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ORIGINAL ARTICLE

β-defensin-2 in breast milk displays a broad antimicrobial activity against pathogenic bacteria☆,☆☆

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Abstract
Objective: To describe the antimicrobial activity of β-defensin-2 produced in the mammary gland and secreted in human breast milk.
Methods: The peptide production was performed by DNA cloning. β-defensin-2 levels were quantified in 61 colostrum samples and 39 mature milk samples from healthy donors, by an indirect enzyme-linked immunosorbent assay (ELISA). Using halo inhibition assay, this study assessed activity against seven clinical isolates from diarrheal feces of children between 0 and 2 years of age. The activity of β-defensin-2 against three opportunistic pathogens that can cause nosocomial infections was determined by microdilution test.
Results: The peptide levels were higher in colostrum (n = 61) than in mature milk samples (n = 39), as follows: median and range, 8.52 (2.6-16.3) μg/ml *versus* 0.97 (0.22-3.78), $p < 0.0001$; Mann-Whitney test. The recombinant peptide obtained showed high antimicrobial activity against a broad range of pathogenic bacteria. Its antibacterial activity was demonstrated in a disk containing between 1–4 μg, which produced inhibition zones ranging from 18 to 30 mm against three isolates of *Salmonella* spp. and four of *E. coli*. β-defensin-2 showed minimum inhibitory concentrations (MICs) of 0.25 μg/mL and 0.5 μg/mL for *S. marcescen* and *P. aeruginosa*, respectively, while a higher MIC (4 μg/mL) was obtained against an isolated of multidrug-resistant strain of *A. baumannii*.

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Conclusions: To the authors' knowledge, this study is the first to report β-defensin-2 levels in Latin American women. The production and the activity of β-defensin-2 in breast milk prove its importance as a defense molecule for intestinal health in pediatric patients.

PALAVRAS-CHAVE
Peptídeos catiônicos antimicrobianos;
hBD2;
Lactação;
Defensinas;
Agentes anti-infecciosos;
Leite materno

β-defensina 2 no leite materno mostra uma ampla atividade antimicrobiana contra bactérias patogênicas

Resumo
Objetivo: Descrever a atividade antimicrobiana da defensina-beta 2 na glândula mamária e secretada no leite materno humano.
Métodos: A produção de peptídeos foi realizada por clonagem de DNA. Os níveis de defensina-beta 2 foram quantificados em 61 amostras de colostro e 39 de leite maduro de doadoras saudáveis pelo teste ELISA indireto. Por um ensaio de halo de inibição, avaliamos a atividade contra sete isolados clínicos diarreicos de crianças entre 0 e 2 anos. A atividade da defensina 2 contra três patógenos oportunistas que podem causar infecções nosocomiais foi determinada pelo teste de microdiluição.
Resultados: Os níveis de peptídeos estavam significativamente maiores nas amostras de colostro (n = 61) que de leite maduro (n = 39), como segue: 8,52 (2,6-16,3 μg/mL) mediana e faixa em comparação a 0,97 (0,22-3,78), p < 0,0001; teste de Mann-Whitney. O peptídeo recombinante foi obtido da alta atividade antimicrobiana demonstrada contra uma ampla gama de bactérias patogênicas. Sua atividade antibacteriana foi demonstrada em um disco contendo entre 1-4 μg, que produziu zonas de inibição entre 18 e 30 mm contra três isolados de *Salmonella* spp. e quatro de *E. coli*. A defensina-beta 2 demonstrou concentrações inibitórias mínimas (CIMs) de 0,25 μg/mL e 0,5 μg/mL para *S. marcescen* and *P. aeruginosa*, ao passo que uma CIM maior (4 μg/mL) foi obtida contra um isolado de cepa multirresistente de *A. baumannii*.
Conclusões: Até onde sabemos, este estudo é o primeiro a relatar níveis de defensina em mulheres da América Latina. A produção e a atividade da defensina 2 no leite materno comprovam sua importância como uma molécula de defesa para a saúde intestinal em pacientes pediátricos.

Introduction

The World Health Organization reports that 6.6 million children under the age of 5 died in 2012. Moreover, 44% of those deaths occurred during the neonatal period. From the end of the neonatal period through the first 5 years of life, the main causes of death are pneumonia, diarrhea, and malaria. Malnutrition is the underlying contributing factor in approximately 45% of all child deaths, making children more vulnerable to severe diseases.[1] Over half of these early child deaths are due to conditions that could be prevented or treated with access to simple, affordable interventions; one of them is breastfeeding.

Solely during lactancy, humans confront the challenge of survival by means of breast milk. Human milk is associated with protection against diarrheal diseases, respiratory-tract infections, and necrotizing enterocolitis (NEC).[2–5] Protection against infection in breastfed infants appears to occur through a variety of complementary acquired and innate defense factors found in human milk, including oligosaccharides and their glycoconjugates,[6] and antimicrobial peptides (AMPs).[7,8]

Among AMPs, the defensins family constitutes an important part of the innate immune response in fluids secretion. Increasing evidence suggests the importance of defensins in the immune response. Results in recent studies on the homeostatic and disease-fighting activities of human defensins point to their key relevance in several pediatric disorders. For example, high β-defensin-2 (hBD2) concentrations reflecting strong intestinal immune responses were associated with moderate courses of NEC.[9] However, infants with severe NEC showed no increase in fecal hBD2 concentrations before and during the disease. These studies suggest that a specific deficiency of innate defense activation in extremely-low birth weight (ELBW) infants rather than an impaired intestinal epithelial barrier leads to a more severe course of NEC.[9] In this context, the intake of hBD2 from breast milk gains importance. Notably, intestinal hBD2 levels correspond to both defensin in breast milk[7,8,10] and secretions of intestinal epithelial cells. The latter are induced by catalytic antibodies contained in breast milk, acting through protease-activated receptor-2 present in the plasma membrane.[11]

The concentrations of total and specific components of human breast milk vary between mothers, diurnally, based on infant's gestational age, and over the course of lactation.[10,12] Presence of intact hBD2 has also been reported in infant feces, which shows that hBD2 tends to