Microbial Pathogenesis 109 (2017) 4–7

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Enhancing effect of 50 Hz rotating magnetic field on induction of Shiga toxin-converting lambdoid prophages

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ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form
8 May 2017
Accepted 11 May 2017
Available online 12 May 2017

Keywords:
Rotating magnetic field
Mitomycin C
STEC
Induction
Stx prophages

ABSTRACT

Studies aimed at investigating factors and mechanism of induction of prophages, a major pathogenesis factor of Shiga toxin-producing *Escherichia coli* (STEC), are considered important to develop an effective treatment for STEC infections. In this study, we demonstrated the synergistic effect of the rotating magnetic field (RMF) of induction $B = 34$ mT and frequency $f = 50$ Hz at a constant temperature of 37 °C and mitomycin C (MMC), that resulted in a higher level of induction of *stx*-carrying lambdoid Stx prophages. This is a first report on the induction of lambdoid Stx prophages in response to the enhancing effect of popular inductor (mitomycin C) under the influence of RMF.

1. Introduction

The Shiga toxin (Stx) is a major pathogenesis factor of Shiga toxin-producing *Escherichia coli* (STEC). The Stx genes (*stx1*, *stx2*) are located in genomes of Shiga toxin converting lambdoid prophages (also referred to as Stx prophages), which are mobile genetic elements [1]. Expression of the *stx* gene is under control of the phage's late genes promoter. According to this phenomenon, the synthesis of the Shiga toxin is normally repressed, and occurs only after induction of Stx prophages by various biological, chemical, and physical factors. A special role is played by the factors damaging genetic material, of which UV radiation and mitomycin C (MMC) are best known [2]. Antibiotics are also considered the inductive factors and for that reason STEC infections cannot be treated with these substances [3].

STEC strains do not have systems of the Shiga toxin secretion, therefore the release of toxins is subsequent to lysis of the cells and the release of progeny phages [4,5]. The significant increase of Shiga toxin production during the induction of prophages suggests that it may play a direct and important role in the modulation of the expression of STEC virulence factors encoded in the genomes of lambdoid phages [5].

The effective and specific treatment of STEC-caused infections has not been developed to date [3]. Therefore, understanding the role of various factors causing the stx prophages induction is essential for developing a treatment which could eliminate enterohemorrhagic *E. coli* without inducing the Stx prophage.

Numerous experimental investigations upon organisms have been carried out with an effort to detect the biological effects of static magnetic fields (SMFs). These studies cover a wide range of topics, including both biological systems and magnetic fields strength. For example, strong SMFs (typically greater than 0.2 T) found versatile applications in biological systems: genotoxic effect and mutation studies [6]; chromosomal damage [7]; DNA damage [8]; proliferation [9]; gene expression [10,11]; metabolic activity [12]; aggregation and cell adhesion [13]. Nevertheless, there are only few studies regarding the effect of rotating magnetic field (RMF) on microorganisms where the increase of functional parameters was observed (e.g. cell metabolism or proliferation parameters) [14–16]. The RMF is a magnetic field that changes direction (ideally) at a constant angular rate. This is a key principle

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http://dx.doi.org/10.1016/j.micpath.2017.05.018
0882-4010/

in the operation of the alternating-current motor. RMFs are mainly utilized in electric rotating machinery e.g. electric generators or induction motors, and therefore electromechanical applications. Furthermore, the RMF arises as a resultant field during the superposition of two or more alternating current magnetic fields (ACMFs) of identical frequency but spatially displaced in phase with respect to one another. This kind of MF is used in electrical motors, measuring instruments, and various AC regulation and control equipment [17]. The knowledge regarding the influence of RMF on cellular processes is still insufficient, and data on its role in the mechanism of the prophages induction still lacks.

Therefore, the aim of this study was to analyse the effect of induction of *stx*-carrying lambdoid Stx prophages from *Escherichia coli* by 1-h exposure to RMF ($B = 34$ mT, $f = 50$ Hz) and popular inductor – mitomycin C (0.5 μg × mL^{-1}).

2. Materials and methods

2.1. Exposure to RMF and mitomycin C

The exposure of Stx prophages-carrying bacterial cells to RMF and mitomycin C (Sigma-Aldrich, USA) was conducted in the experimental set-up consisting of the RMF generators described by Nawrotek et al. [15].

2.2. The biological material

Prior to the study, two *E. coli* strains: MG1655 lysogenic with bacteriophage 933 W Δ*stx*2:catGFP [18], courtesy of Department of Molecular Biology, University of Gdańsk, Gdańsk, Poland, and a wild-isolate labelled W10 (sheep origin, carrying *stx1* and *stx2* genes), deposited in Department of Immunology, Microbiology and Physiological Chemistry, were used. For phage titration, *E. coli* MG1655 was used as a host.

2.3. Optical density (OD) measurements

The optical density of the bacterial cultures was measured for a purpose of phage titration every 30 min at the wavelength of 600 nm in 96 well microtiter plates with 100 μL of each sample using Infinite 200 PRO NanoQuant microplate reader (Tecan, Männedorf, Switzerland). The measurements were carried out in three replicates.

2.4. Monitoring the prophage induction

Bacterial strains were cultured overnight in LB medium (Luria-Bretani, Oxoid, UK) at 37 °C with shaking (180 rpm). Afterwards, the fresh medium was inoculated at a 1:100 ratio with overnight cultures and incubated with shaking at 37 °C to obtain optical density OD = 0.1 at 600 nm. Prepared cultures were vortexed and dispensed in the volume of 10 mL into falcon tubes (15 mL) into four parts. Two inductive agents were used in three experimental setups for each individual strain: RMF (50 Hz, one hour), mitomycin C (0.5 μg × mL^{-1}) and both agents together. The control was cultured without any inductive agent. Samples, that were not exposed to the RMF, were incubated for one hour without shaking at 37 °C. Eventually, the culture was continued at 37 °C with shaking for 7 h.

Samples were collected every 30 min. Phage lysates were prepared. For this purpose, aliquots of 300 μL of the induced bacterial culture were vortexed with 30 μL of chloroform, then centrifuged. Lysates were titrated using double layer agar method. Top-agar medium, preheated to 45 °C, was mixed with overnight culture of MG1655 and suspensions from serial dilutions of phage lysates in TM buffer (Tris-HCl, pH 7.2; $MgSO_4$ 10 mM). The bottom layer was previously supplemented with chloramphenicol (2.5 μg × mL^{-1}, Sigma-Aldrich, USA) [19]. Plates were incubated at 37 °C for 24 h. The phage titre was calculated based on the number of plaques and described as plaque-forming units (PFU). The experiment was conducted in triplicate.

2.5. Analysis of mitomycin C UV-VIS spectra

UV–VIS spectra of MMC aqueous solution have been tested to exclude the impact of RMF on cytostatic structure. Therefore, UV–VIS spectra of mitomycin C aqueous solution were recorded with computer-controlled spectrophotometer V-670 Jasco (Jasco Inc., USA), within the range of 235–600 nm at 25 ± 0.1 °C.

2.6. Statistical analysis

For statistical analysis of the results, the *t*-test was performed. *P* values of <0.05 were considered significant. All statistical analyses were conducted with GraphPad Prism 5.02 (La Jolla, CA, USA) software.

3. Results

3.1. OD of bacterial cultures

Exposure of analysed *E. coli* strains (model and wild isolate) to the RMF of induction $B = 30$ mT, frequency 50 Hz and 1-h exposure caused no significant difference in comparison to the ODs of the control and treated samples. The optical density of model strain simultaneously treated with mitomycin C (0.5 μg × mL^{-1}) and RMF in defined parameters decreased significantly ($p < 0.05$) in relation to the samples treated only with mitomycin C. There were no statistically significant changes ($p = 0.1514$) of OD values for wild isolate in samples treated only with mitomycin C as compared to those treated using both factors. The growth curves are presented in Fig. 1.

3.2. Prophage induction

The cultures of both *E. coli* strains were exposed to mitomycin C in LB broth at 37 °C, whereas the influence of RMF on phage induction in strains was conducted in field generator. Fig. 2 (A and B) shows that the cooperation of mitomycin C and RMF resulted in higher relative phage titre. The phage titre of the reference 933 W phage in response to mitomycin C together with the exposure to RMF after 270 min was 3.37 × 10^7 PFU × mL^{-1}, which was significantly higher than in the control sample induced only with mitomycin C, for which the titre counted as 1.1 × 10^7 PFU × mL^{-1}. Wild type *stx*-converting phage from W10 strain was also induced after mitomycin C treatment. Relative titre of this phage after exposition to mitomycin C for 120 min was 2.46 × 10^5 and increased to 1.3 × 10^6 PFU × mL^{-1} once cultures were exposed to RMF. However, after this time, in the case of bacteriophage W10, a rapid decline in relative phage titre to 6.5 × 10^5 PFU × mL^{-1} was observed, due to the adsorption of released progeny bacteriophages to their host cell (Fig. 2B).

3.3. UV-VIS analysis of mitomycin C

UV-VIS spectroscopy showed the characteristic strong absorption of mitomycin C at 363 nm (Fig. 3). After exposure to the RMF (50 Hz, 1 h), the detected absorption was consistent with the control not exposed to MMC.