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Total proteolytic activity and concentration of alpha-1 antitrypsin in meconium for assessment of the protease/antiprotease balance

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ABSTRACT

Background: During intrauterine life, various proteolytic enzymes and their main inhibitor, alpha-1 antitrypsin, accumulate naturally in meconium. A protease/antiprotease balance is required to maintain the biological stability of the environment in which the fetus develops.
Methods: The pool of active proteases was determined using the EnzChek Protease Assay Kit. The concentration of alpha-1 antitrypsin in meconium was measured by enzyme-linked immunosorbent assay. Serial portions of meconium ($n = 80$) were collected from healthy full-term neonates ($n = 19$).
Results: Mean concentrations of active proteases and alpha-1 antitrypsin were 1.55 [standard deviation (SD) 1.3] mg g^{-1} (range 0.15–6.17) and 3.72 (SD 1.78) mg g^{-1} (range 0.76–8.55), respectively, with significant correlation ($R_s = 0.32$, $p = 0.004$). A significant increase in the concentration of active proteases was found between the first and last meconium portions ($p < 0.05$). The proteases in the last meconium portions had a higher reaction velocity and affinity for the substrate than the proteases in the first meconium portions. The active protease:alpha-1 antitrypsin ratio was <0.5 in all first meconium portions, but was higher in the last meconium portions.
Conclusions: Strong correlation between the concentrations of active proteases and alpha-1 antitrypsin in meconium may indicate their mutual interaction in the intrauterine environment. Alpha-1 antitrypsin maintains the protease/antiprotease balance during fetal development.

Introduction

The homeostatic balance between proteases and antiproteases is an important condition for normal functioning of all living organisms. Activation of proteolytic enzyme cascades and their inhibitors influences a number of physiological and pathological processes, including effects on reproduction and development, host defence, haemostasis, inflammation and cancer [1–3].

The protease/antiprotease balance within the intrauterine environment in which the fetus develops remains poorly understood, as few studies have investigated the individual proteases involved in metabolic processes occurring in the fetus [4–6]. Proteases play a critical role in the initiation of protein digestion, activating proteolytic zymogens in the gastrointestinal tract, but massive proteolytic activity may induce intestinal inflammation. Intestinal proteolysis occurs through the combined actions of luminal and brush border enzymes, as well as enteric bacterial degradation [7].

Assessment of the intrauterine protease/antiprotease balance is significantly limited by the invasive receive of the clinical material. Meconium, the first stool passed by a neonate, is a specific and easily obtained biological material, and its components are accumulated exclusively during life in utero. Meconium is deposited in layers in the fetal intestine, starting from 12 weeks of gestation, and is excreted by healthy neonates in portions over the first days after birth [8].

Little is known about total protease activity, although high concentrations of alpha-1 antitrypsin (alpha-1AT) have been measured in meconium [9]. Alpha-1AT is synthesized and secreted predominantly by hepatocytes, and in smaller amounts by other cells such as neutrophils, macrophages, mononuclear phagocytes and enterocytes. It can also be produced locally. Alpha-1AT is a

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neutrophil elastase inhibitor, but also inhibits other proteases [10–12].

The aim of this study was to demonstrate kinetic changes in total proteolytic activity in serial portions of meconium, and to assess the effect of alpha-1AT concentration on the protease/antiprotease balance in meconium by correlation analysis.

Patients, materials and methods

Patients

Nineteen full-term neonates of normal pregnancies, born in the Clinical Department of Obstetrics, Female Diseases and Gynecological Oncology, Central Clinical Hospital of the Ministry of the Interior, Warsaw, were included in this study. Mean maternal age was 32.6 [standard deviation (SD) 3.4] years (range 26–39).

Characteristics of neonates

The mean gestational age of the 19 neonates (seven females and 12 males) included in this study was 38.6 (SD 1.3) weeks (range 36–41). Eight were delivered vaginally and 11 were delivered by caesarean section. Apgar scores at 1, 3, 5 and 10 min were 10, 10, 10 and 10 ($n = 15$); 9, 10, 10 and 10 ($n = 1$); 9, 9, 10 and 10 ($n = 1$); 8, 9, 10 and 10 ($n = 1$); and 9, 9, 9 and 9 ($n = 1$), respectively. Mean birth weight was 3317.4 (SD 553.5) g (range 2040–4280).

The main inclusion criteria for this study were an Apgar score >9, which was considered as evidence of good health, and passing at least two portions of meconium. Good maternal health during pregnancy was another factor taken into consideration.

Materials

All portions of meconium passed by the neonates were collected in the hospital ward. All meconium portions ($n = 80$) were collected serially from the 19 neonates in this study in a hospital setting under a physician's supervision. Meconium has a characteristic gel-like consistency and dark-green/black colour. The collection of meconium was considered to be complete when the consistency and colour had changed to that of stool. A physician (neonatologist) determined the end of the meconium collection period following a visual inspection of samples. For each sample, all meconium was collected from the nappy using a disposable spatula, and transferred into a plastic container (50 ml). The empty containers were weighed prior to adding meconium and reweighed after filling. The date and time of each collection were recorded. The meconium was kept frozen at −20 °C for approximately 3–5 days to facilitate collection in the maternity ward, and subsequently stored at −80 °C. Prior to assay, the samples were thawed over 12 h at 4 °C. Analytical-grade distilled water was added to each meconium sample in three stages (10 ml, 10 ml and up to a homogenate volume of 45 ml). After each addition of distilled water, the homogenate was shaken thoroughly, using a 358S Shaker at 350 cpm, in a horizontal position for 10, 15 and 30 min, respectively. The homogenates were used for the determination of proteases and alpha-1AT.

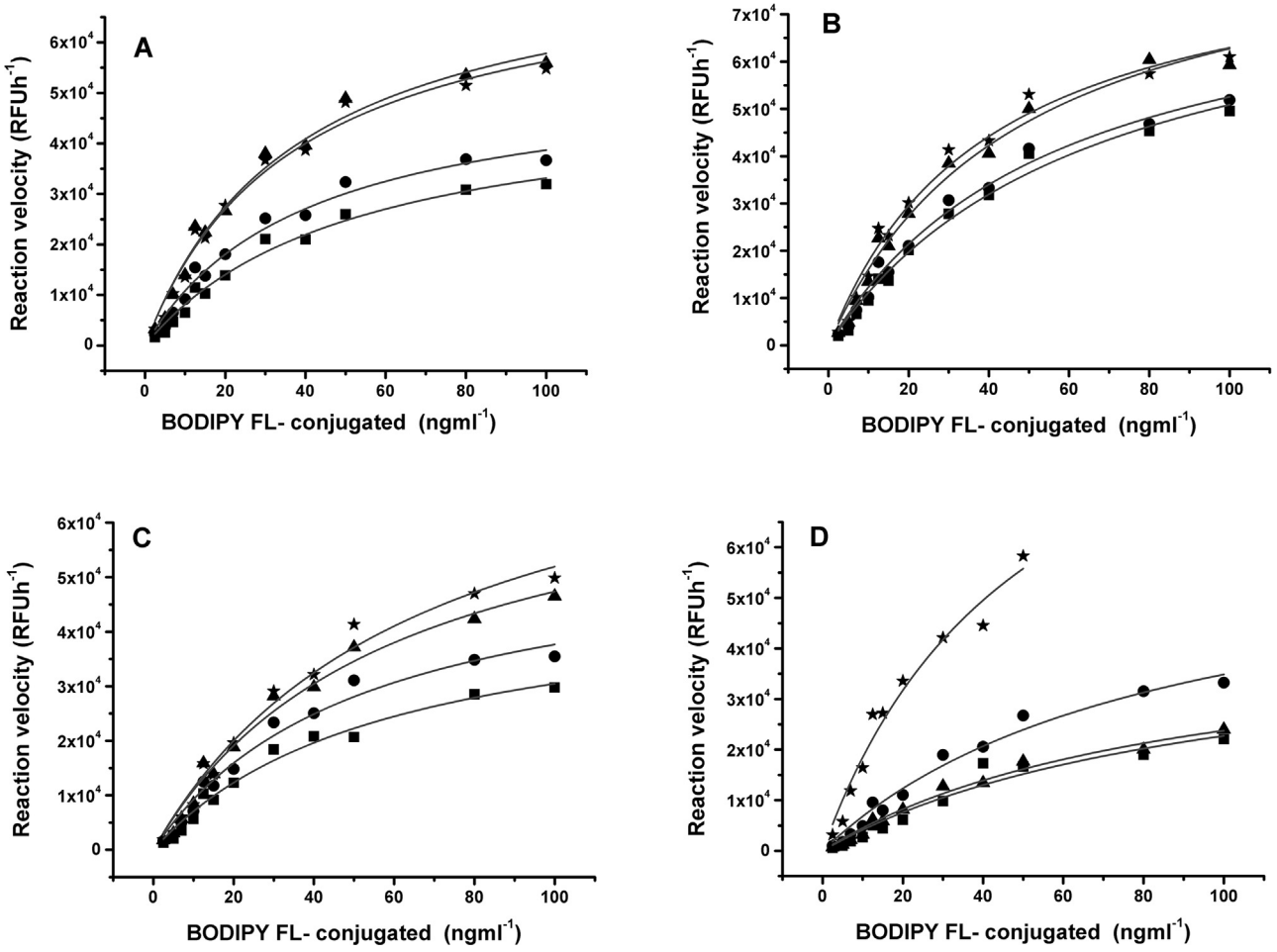

Fig. 1. Reaction velocity (v) as a function of the substrate concentration (BODIPY FL-conjugated) for active proteases present in serial meconium portions from Neonates A, B, C and D. Meconium portions: ■, first; ●, second; ▲, third; ★, fourth. RFU, relative fluorescence units.

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