

EXPERIMENTAL SUBHEPATIC OBSTRUCTIVE JAUNDICE AND BCL-2 GENE EXPRESSION

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Maintaining homeostasis of organs and tissues at all levels of organization of living matter is provided by the balance of processes of cell death and renewal. The increased expression of the Bcl-2 gene, blocking apoptotic death, prolongs cell survival [2, 3]. Objective. To evaluate the role of endogenous intoxication in the regulation of Bcl-2 antiapoptotic gene expression in the dynamics of experimental subhepatic obstructive jaundice.

Materials and methods. The experiment was carried out in accordance with the ethical norms of animal handling and the requirements of the European Ethical Committee Directive 86/609/EEC as of 24.11.1986 and the rules of the "European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes" as of 18.06.1986. The study was performed on 146 white random-bred male rats weighing 250 ± 50 g. Subhepatic obstructive natural jaundice (duration 1, 3, 5 and 10 days) was simulated in rats of the first ($n = 18$), second ($n = 18$), third ($n = 18$) and fourth ($n = 20$) groups by ligating of the common bile duct (CVD) in the liver gate area with the following transection of the duct between two silk ligatures under ether anesthesia. Sham-operated animals served as a control group. All animals were kept in individual cells with free access to water and food. At the end of the experimental period, the animals were decapitated after preliminary ether anesthesia. Total RNA was isolated from 1 ml of whole blood using SV Total RNA Isolation System (Promega, USA) according to the manufacturer's protocol. The quantitative assessment of the isolated RNA samples was performed by spectrophotometric method on NanoPhotometer P360 (Implen, Germany). The level of Bcl-2 gene expression was measured by the real-time PCR (PCR-RB) method using the commercial GoTaq 1-Step RT-qPCR Run kit. The amplification response was performed in two repetitions for each sample using the PCR-PB protocol with the use of intercalating fluorescent agents (SYBR Green). PCR-PCR-RV was performed with the help of the Rotor Gene device ("Qiagen N.V.", Germany). PCR protocol: 37C for 30 min, 95C - 10 min; 40 cycles - 10 s at 95C, 20 s at 60C (15 s at 58C for reference gene), 30 s at 72C. The primers were developed using Primer3 Engine Software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) and synthesized by ALC Prime-Tech (Minsk). Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) was used as a reference gene. The relative level of gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method according to [1]. The experimental data was statistically processed using Statistica 8.0 (StatSoft Inc.) and Prism 5 for Windows (GraphPad Software Inc.) software packages, as well as RStudio integrated development environment with "R" version 3.4. For data processing, the two-sided unpaired Student t-criterion was used in case of normal distribution of data in the sample and equality of sample dispersions. If the hypotheses about the normal distribution of data in the samples were rejected, the two-sided unpaired Wilcoxon-MannWhitney criterion was used to compare the samples by the attribute level and the Flynn-Killin criterion for nonparametric comparison of variations (scales) of distributions. Differences were considered statistically significant at $p < 0.05$.

Results. No statistically significant changes at the level of Bcl-2 gene expression were observed after 24 hours of jaundice. A significant increase in the level of Bcl-2 gene expression is already observed at 72-hours of obstructive subhepatic jaundice - on average 2.6 times higher ($p = 0.0376$). After 5 days from the beginning of the modeling of subhepatic obstructive jaundice activation of the Bcl-2 gene expression increases to

an average of 5.8 times ($p=0.0019$). After 10 days of the experiment, the relative level of Bcl-2 gene expression started to decrease gradually, yet still remaining increased 3.2 times ($p=0.036$) on average.

Conclusion: A 10-days subhepatic obstructive jaundice leads to the development of biliary endogenous intoxication, which is accompanied by an increase in the relative level of expression of the BCL-2 antiapoptotic gene, thus blocking the development of apoptosis, the degree of severity of which depends on the duration of the cholestasis.

References

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