

## A COMPLEX INTERFERON INDUCER POTENTIATES THE FUNCTIONAL MACROPHAGE ACTIVITY DURING STAPHYLOCOCCAL INFECTION

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### Abstract

The antibacterial effect of a new type I interferon inducer, an yeast RNA-tilorone molecular complex (MC), was studied using a model of staphylococcal infection. The MC taken in experimental doses being optimal interferon-inducing ones accelerates the *Staphylococcus aureus* elimination from mice organisms accompanied by increased functional ability (phagocytosis and bactericide activity) of peritoneal macrophages. The conclusion is that the MC antibacterial activity detected is mediated by its interferon-inducing properties.

**Key words:** Interferon (IFN), inducer, yeast RNA, tilorone, *Staphylococcus aureus*, phagocytosis, bactericide activity.

### INTRODUCTION

The interferons (IFNs) are already known to possess antiviral, immunomodulating, and radioprotective effects, their antibacterial properties having been also well documented (see for detail Baron et al., 1981; Spivak et al., 1987; Kuznetsov et al., 1989; Bogdan, 2000). The mechanisms of preventive and therapeutic actions of IFN preparations in bacteria-infected organisms are not yet completely understood; however, in such cases the IFN is thought to be a physiological immunomodulator, its main function being the correction of secondary immunodeficiency-driven disorders (Mayet et al., 1989; Niederau et al., 1992).

Depending on their antigenic and physicochemical properties, mode of induction, efficacy and action mechanisms, the IFNs are divided into two types; IFNs type I (IFN- $\alpha$ , - $\beta$ , - $\omega$ , and - $\tau$ ) are produced by majority of cells as a response to virus infection, double-stranded RNAs or some synthetic low-molecular mass compounds; the IFN type II (immune IFN, i.e. the IFN - $\gamma$ ) is produced by T-cells and natural killer cells as a response to foreign antigens and mitogens (Taylor et al., 1984). It should be noted some of these inducers to possess antibacterial properties (Ho, 1984).

We have earlier shown the tilorone hydrochloride interactions with single-stranded RNA molecules form a molecular complex (MC) containing a significant number of double-helix regions stabilized by tilorone molecules intercalated between complementary base pairs (Karpov, 1997). Such structure assures the MC IFN-inducing effect detected both *in vitro* and *in vivo*

(Karpov, Zholobak, 1995, 1996). Tilorone, one of the MC components, is the first described synthetic IFN inducer of low molecular weight possessing, together with related compounds, both antiviral and anti-tumor activities. Tilorone stimulates also the immunoglobulin production (Chandra et al., 1974; Harrington et al., 1981) increasing the cellular immune response (Megel et al., 1974); so it belongs to immunomodulators. The second MC component is single-stranded yeast RNA without any IFN-inducing or immunomodulating activity.

We have previously found MC to act also as an antiviral compound (Karpov et al., 2001). In this paper we describe our results concerning the effect of this molecular complex on the phagocyte activity as well as on the elimination of bacteria from kidneys in the case of experimental staphylococcal infection.

### MATERIALS AND METHODS

#### INTERFERON INDUCER

The MC formed by yeast RNA and tilorone was prepared as described previously (Karpov, Zholobak, 1996). To prepare the MC, we used a commercial yeast RNA preparation («Biochemreagent», Latvia) and tilorone-HCl («Sigma», USA). The commercial yeast RNA preparation was then purified by triple phenol deproteinization followed by ethanol precipitation according to the routine protocol. Two components were mixed, RNA/tilorone ratio being 1/10 (M/M). These components were dissolved in a Tris-buffer solution (0.01 M Tris-HCl, pH 6.8; 0.05 M NaCl). The MC solutions and MC components were sterilized using 0.22 m membrane filters («Millipore», USA).

#### BACTERIA

In these experiments we used an alive culture of the *Staphylococcus aureus* strain 8325 received from Dr. V.S. Zuyeva (N.Gamaleya Institute of Epidemiology & Microbiology, Russia). Bacterial cultures were grown in standard media at 37 °C during 18 h and then taken for our investigations.

#### MICE

325 non-inbred male mice (aged 6-8 weeks) were used for experiments. The mice were housed in a barrier

environment and fed by standard diet. These animals were inoculated intraperitoneally by *Staph. aureus* strain 8325, the infecting dose being 108 bacteria/ml, i.e. a LD<sub>30</sub>. In 24 h p.i., the MC solution (25 µg/ml, 0.2 ml) was injected in the same way to induce the maximal IFN synthesis (Karpov, Zholobak, 1995, 1996). The number of animals perished during our experiments was registered daily during 10 days.

#### PREPARATION OF PERITONEAL CELLS

Mouse peritoneal macrophages were isolated from normal and infected mice by washing the peritoneal cavity with sterile physiological buffered saline (PBS) containing heparin (2.5 U/mL); the cells were washed twice with the same solution, pelleted by centrifugation (800 g, 10 min), and then suspended at 10<sup>6</sup>/ml in the same medium (Imanishi et al., 1982).

#### CALCULATION OF BACTERIA PERSISTING IN THE ORGANISM

The murine kidneys taken in sterile conditions from infected animals were homogenized in porcelain mortars with quartz sand, 0.15M NaCl having been added. Following homogenization we obtained serial dilutions of this material and put them (0.1 ml of each dilution) on Petri dishes with the beef-extract agar. In 24 h the colonies grown on this medium at 37 °C were calculated (CFU).

#### EVALUATION OF PHAGOCYtic ACTIVITY

The phagocytic activity was evaluated taking into consideration the percent of phagocytosis (quantity of phagocytizing cells among 100 ones) and phagocytal number (mean quantity of microbe cells swallowed up by a single phagocyte) (Verhoef and Waldvogel, 1985). To study the phagocytosis process, a 18-hour-old *S. aureus* strain 8325 culture was used, the phagocyte/bacteria ratio being 1/100.

#### EVALUATION OF BACTERICIDE ACTIVITY

Bactericide activity of macrophages was determined by the nitroblue tetrazolium (NBT) reduction test (deShazo et al., 1987). Cell suspensions (0.2 ml, 5x10<sup>6</sup> cells/ml) in the medium 199 supplemented with bovine serum (10 %) were put on cover slides and incubated in humidified thermostats during 10-15 min (37 °C, 5% CO<sub>2</sub>). NBT solution (0.2 %, 0.2 ml) was then added to phagocytes alone (for spontaneous test) or together with alive or inactivated *Staph. aureus* cells (0.2 ml, 10<sup>9</sup> cells/ml) (for stimulated test). Following incubation at the same conditions fluids were aspirated, all slides carrying bound phagocytes were washed in Hanks solution, air-dried and stained in safranin solution (0.1 %) during 30 sec. The bactericide activity was estimated by calculation of cell quantity (%) containing dark-blue diphormazane granules.

The functional reserve of the cell was evaluated as the difference between the NBT-value stimulated by the *Staph. aureus* and the spontaneous one.

#### STATISTICS.

The data presented here are mean values of four experiments; each point was obtained using 6 animals. The significance of differences for all parameters was estimated by Student's T-criterion.

#### RESULTS AND DISCUSSION

The first step of this work was the study of the MC being an interferon inducer and of its components on the *Staph. aureus* strain 8325 persistence in experimental animal organism. The MC introduction was found to be followed by increased bacterial elimination from the organism (Fig.1).

The development of staphylococcal infection is accompanied by the reproduction of bacterial cells in infected organism, its maximum being reached at the third day post infection. The MC introduction in such situation led to significant drop of bacterial cell quantity. At the third day post infection the number of *Staph. aureus* cells in infected murine kidney dropped by 4.8 times versus control values. It is noteworthy the tilorone itself - a low molecular mass MC component - increased also bacterial cells elimination, this elimination being, however, lower comparing to the MC effect (by 3.2 times). Another MC component - high molecular mass yeast RNA - showed no similar action.

Any staphylococcal infection was proved to be accompanied by suppressed macrophage activity; such suppression may sometimes lead to chronic form of infection. So we investigated also the effect of MC introduction to infected animals on the functional activities (both phagocytic and bactericide ones) of peritoneal macrophages.

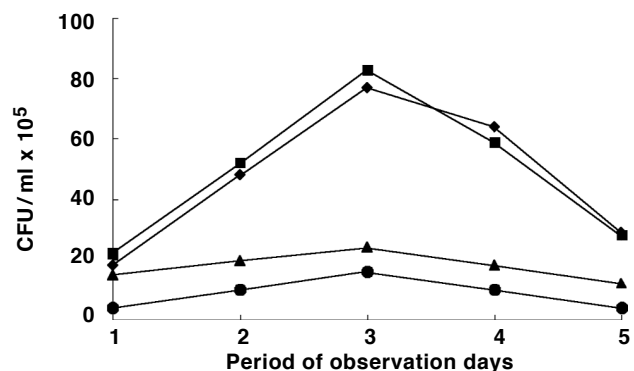


Fig.1. Elimination of bacteria from murine kidneys, the animals having been infected by *Staph. aureus*; the infected animals are treated by the MC (circles) and its components - tilorone (triangles) and yeast RNA; the components are used in concentrations adequate to their contents in the MC. Control infected animals are not treated (rhombi).

The data presented in the Figure 2 demonstrate the suppression of peritoneal macrophage cell effector functions in infected animals during the whole observation period; it is evident from percent of phagocytosis (Fig.2A) and phagocytal number values (Fig.2B). Simultaneously, the MC use caused a marked increase of

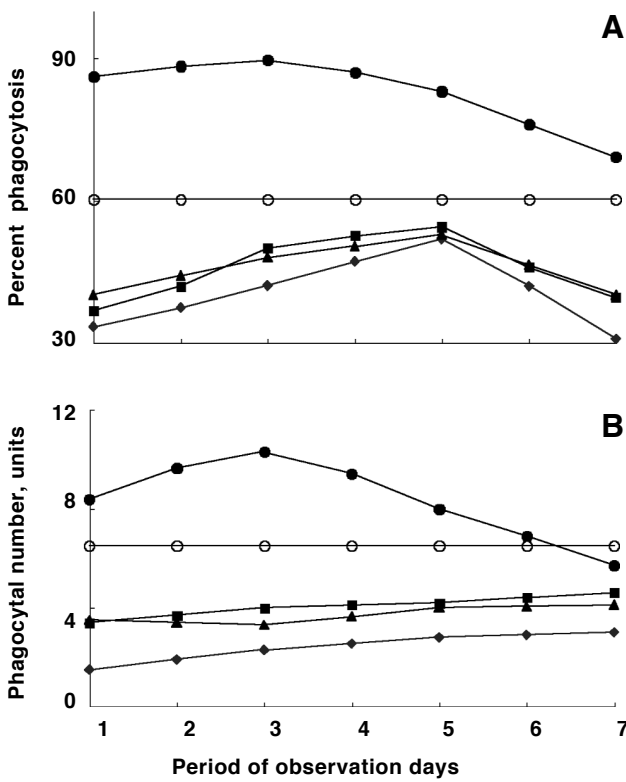


Fig.2. Phagocytic activity of peritoneal macrophages during staphylococcal infection following the action of the MC (closed circles) and its components - tilorone (triangles) and yeast RNA (squares). Control I (open circles) and control II (rhombi) are presented by intact animals and by Staph.aureus-infected mice, respectively. (A) Granulopectic index. (B) Phagocytal number.

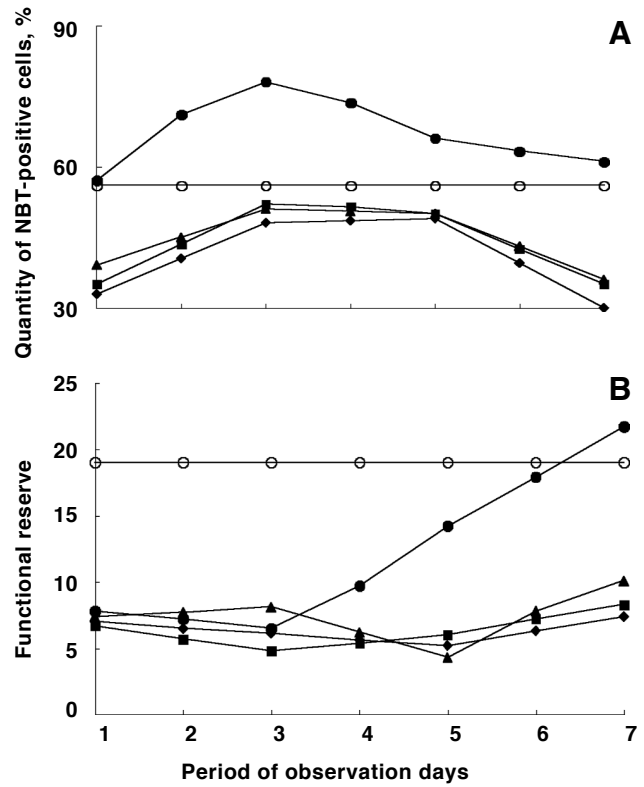


Fig.3. The effect of the MC (closed circles) and its components - tilorone (triangles) and yeast RNA (squares) on the bactericidal activity of peritoneal macrophages during the staphylococcal infection. Control I (open circles) and II (rhombi) are intact animals and Staph.aureus-infected mice respectively. (A) Spontaneous NBT-test. (B) Functional reserve.

these both values versus the values found for control infected mice, the maximal values were seen at the third day following MC administration. It is noteworthy the introduction of single MC components was also followed by some higher phagocytic activity of macrophages, being, however, lower comparing to control uninfected animal activity.

An important functional index of macrophage activity is macrophage ability for active oxygen form accumulation. From the data given in the Figure 3 the staphylococcal infection is seen to take place together with the increased spontaneous bactericide cell activity (according to the NBT-test results) comparing to such activity found in intact control animals. Similarly to phagocytic activity values, the spontaneous bactericide activity following the MC introduction was higher (its maximum was reached at the third day) and superior comparing to intact animals. As in previous experiments, the administration of single MC components showed no marked effect on bactericide activity. During these experiments the functional reserve of the cells being a marker of macrophage potential ability for active oxygen form accumulation was always significantly suppressed in infected animals (Fig.3B) by 4.5 times comparing to control ones. The MC use in these conditions led to the increase of this reserve beginning from the third day post MC introduction, the marker becoming a normal one on the sixth day.

Any of MC components demonstrated no effect on this index.

The index of functional reserve of cells being a marker of macrophage potential ability for active oxygen accumulation (Fig.3B) is significantly suppressed (by 4.5 times) in infected animals versus control ones during the whole experiment period. The MC administration in such conditions led to the increase of this marker beginning from the third day of MC injection; the index studied became normal on the sixth day. As usually both MC components had no effect on this index. In healthy mice the MC administration led to some stimulation of functional reserve (by 30 %) comparing to healthy non-treated mice.

Some IFN type I inducers of low molecular mass are proved to inhibit the development of bacterial infections (Ho, 1984). There are also some data concerning protective effect of exogenous IFN and IFN inducers against other agents of non-viral infections including *Chlamydiae*, *Protozoa*, *Rickettsiae*, *Fungi* (Bogdan, 2000). Our results suggest the MC studied here to possess also such properties. The administration of this preparation to infected animals leads to increased staphylococci elimination from the organism. The tilorone itself, a low molecular mass substance being a MC component, also accelerates markedly the bacterial elimination, its antibacterial activity being, however, significantly lower (synergic ef-

fect); the yeast RNA, a high molecular mass component of the MC, shows no effect on bacterial elimination. It might have been explained by a potent staphylococcal exonuclease system present in many bacteria and enabling exogenous nucleic acid degradation. The tilorone's antibacterial effect in this case may be due to its side action not induced by the IFN system activation. The tilorone is known to accelerate the immunoglobulin synthesis (Chandra et al., 1974; Harrington et al., 1981) expressed by the increased IgM and IgG production as well as of antibodies dependent on T- and B-cells; besides, the tilorone stimulates the increasing of IgE titers. The tilorone is also proved to influence favorably on cellular immune response (Megel et al., 1974). As the tilorone doses used in infected animals are about 500 times lower comparing to its optimal IFN-inducing dose (Karpov and Zholobak, 1996) these factors are namely thought to enable the observed elimination of staphylococci.

The effectors function of phagocytes is shown to be a probable mechanism responsible for antibacterial activity of IFN preparations and of its inducers (Spivak et al., 1987). The MC investigated here presents no exception. Its use is accompanied by a significant increase of cell phagocytic and bactericide functions in treated animals versus these functions both in infected and in intact ones. Such MC property belongs to the most important factors causing the observed promotion of bacteria elimination from the organism. The administration of the tilorone dose adequate to its dose contained by the MC is followed by no marked increase of phagocytic or bactericide cellular activity. The tilorone injection in dose adequate to its quantity in MC was not followed by significant additional phagocytic and bactericide cell activity versus activity found in control non-infected animals. So the MC effects observed here are among the properties of this compound itself; they are not mediated by activities of its components.

The MC injection to intact animals having caused no phagocytosis index stimulation led, however, to increased number of engorged bacteria per cell. The promotion of bactericide cell activity in MC-treated animals was due not to increased spontaneous bactericide activity, but to increased potential ability of macrophages to accumulate active oxygen forms. Such response of immune cells in healthy animals induced by MC injection proves this compound to act as an immunomodulating substance.

The MC belongs to IFN inducers, the IFN being the most active during initial infection stages; so we have studied the MC effect introduced simultaneously with *Staph. aureus* inoculation to mice. We think the IFN-inducing MC activity to be the main factor responsible for its antibacterial efficacy due to the IFN system activation and to a series of antibacterial mechanisms triggered by this system.

The MC stimulates the active bacteria elimination in animal kidney as well as the macrophage-mediated immunity during the whole infection period studied. So we think it would be useful to administrate the MC during the infection peak accompanied by maximal bacteria reproduction in kidney.

We have earlier shown the MCs appeared following tilorone-single-stranded RNA interactions to possess a lot of double-stranded regions stabilized by tilorone molecules intercalated between complementary base pairs (Karpov, 1997). It is namely this structure to assure the MC IFN-inducing effect both *in vitro* and *in vivo*. It is namely this MC property seems to be the main factor determining the antibacterial effect caused by IFN system activation and by further triggering of antibacterial mechanisms by this system.

The data presented here demonstrate some molecular complexes to be promising antibacterial preparations for clinical use.

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