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ORIGINAL ARTICLE

Effect of metronidazole supplemented with hydroquinone on the adhesion of *Lactobacillus acidophilus* in ovine vaginal cells

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Abstract This work demonstrates that the addition of metronidazole together with a ubiquitous quinone compound reduces adherence of *Lactobacillus acidophilus* to ovine vaginal cells.

Spectrophotometric and voltammetric studies have shown that neoformed compounds were observed in these systems; there were also changes in their electroactive composition, and the oxidant status had a significantly higher value compared to the control ($p < 0.05$). Based on reduction potential (E; mV), the distribution of electroactive compound concentrations suggests that the compounds with low reduction potential induce this behavior, which would indicate that the addition of metronidazole with a ubiquitous quinone compound to the vaginal system might increase the reductive capacity of these systems.

This work shows that the study of behavior and fluctuations of the redox compounds that compose the vaginal environment, in terms of concentration and species of redox molecules, must be hierarchized in order to better understand the early stages of colonization by microorganisms.

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PALABRAS CLAVE
Adhesión microbiana;
Metronidazol;
Células vaginales;
Ovinos;
Redox;
Voltametría

Efecto del metronidazol suplementado con hidroquinona en la adherencia de *Lactobacillus acidophilus* en células vaginales ovinas

Resumen Este trabajo demuestra que la incorporación de metronidazol conjuntamente con un compuesto quinónico ubicuo disminuye la adherencia de *Lactobacillus acidophilus* a células vaginales ovinas.

Los estudios espectrofotométricos y voltamétricos mostraron que en estos sistemas aparecieron compuestos neoformados y que hubo modificaciones en la composición electroactiva; asimismo, el estatus oxidante presentó un valor significativamente superior con respecto al control ($p < 0,05$). Según los potenciales de reducción (E; mV), la distribución de las concentraciones de los compuestos electroactivos muestra que los compuestos con bajos potenciales de reducción inducen este comportamiento. Esto indicaría que la incorporación de esta mezcla al sistema vaginal aumentaría su capacidad reductora.

El trabajo muestra que el estudio del comportamiento y las fluctuaciones de los compuestos redox que componen el ambiente vaginal, en términos de concentración y especies moleculares, debe ser jerarquizado para comprender mejor las primeras etapas de la colonización de este ambiente por parte de los microorganismos.

Introduction

Microbial adhesion to surfaces is the first step of the early processes not only in beneficial but also in pathogen microorganism installation in many diverse ecological niches[24]. At this first stage of bacterial adherence processes there are physicochemical conditioners acting in non-specific interactions (such as electrostatic repulsion and Van Der Waals' force)[23,24]. Moreover, it has been shown that in cell–cell adhesion processes there is intervention of molecules that can undergo intra- and inter-molecular interactions with molecules or ubiquitous functional groups, such as H_2O_2 or thiols, through chemical reactions as well as redox reactions[5,9,20]. Based on these facts it is evident that the factors affecting these physicochemical variables must be especially considered in order to characterize this biological scenario[8,13]. Studies on bacterial adherence in bovine vaginal cells showed that the presence of oxidant compounds, such as periodate ions, may affect this process[11], which suggests that the redox status of an extracellular environment, defined in terms of intensity (reduction potential; Eh) and capacity (number of electroactive compounds)[14], might be a significant proximity factor[13] in microorganism adherence to the vaginal mucosa.

Metronidazole (MTZ) (Fig. 1a) is an antibiotic used in the digestive, reproductive and skin systems. The mechanism of action consists in inhibiting the synthesis of nucleic acids. MTZ and other nitroimidazole derivatives are active redox compounds which perform a significant antibiotic activity based on intracellular reduction from a nitro group to a nitro radical ($R{-}NO_2 + e^- \rightarrow R{-}NO_2^{\bullet -}$)[2], which is the reason why they are widely used in various microbial ecosystems inside the animal's body, including the vaginal environment[10,19].

Quinones are molecules that play important roles in living organisms, such as the e^- transfer in photosynthesis and in vitamin K. They are mostly benzoquinones, naphthoquinones and anthraquinones. Their important feature for this work is

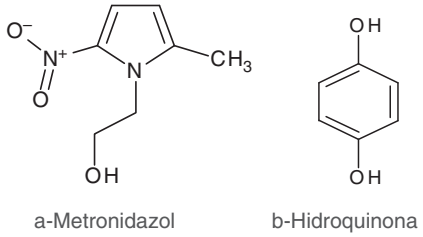

Figure 1 (a) Molecular structure of Metronidazol; (b) molecular structure of Hidroquinona.

their ability to act reversibly in redox processes. Currently, natural and synthetic quinone derivatives are being strongly studied because of their chemotherapeutic activity against diseases such as cancer; furthermore, they have antimicrobial, anti-inflammatory and antioxidant properties[1,21].

Independently of its antibiotic nature, when MTZ is in the vaginal-mucus-physicochemical-environment, it may act as a redox effector interacting with other ubiquitous redox compounds such as quinines (Fig. 1b), and in biological processes that are affected by redox conditions, for example in microbial adherence to vaginal cells.

This work aimed to assess the effect of adding MTZ alone and MTZ with hydroquinone (H_2Q) (as a model of ubiquitous redox compounds in biological systems) on *Lactobacillus acidophilus* adherence to ovine vaginal cells suspended in simulated vaginal fluid, in order to hierarchize the study of the electrochemical scenario as a systemic view.

Materials and methods

Ovine vaginal cells

Ovine vaginal epithelial cells (VECs) were taken with a sterile swab from the vaginal cavity of experimental animals.

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