil is connected with the input end of the amplifier, and the receiving coil is connected with the amplifier output end. The signal amplifier is powered by 24 V DC, and the internal processing is ± 12 V dual power supply. The amplifier IC adopts AD620. The transmitting and receiving coils are wound on a cylinder with diameter of 60 mm and 70 mm. The coil resistance is 14.5 Ω and the inductance is 3.9 MH. The transmitting coil is connected with the input end of the amplifier, and the receiving coil

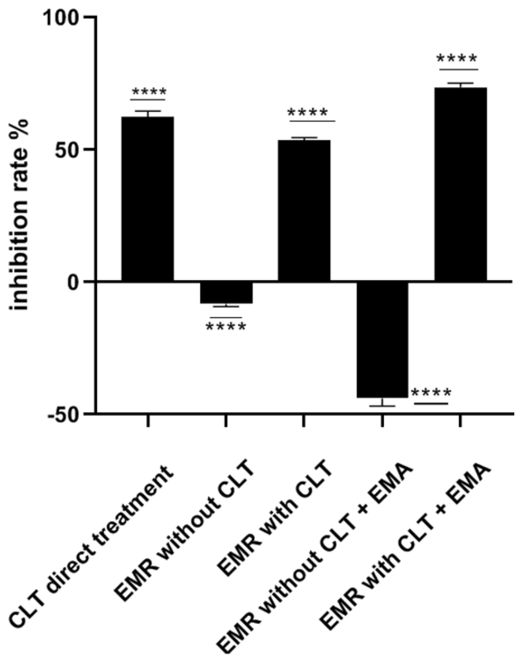

Figure 2. The synergistic effect of CLT information in EMR and EMA on yeast growth. The activity of the control was defined as "1". bars, SD (n = 3). ****, P<0.0001.

is connected with the amplifier output end. The signal is amplified 500 times by the amplifier and then output to the receiving coil. The transmitting coil of EMA was placed approximately 3 m away from the transmitting coil of EMR. Only in synergistic experiment, the EMA apparatus was switch-on. The structure information of the drug powder is transmitted to the receiving end of EMR or the transmitting end of EMA device through the resonance of EMR. The structural information acts on the yeast cells at the receiving end through the amplification function of EMA.

Experimental procedures

A glass beaker with or without CLT powder was placed on the transmitting coil of EMR. The logarithmically grown cells were harvested by centrifugation; suspended in fresh YPD medium to obtain a cell culture of 2 × 10^7 cells/mL; added 20 ml cell culture was added to each quartz triangular flasks of 50 ml. In the EMR-mediated CLT experiment, one flask with cell culture was pleased on the receiving coil of EMR, while another flask with cell culture was placed outside the field used as the control. In EMR and EMA synergistic experiment, nothing was placed on the receiving coil of EMR; one flask with pure water was placed in the transmitting coil of EMA, and one flask with cell culture was placed in the receiving coil of EMA; while another flask with cell culture was placed outside the field used as the control. Self-resonance frequency in the two kinds experiments was 3.4 MHZ. The biomass of yeast cells is our index to measure the experiment, biomass growth represents that the treatment method can promote the growth of yeast cells, otherwise it indicates inhibition. The biomass of the yeast cells was measured by optical density (OD) in the experiment.

Yeast cells have characteristic absorption peak under light of 600 nm wavelength. At the same time, we count the number of cells under 1 unit OD through the cell counting plate (1 OD = 2 * 10^7 cell/ml). In this way, the number of yeast cells (biomass) can be obtained indirectly through the OD value. For each experiment, after 24 h cultivation at 25°C, optical density (OD) was measured at 600 nm (UV-2800, UNICO, shanghai, China). The inhibition rate was calculated compared to the control, which was evaluated as: Inhibition rate = (OD_{600}-sample/OD_{600}-control - 1) × 100%.

Statistical analysis

Values of different measurements were normalized to a respective mean control value from untreated samples and expressed as percent control. All data are expressed as mean ± standard deviation (SD). They were analyzed by analysis of variance (ANOVA) and Least Significant Difference (LSD) using GraphPad InStat software, where P<0.05 was considered statistically significant.

Results

Synergistic effect of EMR and EMA mediated CLT on yeast growth

To investigate whether CLT acts on yeast cells through the synergistic effects of EMR and EMA, a series of experiments were conducted. The results were shown in **Figure 2**. First, CLT powder was directly added to yeast cells, the growth of yeast was significantly inhibited, and the inhibition ratio reached 62.4%. Then, an empty beaker (without CLT) was placed on the receiving coil of the EMR. When the EMR was on (EMA off), yeast cell growth increased by 8.3%, when both EMR and EMA worked together, the promotion effect was 43.0%. Finally, a beaker containing CLT was placed on the receiving coil of EMR, EMR alone or EMR and EMA working together all significantly inhibited yeast cell growth, with an inhibition ratio of 53.4% and 73.6%, respectively. These results indicated that there was a synergistic effect between EMR and EMA. On the one hand, EMA can enhance the growth-promoting effect of EMR and on the other hand, it can increase the growth-inhibiting effect of EMR-mediated CLT.

Theoretical analysis

It has been found that extremely low frequency EMS can inhibit the growth of yeast [12], and these frequencies are usually below 50 Hz, while high frequency EMS has little influence on yeast. Recently, we found that EMS with frequencies between 3.1-3.9 MHz increased the activity of *S. cerevisiae* cells (data not shown), which maybe the reason why EMR formed by an input of 3.4 MHz frequency signal promoted the growth of *S. cerevisiae* cells by 8.3%. The frequency of 3.4 MHz is very close to the cellular electrical oscillations frequency of *S. cerevisiae* cells found by Hölzel [13]. He examined the EMF signal of *S. cerevisiae* and found several cellular electrical oscillations frequencies of 1.5, 2.6, 3.5 and 5.7 MHz, suggesting that these levels of radiofrequency may resonant with yeast cells. The resonant interactions between EMF and the vibrational modes of cellular structures are considered to be the mechanism of biological effects of EMF.

There are four rings in the molecular structure of CLT, three of which contain high density charge and conjugated electronic structure. Under the action of EMR, the perturbation caused by rotational motion of the free electrons in the ring structures may release a unique EMS, they represent the characteristics of CLT. According to the quantum field theory [14, 15], the free electron spin waves in the CLT molecular structure (similar to the spin waves in ferrimagnets [16, 17]) are in quantum coherence with the electron spins in the water molecule, and the structural information of CLT is transmitted to the water (or yeast culture medium, which is mainly water), and then the information acts on yeast cells to produce the same effect as that of CLT directly contacting yeast cells.

In our study, CLT is selected mainly because of its ring structure and conjugated electronic structure. These characteristics make it easier for CLT information to be transmitted to the receiver through the resonance of EMR. We believe that the material structure information in EMR can be captured by EMA, its properties are similar to electromagnetic waves, and can be amplified and transmitted by electromagnetic amplifiers, which significantly increases the action distance of this resonance information.

Previous studies on EMR have shown that water can store and transmit EMS that represents the material information [1, 7, 18, 19], and our study reveals that the function of EMA is to receive, transmit and amplify EMS. The EMA transmitting coil received the EMS with CLT structure information at a distances of 3 m from the EMR, the signal amplifier amplified the EMS and sent it to the EMA receiving coil, finally, the EMS acted on yeast cells. In this study, both the promotion of EMR and the inhibition of EMR-mediated CLT on yeast growth were significantly amplified, from 8.3% to 43.0% and 53.4% to 73.6%, respectively. Because it is no longer limited by the resonance distance of EMR (the resonant distance between the EMR transmitting coil and receiving coil is about 0.5 m at the experimental power), the working distance of the signal is significantly increased to 3 m in the EMR and EMA synergistic system. Due to laboratory space limitations, longer distances were not tested. It can be seen that the main function of this synergistic system is signal amplification and signal long-distance transmission, which provides great convenience for the application of this technology.

Conclusion and perspectives

The free electrons in the CLT structure are excited by EMR, which makes it resonate and produce the characteristic frequency signal of CLT structure information. This signal can act on yeast cells in a remote and non-contact manner, producing growth inhibition similar to that of CLT directly applied to yeast cells. Under the synergistic effect of EMR and EMA, the growth inhibition of EMR-mediated CLT was significantly enhanced, at the same time, the growth promotion effect of EMR was amplified. The results show that the synergistic effect of EMR and EMA can effectively amplify and transmit material signals over longer distances and produce biological effects.

Next, studies can be carried out on other substances with similar molecular structure of CLT. New techniques can be used to unravel the mechanism to provide new means and methods for biological science research. In the potential application of EMR remote interaction technology in the future, higher information intensity and longer action distance are of great significance to modern medical research and practice.

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Disclosure of conflict of interest

The manuscript has not been published before and is not being considered for publication elsewhere. All authors have contributed to the important intellectual content of the manuscript, and have read and approved the final manuscript. We declare there is no conflict of interest.

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