Can new probiotic isolate of bovine milk reverse aflatoxin M1-induced neutrophil oscillation?

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Introduction

In high yielding dairy cow infection is a big problem, and protection of animals from pathogens is a tough challenge for the bovine and dairy industries and public health. A aflatoxin (AFM) is a potential granulotoxic, and in AFM-positive milk the metabolite appears everywhere, especially in mammary gland. Neutrophils are pivotal for defense against mastitis; they have numerous potentials to finally eliminate engulfed pathogens. We have recently observed the antagonistic properties of a Lactobacillus (L.) fermentum, isolated from milk of healthy dairy cows, against Staphylococcus aureus (S. aureus). The main purposes of this study were: 1) To assess the effect of AFM on neutrophils killing activity against S. aureus in healthy dairy cows, and 2) to determine whether L. fermentum reverses the diminished effect of the AFM on neutrophils in healthy dairy cows.

Materials and Methods

Cows, blood samples and analyses: Healthy mid-lactating dairy cows (n=6) were used as a source of neutrophilic blood samples. The cows were aseptically collected for neutrophil isolation and neutrophil functional analyses. The isolated blood neutrophils were exposed with: 1) only 25 ng/ml AFM1 2) AFM1 plus overnight grown L. fermentum 3) overnight grown L. fermentum and 4) none of them for 3 hours; their capacity to kill S. aureus was then monitored by a bacterial assay, using in vitro challenge of S. aureus. Newbold 305, which are routinely isolated from clinical cases of mastitis, with above mentioned conditions for 1 h and then cfu counting of the S. aureus was monitored by a bacterial assay, accordingly.

Bactericidal assays: Briefly, 100 µl live S. aureus (5x10^6 / ml) were added to 500 µl viable neutrophils (5x10^6 / ml) already exposed with/without AFM1, L. fermentum, AFM1 plus L. fermentum, or to 500 µl of normal saline containing no neutrophils, incubated for 1 hour, and finally serially diluted for plating out on the blood agar media (see Scheme 1).

To see the effect of four above mentioned treatments on phagocytosis and killing activities on the S. aureus, after dilution and plating out, they were mixed with a sterile plastic loop in triplicates onto Columbia sheep agar blood (Biokar Diagnostic, Beauvais, France) in 5% CO2 at 37°C for 24 h, and colony counts were performed. The log CFU/ml of each sample was calculated, and results from the bacteriological assay are expressed as and finally the percentage of killed (% killing) of S. aureus4 (see the formula in the Scheme 1).

Results and discussion

The bactericidal activity of neutrophils against S. aureus in AFM1-treated group was -23 %, and -43 % in control group. Similar pattern was observed on phagocytosis activity, that is, the phagocytosis of S. aureus by AFM1-exposed neutrophils significantly diminished (P < 0.01; figures 1 and 2).

The phagocytosis capacity of neutrophils against S. aureus in AFM1-treated group was significantly lower compared to other groups. Interestingly, this adverse effect of AFM on neutrophils' engulfing and killing capacities was reversed when co-cultured with L. fermentum (see figures 1 and 2). Further, both the killing activity and phagocytosis activity of neutrophils against this pathogenic superbug, S. aureus, were remarkably improved with the application of L. fermentum (see figures 1 and 2). This inhibiting the adverse effects of AFM on neutrophils' killing capacity with pre-exposing of neutrophils with the L. fermentum and/or co-culturing with L. fermentum is interestingly encouraging.

Our observation confirmed the fact that AFM1 is immunotoxic or antiphagocytic in dairy cows, especially affecting on the pivotal cellular part of circulating innate defence system. The killing of S. aureus by AFM1-treated neutrophils was markedly minimal and improved significantly with application of the L. fermentum. Observed improved phagocytic activity of neutrophils sheds fresh light on the application of this lactobacillus as a good probiotic in farm animals, to prevent immune suppression in immunocompromised periparturient dairy cows.

Since neutrophils' oscillation happens in dairy cows around peripartum period, and AFM1 accelerates oscillatory events, finding potential immunostimul is essential and L. fermentum can be considered as a good candidate for that purpose.

Further studies are needed to explain the cellular and molecular mechanisms happening in AFM1-L. fermentum – neutrophils interactions.

References


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