Butyric acid, the product of bacterial fermentation of complex carbohydrates in the large intestine, is a preferred energy source for normal colonocytes and stimulator of their proliferation in vivo. Butyrate has also been shown to inhibit proliferation, augment differentiation and induce apoptosis in a number of colorectal tumor cell lines (1). Reactive oxygen species (ROS) are involved in many physiological and pathological processes, such as cell proliferation control, apoptosis and gene expression induction. An overproduction of ROS or their limited inactivation may cause cellular damage resulting in genomic changes and carcinogenesis process induction. Among several enzymatic and non-enzymatic defense mechanisms that have evolved against reactive oxygen species, superoxide dismutase (SOD) is thought to provide a primary line of defense by catalyzing the dismutation of the one-electron reduction product of oxygen $O_2^·$ to $H_2O_2$ and $O_2$, and glutathione peroxidase (GPx) is involved in degradation of $H_2O_2$ to $H_2O$ (2). The aim of this study was to evaluate the impact of sodium butyrate (NaB) on antioxidative enzymes GPx and SOD activity in colon cancer cell line Caco-2.

**EXPERIMENTAL**

Caco-2 cells were purchased from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The cells were grown in Minimum Essential Medium (MEM) supplemented with 10% FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, and 10 mM HEPES. The cell cultures were maintained at 37°C in an atmosphere of 5% CO$_2$/95% air. In order to evaluate the impact of NaB on GPx and SOD activity, the cells were plated into tissue culture dishes at an initial density $1 \times 10^6$ cells/dish. The cells were incubated for 3 days to adhere to the plates, and then they were exposed for 4 days to 1mM and 10 mM NaB. Afterwards, the adherent cells were washed with phosphate-buffered saline, harvested and sonicated. Cell supernatants were used for GPx and SOD activity assay with the use of Ransod and Ransel kits (Randox). The Bradford Reagent (Sigma) was used to determine concentration of proteins. Morphology of cells nuclei was evaluated based on their characteristic changes visualized after staining with 5 µg/mL acridine orange and observed under fluorescence microscope.

**Figure 1.** Effect of different concentrations of NaB on SOD (A) and GPx (B) activity in Caco-2 cell lines. Colonocytes were incubated with NaB for 4 days. Each bar represents the mean ± S.D.; (n = 3); p < 0.05 (ANOVA).
RESULTS AND DISCUSSION

Epidemiological and experimental studies support the involvement of short chain fatty acid in colon physiology and the protective role of butyrate on colon carcinogenesis (1). Among the possible mechanisms by which butyrate may exert its anti-carcinogenicity, its antioxidant activity has been recently suggested. Rosignoli et al. (3) studies showed that pre-incubation of the cells with physiological concentration of butyrate reduced H2O2-induced damage in human colorectal cells. Courtois et al. (4) showed that butyrate inhibited membrane lipid peroxidation in Caco-2 cells which has been induced by coadministration of LPS or iron ascorbate. Our study showed differential influence of NaB on antioxidative enzymes SOD and GPx activity in colorectal Caco-2 cell lines. The cells incubated with 1 mM NaB were characterized by higher GPx activity compared with a control, however, 10 mM NaB caused two-fold decrease in GPx activity (Figure 1A). Decreased GPx activity at 10 mM NaB concentration probably resulted from GPx protein damage. The highest SOD activity was observed at 10 mM concentration of NaB (Figure 1B). At lower concentration of NaB there was no statistically significant difference between a study probe and a control probe.

Cellular differentiation of NaB-treated cells was evaluated by measuring of alkaline phosphatase activity. The alkaline phosphatase is a brush border membrane-associated hydrolase. Its activity in human colorectal cancer cell lines provides useful information regarding the differentiation of these cells. NaB augmented differentiation of colonocytes. ALP activity in Caco-2 cell lines cultured with 1 mM NaB for 4 days was two-fold higher compared to control. The highest activity of the enzyme was found in cells incubated with 10 mM NaB (Figure 2). Dzierewicz et al. (5) as well as Hague et al. (6) reported that incubation of colorectal cancer cells with high concentrations of NaB caused mass cell apoptosis and their detachment from the substrate. Therefore we hypothesize that cells that remained alive after 4 days in the presence 10 mM NaB were a selected population of cells relatively resistant to proapoptotic activity of butyrate. Mariadason et al. (7) revealed that differentiated Caco-2 cells are relatively resistant to NaB and this could explain high activity of ALP in colonocytes treated with 10 mM NaB. We hypothesize that high activity of SOD in differentiated cells could partially explain their resistance to proapoptotic action of NaB.

The results of our study showed that butyrate induced differentiation and apoptosis of Caco-2 cells are accompanied by alterations in antioxidative enzymes activity.

REFERENCES