DENSITOMETRIC ANALYSIS OF ALKALINE PHOSPHATASE IN THE DUODENAL AND JEJUNAL ENTEROCYTES OF 8-WEEK-OLD PIGLETS WITH RETARDED GROWTH

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The activity of alkaline phosphatase in the duodenal and jejunal enterocytes was determined in 8-week-old clinically healthy piglets with normal (12 animals, average body weight 15.30 ± 0.49 kg) and retarded growth (12 animals, average body weight 5.30 ± 0.75 kg). A densitometric analysis was used. A marked decrease in an average density of the alkaline phosphatase (P < 0.001) was observed in the animals with retarded growth. This was in contrast with the results from piglets with normal growth. The obtained results point to a relationship between the amount of the investigated enzyme found in the microvillous zone of enterocytes and the functional state of enterocytes.

Key words: piglets, alkaline phosphatase, densitometric analysis, enterocytes.

The role of the enzyme histochemistry is to determine localisation of these compounds in tissues, cells and cellular organelles. Quantification of enzyme activity is possible by the semiquantitative and quantitative methods in which densitometer image analysis is used. These methods are frequently used in different clinical fields. Of course, the possibilities are wider; e.g. in nephrology, myopathology, oncology and toxicology (2, 3, 5, 10).

Failure of absorption of nutrients from the intestinal tract results in clinical manifestations generally called malabsorption syndrome. The clinical signs most evident in animals are persistent gastrointestinal upset, change in eating habits and weight decrease. In some cases steatorrhea may be observed.

The objective of this study was to determine the activity of alkaline phosphatase (AP) in the duodenal and jejunal enterocytes in 8-week-old healthy piglets with normal growth and piglets with retarded growth, by the use of a densitometric analysis.

Material and Methods

Experimental animals. Twenty-four 8-week-old Slovak White x Landrace crossbreed piglets reared on a large-capacity farm were used in this experiment. The animals showed no clinical symptoms of sickness and had negative bacteriological
findings. The animals were divided into two groups of 12 animals each. The control group (1st group) consisted of clinically healthy piglets with normal growth (mean body weight 15.30 ± 0.49 kg). Piglets with retarded growth (mean body weight 5.30 ± 0.75 kg) were included into the 2nd group.

**Sampling.** Piglets were sacrificed by the jugular incision, and samples of the duodenal and jejunal were taken. The samples were frozen in cold petroleum ether at –20 °C. Sections of thickness 7 µm were cut in a cryostat (Cryocut 27 000) at a cabinet temperature -21 °C. Sections were picked onto clean glass slides (0.96 to 1.06 mm of thickness) and stored in the cryostat cabinet until used. The cryostat sections were allowed to dry for 5 min at 37 °C and incubated for alkaline phosphatase. From each tissue segment six sections were cut for the enzyme assay.

**Enzyme incubation.** The demonstration of alkaline phosphatase activity was performed using a modified simultaneous azo-coupling method (9). The incubation medium contained naphthol-AS-MX-phosphate (Fluka, Germany), stable diazonium salt Fast Blue BB (Sigma, USA), and veronal acetate buffer (pH 9.2). The incubation was performed at 37 °C for 30 min.

**Postfixation.** After incubation, the sections were rinsed with distilled water in order to stop the reaction. The postfixation of the section was performed in a solution of 4% (v/v) formaldehyde for 10 h at room temperature. The sections were rinsed in distilled water and mounted in glycerine jelly.

**Densitometric analysis.** Enzyme activity was analysed cytophotometrically with the Vickers M85a microdensitometer. The measurements were carried out by means of an x 40 objective, and effective scanning area of 28.3 µm² and scanning spot of 0.5 µm. The integrated absorbance was measured at a wavelength of 480 nm. The mask was set over at least 30 brush border areas along the villus length (from the cryptal parts to the tip) in the jejunal sections. AP activity was calculated as the absorbance values recorded by the instrument in min/mm³ brush border ± SD. The data obtained were used to determine the average density of reaction product (Dx) in the investigated samples.

**Statistical analysis.** The results were analysed statistically by one-way analysis of variance (ANOVA). Significance of the differences between the means was calculated by Tukey’s test.

**Results**

The microdensitometric evaluation of AP activity is summarised in Table 1. Taking into consideration the values of average density (Dx), in the piglets with normal growth, a considerable (P<0.001) decrease in the microdensitometric parameter of alkaline phosphatase activity was observed in the piglets with retarded growth.

Activity of alkaline phosphatase in the microvillous zone of enterocytes in all experimentally animals are given in Figs 1 and 2. Comparison of the group 2 (piglets with retarded growth) with its control (healthy piglets with normal growth) revealed that the activity of AP was significantly decreased (P<0.001) in duodenal as well as jejunal enterocytes. Figs 1 and 2 show the AP activity along the villus axis of the jejunum. The final reaction product observed in the brush border of the jejunal epithelial cell showed a substantially higher activity in healthy piglets with normal growth (Fig. 1) than in the piglets with retarded growth (Fig. 2).
Table 1
Microdensity of duodenal and jejunal enterocytes in 8-week-old clinically healthy piglets with normal and retarded growth.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Piglets with growth</th>
<th>Duodenum Dx(^1) (\bar{X} \pm SD)</th>
<th>Jejunum Dx (\bar{X} \pm SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline</td>
<td>normal</td>
<td>85.29 (\pm 4.32)</td>
<td>79.36 (\pm 3.29)</td>
</tr>
<tr>
<td>phosphatase</td>
<td>retarded</td>
<td>33.28 (\pm 2.43^*)</td>
<td>30.73 (\pm 4.28^*)</td>
</tr>
</tbody>
</table>

\(^1\)average density of reaction product, \(^2\)standard deviation, \(^*\)P<0.001

Figure 1
The light micrograph of jejunal alkaline phosphatase activity in a 7 \(\mu\)m cryostat section in healthy piglets with normal growth (obj. 10, diaphragm 6.3). The final reaction product is found in the brush border of the cells along the villus axis.

Figure 2
The light micrograph of jejunal alkaline phosphatase activity in a 7 \(\mu\)m cryostat section in piglets with retarded growth (obj. 10, diaphragm 6.3). The final reaction product is found in the brush border of the cells along the villus axis.
Discussion

The brush border of enterocytes contains many hydrolytic enzymes, e.g. disaccharidases, peptidases, phosphatases (4). The alkaline phosphatase is a brush border representative involved in the active uptake of nutrients (1, 8, 12). The alkaline phosphatase activity in the small intestine displays circadian fluctuations closely related to food intake. It markedly decreases after food deprivation as well as, when food-deprived rats are given, an increased food ratio (11).

Thompson (13) observed, that malabsorption syndrome usually results from short atrophic villi with flattened epithelium at the surface, and the lesion may be caused by many etiological factors.

The results obtained in our experiment point to a high activity of alkaline phosphatase in normal piglets which is in accordance with the results obtained by Lenhardt and Dudriková (6). A decreased alkaline phosphatase activity was recorded in piglets with retarded growth. These results are similar to those obtained from piglets with spontaneous and experimental viral, bacterial, and protozoal enteritis (7).

Disturbance of the functional state of the digestive tract, accompanied by malabsorption and body weight decrease, occurs during a reduction of alkaline phosphatase activity in the microvillous zone of enterocytes caused by the disease mentioned above. The decrease of alkaline phosphatase activity in our experiments allows assuming a similar degree of functional conditions in the digestive tract in animals with retarded growth.

The obtained results point to a relationship between the amount of the investigated enzyme found in the microvillous zone of enterocytes and the functional state of enterocytes. The data can be used in the diagnosis of the growth disturbances in food-producing animals.

References


