Expression of Intracellular Molecular Apoptosis Regulator Bcl-2 in the Liver in Isolated and Combined Exposure to 24-h Illumination and Industrial Frequency Magnetic Field

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We studied the effect of isolated and combined exposure to 24-h illumination and industrial frequency magnetic field on the expression of intracellular molecular apoptosis regulator Bcl-2 in the liver. Previous experiments showed enhanced expression of Bcl-2 protein in liver cells in animals maintained under conditions of 24-h illumination, whereas exposure to industrial frequency magnetic field had practically no effect on this process. Under conditions of combined exposure to 24-h illumination and industrial frequency magnetic field, magnetic field partially suppressed the expression of Bcl-2 induced by 24-h illumination.

Key Words: *liver; 24-h illumination; industrial frequency; apoptosis; Bcl-2*

According to modern views, apoptosis (programmed cell death) can be triggered by various internal and external signals [6-9,11,12]. Elimination of old or excessive cells and cells with differential and genetic abnormalities in organs is effected via activation of apoptosis [1,3,4]. It is known that viability of cells during pathogenic exposure depends on the ratio between activators and inhibitors of apoptosis, The key role in the regulation of apoptosis is played by proteins of the Bcl-2 family (products of the corresponding genes).

Electromagnetic fields are a factor of environmental pollution leading (according to WHO data) to the development of cancer, Alzheimer disease, immunodeficiency, *etc*. Shifts in biological rhythms (desynchronosis) developing due to changes in the illumination regimen (night duties, long-distance flights, shift works, *etc*.) are also unfavorable factors and the effects of these antropogenic factors are increasing in industrial society. However, the effect of industrial frequency magnetic field (MF) against the background of disturbed illumination regimen on apoptosdis processes was never studied.

We studied the effect of individual and combined exposure to 24-h illumination (24-IL) and MP on Bcl-2, an important apoptosis regulator in the body.

MATERIALS AND METHODS

Experiments were carried out on mature Wistar rats (body weight 200 g, age 2.5 months, 7-8 animals per group). The animals were divided into 4 groups. Group 1 (controls) comprised animals maintained under normal conditions (day/night alternation), group 2 animals were kept under conditions of 24-IL for 14 days, group 3 rats were exposed to MF

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for 14 days under conditions of normal day/night regimen, and group 4 animals were exposed to both 24-IL and MF for 14 days.

We used single-step immunohistochemical method of detection of antiapoptotic protein Bcl-2 on paraffin sections (a variant of streptavidin-avidin method) [2,4]. Stained sections were examined under a MS300A microscope. Images obtained using a Baumer optronic CX13c digital camera were processed using Video-test Morfo-4 software.

Throttles for fluorescent lamps were used as the source of MF (exposure 23 h per day for 14 days). The magnitude of aperiodic MF amplitude oscillations (in the near-hertz range) during experimental exposures was 5-10 μT. Fluorescent lamps served as the source of 24-h illumination (300 lux illumination intensity).

The animals fed standard ration and had free access to water and food. After 14 days, the animals were decapitated.

The data were processed statistically using the Student and Mann—Whitney tests (SPSS software).

RESULTS

The most intensive staining of liver sections was observed in animals maintained under conditions of 24-IL. In the majority of hepatic lobules, the marker was located in hepatocytes and sinusoidal cells. Enhanced expression of Bcl-2 was observed in lymphocytes, bile duct epithelium, and endothelium of interlobular arteria and veins, central and sublobular veins (Fig. 1, *a*).

In the majority of preparations from animals exposed to MF (Fig. 1, *b*), mosaic pattern of staining (lobules with high and low affinity to the dye) was noted. The most intensive staining for Bcl-2 was found in hepatocytes and sinusoidal cells located near the central veins and near the triads. Areas with destructed liver cells and disturbed cord structure were found. These areas were characterized by more intensive staining.

In animals exposed to 24-IL+MF, staining of liver sections was less intensive (Fig. 1, *c*) compared to the control and effect of MF alone. In the majority of hepatic lobules, the marker was located in hepatocytes and sinusoidal cells Enhanced expression of Bcl-2 was observed in lymphocytes, bile ducts, and in the endothelium of interlobular arteria and veins, central and sublobular veins.

The area of immunohistochemical staining for Bcl-2 protein was maximum in liver cells of animals maintained under conditions of 24-IL (Fig. 2). Exposure to MF did not change this parameter compared to the control. Combined 24-IL+MF exposure significantly increased the area of Bcl-2 expression compared to the control, but this increase was less pronounced than in rats kept under conditions of 24-IL.

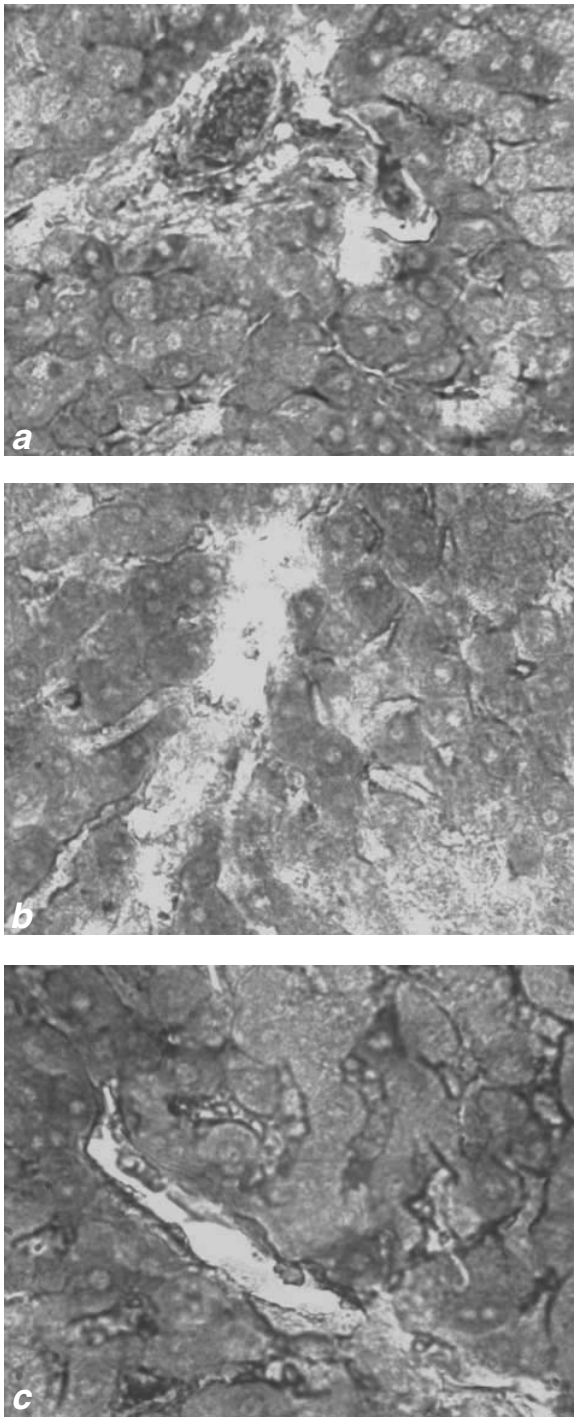

Fig. 1. Detection of antiapoptotic protein Bcl-2 in the liver of rats exposed to 24-IL (*a*), MF (*b*), and 24-IL+MF (*c*). Indirect streptavidin-avidin method, ×400.

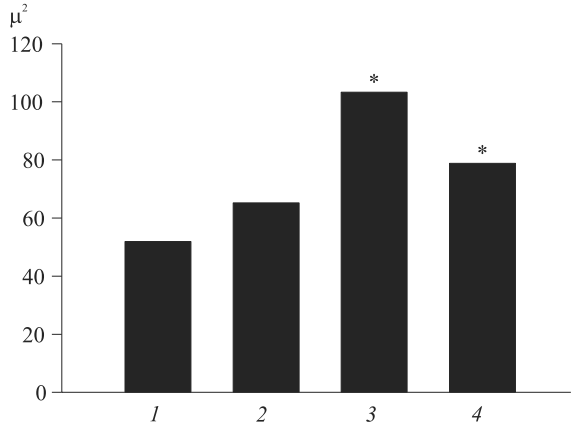

Fig. 2. Changes in the area of histochemical staining of Bcl-2 protein on liver preparations from animals after individual and combined exposure to 24-IL and MF. *1*) control; *2*) MF, *3*) 24-IL; *4*) 24-IL+MF. **p*<0.05 compared to the control.

The causes and mechanisms of the formation of apoptotic signal, its transmission, and realization vary [3,4,9,11,12]. Antiapoptotic proteins, *e.g.* proteins of the Bcl-2 family, counteract the proapoptotic signals [6,8,9]. Enhanced expression of Bcl-2 protein in organs and tissues of the body is usually associated with the appearance of an environmental factor (or a complex of factors) capable of inducing apoptosis, *e.g.* oxidative apoptosis activation pathway [1,3,5,10]. The fact that in our experiments 24-IL and 24-IL+MF induced the synthesis of Bcl-2 protein in liver cells attests to the effect of proapoptotic signals on these cells. This assumption is indirectly confirmed by elevated activity of endonucleases, enzymes promoting DNA fragmentation, in hepatocytes under the effect of MF. This leads to emergency activation of the Bcl-2-dependent antiapoptotic defense system. Combined exposure to 24-IL+MF led to less pronounced production of Bcl-2 protein in the body (compared to 24-IL alone). These findings suggest that MF modulates (reduces) the antiapoptotic effect of 24-IL. It can be hypothesized that combined exposure to adverse factors reduces activity of defense (*e.g.* antiapoptotic) mechanisms.

Thus, in animals maintained under conditions of 24-IL for 14 days, expression of Bcl-2 protein in liver cells increases, while 14-day exposure to MF did not change this parameter. In animals exposed to 24-IL+MF, MF partially suppressed the expression o Bcl-2 protein.

Disturbed light-darkness regimen leading to external and internal desynchronosis modifies the effects of adverse ecological factors (MF), which should be taken into account in the development of hygienic recommendations and during prophylactic medical examinations of individuals exposed to combinations of desynchronizing and other technogenic factors.

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