Liver cancers and glioblastoma are both resistant to chemotherapy. The most effective treatment includes the combination of surgery, chemotherapy and radiation. Sodium butyrate (NaB) demonstrates a high efficiency and low toxicity. It inhibits proliferation and cell cycle of the cancer cells. The aim of this study was to investigate the effect of sodium butyrate on HepG2 and C6 cell line viability. Hepatocellular cancer (HepG2) and glioblastoma cell line (C6) were cultured in DMEM/Ham’s F12 medium with 10% FBS and antibiotics. Different NaB concentrations (0-10 mM) were tested. The control consisted of cells without tested substance. The incubation times were 24 and 48 h. Cell viability was studied using Trypan Blue exclusion test. Inhibitory influence of sodium butyrate on cell viability in both examined cell lines was confirmed. Strong correlation between NaB concentration and cell viability after 24 h was noticed (correlation coefficient was 0.94 and 0.98 for C6 and HepG2, respectively). IC₅₀ values after 24 h were 8.44 mM and 6.17 mM for C6 and HepG2, respectively. The strongest effect was observed after 48 h of incubation with NaB. IC₅₀ values were 3.44 mM and 1.47 mM for C6 and HepG2 (correlation coefficients after 48 h were 0.91 and 0.631 for C6 and HepG2, respectively). C6 line was more resistant to NaB than HepG2. Both cell lines were sensitive to NaB treatment, which gives the promise that NaB can be used against a broader spectrum of neoplasms in the future.

Keywords: sodium butyrate, cytotoxicity in vitro, liver cancer, glioblastoma

The aim of this study was to investigate the effect of sodium butyrate on HepG2 and C6 cell line viability.

EXPERIMENTAL

Cell lines
Two cancer cell lines were used in the experiment: liver cancer cell line HepG2 and glioblastoma cell line C6. HepG2 cell line was obtained from ATCC. The C6 cell line was kindly provided by Prof. A. Grzanka, Nicolaus Copernicus University in Bydgoszcz (Poland). Cells were grown in sterile tissue culture plastics from Greiner (Germany) with growth surface 25 cm² or 24-well plates.

Both cell lines were cultured in a medium which contained Dulbecco’s Modification of Eagle’s Medium (DMEM) and Ham’s F12 Medium (3:1). DMEM/Ham’s F12 medium was supplemented with 10% fetal bovine serum (FBS), amphotericin B (5 µg/mL), penicillin/streptomycin + L-glutamine (100 U/mL / 100 µg/mL). DMEM/Ham’s F12 medium, fetal bovine serum (FBS) and antibiotics solution were obtained from PAA (Austria). Cells were cultured at 37°C in a humidified atmosphere comprised of 5% CO₂ and 95% air.
To investigate effect of different sodium butyrate concentrations (0-10 mM), HepG2 and C6 cells were seeded on 24-well plates at a density of $5 \times 10^4$ per 1 cm$^2$. Sodium butyrate was purchased from Sigma (Germany).

Cells grew in complete medium for 24 h. Then the culture medium was removed and sodium butyrate was added in tested concentrations. The incubation time with sodium butyrate was 24 and 48 h. The proper sodium butyrate concentration was achieved by dissolving it in complete culture medium just before the use. The control cells were cultured without NaB.

After 24 and 48 h of incubation, NaB was removed and each well was washed 2 times with buffered saline to remove the whole culture medium. Then, HepG2 and C6 cells were detached by trypsinization (Trypsin/EDTA, PAA, Austria). The number of viable cells was determined by staining cell population with 0.4% trypan blue (Sigma, Germany). Cells were counted in Neubauer chamber under inverted microscope (Nikon, Japan). The results obtained with trypan blue exclusion test cor-

<table>
<thead>
<tr>
<th>Inhibitory concentration</th>
<th>24 h incubation</th>
<th>48 h incubation</th>
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<tbody>
<tr>
<td></td>
<td>C6</td>
<td>HepG2</td>
</tr>
<tr>
<td>IC$_{10}$</td>
<td>1.44 mM</td>
<td>1.33 mM</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>8.44 mM</td>
<td>6.17 mM</td>
</tr>
<tr>
<td>IC$_{90}$</td>
<td>1.89 mM</td>
<td>1.67 mM</td>
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*NA – not assessed.

Figure 1. HepG2 and C6 cell viability after 24 h of incubation with sodium butyrate.

Figure 2. HepG2 and C6 cell viability after 48 h of incubation with sodium butyrate.
related to MTT assay in monolayer cultures (11). Cytotoxic effect of sodium butyrate was calculated in relation to control. Viability was shown as percent of living cells in the experimental groups to control. Correlations between cell viability and NaB inhibitory concentrations (IC_{10}, IC_{50}, IC_{90}) were determined.

RESULTS AND DISCUSSION

In this study two cancer cell lines were examined (HepG2 and C6). Both of them are examples of chemotherapy resistant tumors. Hepatocellular carcinoma (HepG2) is an in vitro model of human liver cancer. Glioblastoma cell line (C6) has been commonly used as an in vitro glial model system due to its glial like properties (12).

The goal of anticancer treatments is to selectively eliminate tumor cells with minimal general toxicity (9). Sodium butyrate, which is naturally occurred agent, has various pleiotropic stimulatory effects at physiological concentrations. Therefore, it is used as a tool for cell growth experiments (4). Normal colonic tissue tolerates butyrate concentration of 10-60 mM (13). Gibson et al. reported that butyrate suppressed proliferation of neoplastic cells but had no effect on normal epithelial cells (14).

Sodium butyrate was one of the first clinically used histone deacetylase inhibitor (4, 8). In this experiment, its cytotoxicity was also proved. Histone deacetylase inhibitors such as NaB have been shown to express anticancer activity. NaB inhibits cell growth and induces apoptosis (4, 15). These properties make it a good candidate for cancer management. Previous works reported that NaB decreased cell growth in a dose depended manner (8). HepG2 and C6 cells viability after 24 and 48 h of incubation with sodium butyrate was lower than in control samples. Figures 1 and 2 also present dose depend inhibition of NaB on the tested cells. Viability of examined cells decreased with increasing incubation time. On the basis of diagrams IC_{10}, IC_{50}, and IC_{90} values were calculated (Tab. 1). Nearly the same values of IC_{10} after 24 h of incubation for C6 and HepG2 were observed. Correlation coefficients after 24 h NaB treatment were 0.94 and 0.98 for C6 and HepG2, respectively. There were no differences between viability of both cell lines after 24 h of incubation with NaB. Cytotoxic effect of NaB was stronger after 48 h of incubation. These results confirm those obtained by Hirsch et al. who clearly reported association between increasing exposure time and decrease of the cell viability (8). In this study it was shown that the hepatocellular cancer line (HepG2) was more sensitive to sodium butyrate. IC_{10}, IC_{50}, and IC_{90} values for glioblastoma cell line after 48 h incubation time with sodium butyrate were twice as big as for hepatocellular cancer line. A strong correlation between NaB concentration and cell viability was observed, which is the proof that NaB acts in a dose dependent manner. After 48 h treatment correlation coefficients were 0.91 and 0.631 for C6 and HepG2, respectively. There is no efficient treatment of hepatocellular carcinoma and glioblastoma, till now. However, recently histone deacetylase inhibitors such as sodium butyrate are promising antineoplastic factors for adjuvant chemotherapy due to their ability to induce cell differentiation, growth arrest and apoptosis. NaB might be very useful for cancer treatment having in mind its mild toxicity and presence in biological systems (16).

REFERENCES

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