HEPATOTROPIC ACTIVITY OF A BETULONIC ACID BASED COMPOUND

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Using the model of cyclophosphamide (CP)-induced immunosuppression in C57BL/6 mice, the hepatotropic effects of a conjugate of betulonic acid with 9-(4-methylpiperazin-1-ylmethyl)-2-(1,2,3-triazolyl) oreozelone (BABC) have been studied. In the liver of treated animals the expression of genes for cytochromes (*CYP 1A1*, *CYP 1A2*, *CYP 3A44*, *CYP 2B10*, *CYP 2C29*, *CYP 17A1*), *PPARA*, and cytokines (*TNF-α*, *IL-1β*, *IL-12α*, *IL-10*) and the relative levels of NF-κB p65, GST-π, and NAT-1 proteins were determined. On day six after administration of the compound and CP to animals a significant (3.2-fold) increase in the expression of the *CYP 2B10* as compared to the control group was observed. Treatment of mice with the compound and CP also caused a 2.4-fold increase in the mRNA level of the pro-inflammatory TNF-α gene as compared to the group of animals receiving CP. Administration of the studied compound to intact animals was accompanied by a 2.5-fold increase in the *IL-1β* expression and a 1.8-fold decrease in the *IL-10* expression as compared to the control group. An increase in the expression of pro-inflammatory cytokine genes in the liver of animals treated with the compound was accompanied by an increase in the content of NF-κB p65 (by 1.6 times), as well as an increase in the relative amount of NAT-1 protein (by 2.7 times) as compared to control animals.

Key words: betulonic acid; furocoumarins; liver; proteins; cytochromes; cytokines

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INTRODUCTION

Betulonic acid (BA) and its derivatives are widely known for their immunomodulatory and antioxidant properties. Some BA derivatives exhibit a hepatoprotective effect. BA amides, containing piperidine nitroxide or pyrrolidine nitroxide fragments as substituents (C28), have anticytolytic properties, counteracting developing liver fibrosis development [1]. At the same time, they had a cytotoxic effect on some cell lines [2].

The anti-inflammatory properties of BA have been best studied. However, it was found that some modifications (e.g., introduction of an additional substituent at the C9 position of furocoumarin oreozelone in the BA conjugate), led to the loss of its anti-inflammatory activity [2]. We have shown that the BA based compound, the BA conjugate with furocoumarin 9-(4-methylpiperazin-1-ylmethyl)-2-(1,2,3-triazolyl) oreozelone (BABC) has a potential immunotropic effect, increasing the expression of *GM-CSF* in the bone marrow of animals; it also restores the relative content of T lymphocytes in the blood serum after the induction of immunosuppression [3]. This feature of BABC makes it attractive for the use as an agent restoring the functionality of the immune system between chemotherapy courses. An equally important task is to protect the liver from the hepatotoxic effects of drugs used in chemotherapy. In this context, convincing evidence exists that drug metabolism is often associated with hepatotoxicity. The previously discovered hepatoprotective properties of BA [1] still remain unexplored in relation to the BA interaction with the drug metabolism system.

In this study we have investigated the effect of BABC on the expression of genes and proteins in the liver. The hepatotropic effects of BABC were evaluated using the model of cyclophosphamide (CP)-induced immunosuppression.

Abbreviations used: ALP – alkaline phosphatase; AT – antibody; *β-act* – gene encoding the beta-actin protein; BA – betulonic acid; BABC – betulonic acid based compound; BSA– bovine serum albumin; CP – cyclophosphamide; *CYP* – gene encoding an enzyme of the cytochrome P450 superfamily; DMSO – dimethyl sulfoxide; *GAPDH* – gene encoding glyceraldehyde-3-phosphate dehydrogenase; *GM-CSF* – gene encoding granulocyte-macrophage colony-stimulating factor; GST – glutathione-S-transferase; HCC – hepatocellular carcinoma; HCV – viral hepatitis C; HIF-α – hypoxia-inducible factor alpha; Ig – immunoglobulin; IL – interleukin; NAT-1 – N-acetyltransferase-1; NF-κB – nuclear factor kappa-bi; PCR – polymerase chain reaction; PPAR-α – peroxisome proliferator-activated receptor alpha; RT – reverse transcription; SDS – sodium dodecyl sulfate; TBS – Tris-buffered saline; TLR – toll-like receptors; TNF-α – tumor necrosis factor alpha; VEGF – vascular endothelial growth factor.

It is known that enzymes of the CYP450 superfamily are involved in CP metabolism: these enzymes catalyze 4-hydroxylation and activation of the drug (Fig. 1). The enzymes CYP 2B6 and CYP 2C9 play the main role in this conversion [4]. One of the CP metabolites is acrolein. It lacks the antitumor activity, but leads to side effects in the urinary tract. Common side effects reported in several studies and clinical trials of CP include hemorrhagic cystitis, amenorrhea, myelosuppression, alopecia, and bouts of nausea and vomiting [5].

The metabolism of BA and its impact on the drug metabolism system remain poorly investigated. Rendick and Guengerich have shown that only 5–6 human cytochromes P450 (CYP 1A1, CYP 1A2, CYP 3A4, CYP 2B6, CYP 2C9) are involved in the metabolism of 90-95% of xenobiotics [6]. Therefore, we assessed the orthologous proteins encoded in mice by the genes *CYP 1A1*, *CYP 1A2*, *CYP 3A44*, *CYP 2B10*, *CYP 2C29* as potential participants of the metabolism of BABC and CP [7–12].

In addition, we have also investigated expression of two genes encoding protein products involved in metabolism: *PPARA* and *CYP 17A1*.

PPARA encodes a transcription factor that regulates lipid metabolism in the liver. PPAR-α is activated under conditions of energy deficiency and it is required for the process of ketogenesis, a key adaptive response to prolonged starvation [13].

CYP 17A1 encodes the enzyme 17-α hydroxylase, which catalyzes hydroxylation of pregnenolone and progesterone with formation of 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively. These molecules are precursors in the synthesis of male sex hormones and cortisol, respectively [14]. However, results of recent studies suggest that hepatic 17-α hydroxylase plays a role in carbohydrate metabolism, in particular in maintaining the concentration of glucose and ketone bodies during fasting [15].

The effect of BABC on organ immunity was assessed by the expression of cytokine genes involved in the inflammatory response (*TNF-α*, *IL-1β*, *IL-12α*, and *IL-10*).

TNF-α encodes tumor necrosis factor alpha, a proinflammatory cytokine produced primarily by activated macrophages and lymphocytes. TNF-α plays a protective role against infectious and oncological diseases [16].

IL-1β is another important inflammatory mediator. This cytokine is synthesized primarily in macrophages and monocytes. Its production can, for example, occur after the interaction of toll-like receptors (TLRs), located on the membrane of macrophages, with pathogen-associated molecules. A cascade of reactions occurring as a result of TLR activation leads to the mobilization of the transcription factor NF-κB and the initiation of transcription of the *IL-1β* gene [16, 17].

IL-12α encodes one of the subunits of IL-12, a proinflammatory cytokine produced by activated inflammatory cells: macrophages, dendritic cells, activated lymphocytes, as well as endothelial cells and keratinocytes. The cytokine activates differentiation of T lymphocytes and NK cells and increases their cytotoxic activity; IL-12 also induces production of other proinflammatory cytokines in responsive cells [16].

IL-10 is an anti-inflammatory cytokine, a key regulator of the immune response; it is produced by type 2 T helper cells and T lymphocytes. IL-10 inhibits production of NF-κB as well as pro-inflammatory cytokines (including IL-1β) in leukocytes. At the same time, IL-10 polarizes the immune response towards the humoral immune response by enhancing the production of antibodies [16, 18].

In addition to gene expression, we have evaluated the relative content of the following proteins in the liver of experimental animals.

GST-π (glutathione-S-transferase-π) is a phase 2 enzyme of xenobiotic metabolism, catalyzing the reaction of transfer of reduced glutathione to a substrate molecule. Various xenobiotics exhibit different effects on GST-π production; in this work, we have used dimethyl sulfoxide (DMSO) and CP, which enhance the expression of this enzyme [19]. GST-π is involved in the last stage the metabolism of CP

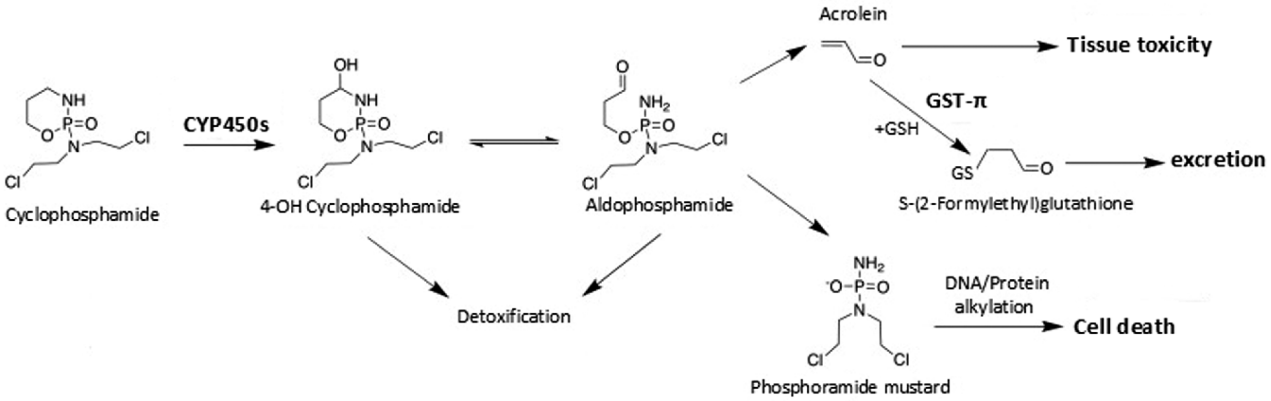

Figure 1. The scheme of cyclophosphamide metabolism.

by attaching a glutathione molecule to acrolein, thus preventing the development of acrolein-dependent toxicity [20].

NF-κB p65 is a transcription factor that forms a heterodimer with NF-κB p50. In its inactive state, it is located in the cytoplasm in a complex with the κB inhibitor. After certain signals (e.g. TLRs binding to components of potential pathogens), the NF-κB-inhibitor complex dissociates and NF-κB migrates into the nucleus, triggering expression of many genes involved in inflammation and antibody production. NF-κB production is enhanced in response to major proinflammatory cytokines such as IL-1β and TNF-α [16, 21].

NAT-1 is one of the main phase 2 enzymes of xenobiotic metabolism. Its main role is acetylation of aromatic amines. In this reaction acetyl-CoA acts as a NAT-1 cofactor [22]. A list of drug substrates for NAT-1 includes dapsone, isoniazid, and sulfonamides [23]. The choice of this protein for analysis is due to the fact that BABC contains aromatic and heterocyclic fragments with a nitrogen atom.

MATERIALS AND METHODS

BABC, the conjugate of BA and furocoumarin 9-(4-methylpiperazin-1-ylmethyl)-2-(1,2,3-triazolyl) oreozelone (Fig. 2), was synthesized in the Laboratory of Medicinal Chemistry of N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS [3].

The creation of the experimental model has been described in our previous work.

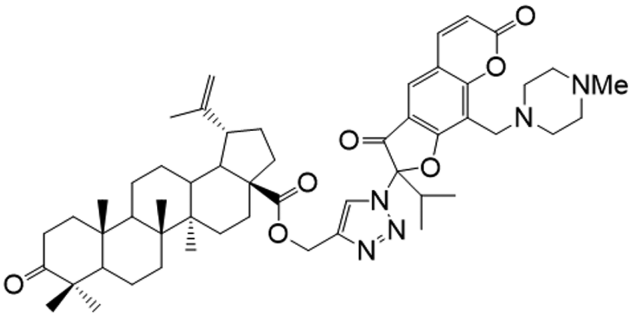

Figure 2. Structural formula of BABC.

Experimental Animals

Experiments were carried out using 3-month-old male C57BL/6 laboratory mice *Mus musculus*, obtained from the vivarium of the Research Institute of Neuroscience and Medicine of the Siberian Branch of the Russian Academy of Sciences. The animals were divided into groups of 6 individuals in each group. The test compound (BABC) was dissolved in DMSO and then diluted in a 1% suspension of starch in water; the concentration of DMSO in the injected mixture was 1%. On day 6 after BABC administration mice were killed by carbon dioxide inhalation and liver samples were isolated (Table 1).

Gene Expression Analysis

Isolation of RNA from biological material was carried out using TRI-REAGENT (MRC, USA), according to the manufacturer's protocol. To process one organ fragment, 1000 μl of the reagent was taken; the cell lysate was extracted twice with 500 μl of a mixture of chloroform and isoamyl alcohol (24:1); phases were separated by centrifugation at 10,000 *g* for 5 min. Next, 500 μl of isopropyl alcohol was added and the total RNA was pelleted by centrifugation at 12,000 *g* for 10 min, and the sediment was washed twice with 80% ethanol; dried and dissolved in double-distilled water containing an RNA secure reagent (Ambion, USA). RNA preparations were stored at -70°C. Reverse transcription was performed using the M-MuLV-RH RT reagent kit (Biolabmix, Russia), according to the manufacturer's protocol: 2 μg of total RNA was taken per reaction, primers with random sequences and oligo-dT primers were added, heated for 5 min at 70°C and transferred to an ice bath for 2 min. Next, a mixture of the remaining reagents (RNA-dependent DNA polymerase, RT buffer, deoxynucleotide triphosphates) was added, incubated for 10 min at room temperature and transferred to a thermostat (42°C) for 2 h. The reaction was stopped by heating for 10 min at 70°C. Quantitative PCR with real-time detection was performed using BioMaster HS-qPCR SYBR Blue (2×) (Biolabmix). The following conditions were selected to allow amplification of the genes selected for the study:

Table 1. The scheme of *in vivo* experiments

Group	Number of animals	Day 1	Day 2	Day 6
Control	6	1% starch + 1% DMSO + H_2O (enterally via tube)	—	Removal of animals from the experiment, preparation of liver samples
CP	6	1% starch + 1% DMSO + H_2O (enterally via tube)	CP (200 mg/kg) intraperitoneally	
BABC + CP	6	BABC (12 mg/kg) in 1% DMSO + 1% starch + H_2O (enterally via tube)	CP (200 mg/kg) intraperitoneally	
BABC	6	BABC (12 mg/kg) in 1% DMSO + 1% starch + H_2O (enterally via tube)	—	

95°C — 5 min; 45 cycles — (95°C — 10 s, 15 s at the appropriate annealing temperature, 72°C — 15 s). *GAPDH* and *β-act* were used as reference genes (Table 2).

Western Blot Analysis

Each liver sample (10 mg) was homogenized and denatured in 1 ml of buffer containing 0.0625 M Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, heated for 15 min at 100°C on the thermostat. Next, β-mercaptoethanol (2% final concentration) and bromophenol blue (0.0005% final concentration) were added to the lysates.

Protein separation by polyacrylamide gel electrophoresis was carried out according to the Laemmli method. Samples of three compared groups ("Control", "CP", "CP+BABC" or "Control", "CP", "BABC") were applied to one gel — three samples from each group per membrane, two membranes for studying the content one protein. After electrophoresis, the proteins were transferred to a nitrocellulose membrane using the semi-dry transfer method (Fastblot device, Biometra; Germany); current strength — 1.5 mA per 1 cm^2 of membrane). Then the membrane was washed with TBS 3 times for 5 min each and the nonspecific sorption centers were blocked by incubating the nitrocellulose membrane in a solution of 5% bovine serum albumin (BSA) in TBS for 1 h at room temperature. After another wash with TBS 3 times for 5 min at 25°C, the membrane was incubated with an antibody (AB) solution against the mouse proteins selected for the study. AB was diluted 1000 times in a TBST solution containing 1% BSA, 0.05% Tween-20, and TBS (×1) for 1.5 h at room temperature with stirring.

During the experiment, the following primary ABs were used: anti-NF-κBp65 (Millipore (USA); concentration 1 mg/ml, host — rabbit), anti-NAT1 (BD Transduction Laboratories (USA); concentration 250 μg/ml, host — mouse), anti-GST-π (BD Transduction Laboratories; concentration 250 μg/ml, host — mouse).

The content of the studied proteins was normalized to the level of β-tubulin, β-actin, and histone H2b proteins. For this purpose, we used ABs to the corresponding proteins from the commercial Western blot loading control kit (ImgenEx, USA), diluted according to the manufacturer's protocol.

Table 2. Target genes, oligonucleotide sequences and their annealing temperatures used in the evaluation of gene expression

Gene	Primer sequences	Annealing temperature
GAPDH	F: 5′-TAGACAAAATGGTGAAGGTCGG-3′ R: 5′-CCTGGAAGATGGTGATGGG-3′	57–62°C
β-act	F: 5′-CCTGAGGAGCACCCTGTG-3′ R: 5′-GGAGAGCATAGCCCTCGTAG-3′	57–62°C
IL-12α	F: 5′-AGTGTGGCACTGATGCTGATG-3′ R: 5′-GTAGCCAGGCAACTCTCGTT-3′	62°C
IL-10	F: 5′-TGGGTTGCCAAGCCTTATCG-3′ R: 5′-CTCTTCACCTGCTCCACTGC-3′	62°C
IL-1β	F: 5′-TGCCACCTTTTGACAGTGATGA-3′ R: 5′-ATCAGGACAGCCCAGGTCAA-3′	58°C
TNF-α	F: 5′-TCAGTTCTATGGCCCAGACC-3′ R: 5′-ACCACTAGTTGGTTGTCTTTGAG-3′	61°C
CYP 1A1	F: 5′-TATCTCGTGGAGCCTCAT-3′ R: 5′-ATCTCTTGTGGTGCTGTG-3′	57°C
CYP 1A2	F: 5′-CCCTTCAGTGGTACAGATG-3′ R: 5′-GACAGGTGTGGGTTCTTC-3′	57°C
CYP 3A44	F: 5′-TTGTGGAGGAAGCCAAAAAGTTT-3′ R: 5′-TGAGAAGAGCAAAGGATCAAAAAAGT-3′	57°C
CYP 2B10	F: 5′-CAATGTTTAGTGGAGGAACTGCG-3′ R: 5′-CACTGGAAGAGGAACGTGGG-3′	60°C
CYP 2C29	F: 5′-CAGATGTCACAGCTAAAGTC-3′ R: 5′-TTTAATGTCACAGGTCACTG-3′	57°C
PPARA	F: 5′-AGCAACAACCCGCCTTT-3′ R: 5′-GACCTCTGCCTCTTTGTCTTC-3′	57°C
CYP 17A1	F: 5′-GATCGGTTTATGCCTGAGCG-3′ R: 5′-TCCGAAGGGCAAATAACTGG-3′	57°C

Then the membrane was washed with TBS 3 times for 10 min at 25°C and kept in a solution of anti-mouse (or rabbit) IgG ABs conjugated with ALP for 1 h at 25°C. Secondary ABs were diluted in TBS 2000 times.

During the experiment, the following secondary ABs were used: Goat anti-mouse IgG alkaline phosphatase conjugated sc-2008 (Santa-Cruz Biotechnology, USA); concentration 400 μg/ml); Bovine anti-rabbit IgG alkaline phosphatase conjugated sc-2372 (Santa-Cruz Biotechnology; concentration 400 μg/ml).

Then the membrane was washed again in TBS 3 times for 10 min at 25°C and stained with a mixture of alkaline phosphatase substrates — naphthol AS-MX phosphate (0.2 mg/ml) and Fast Red Salt (1 mg/ml) dissolved in buffer, containing 50 mM Tris-HCl, 75 mM NaCl, 5 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 8.0.

The results obtained were processed densitometrically using the ImageLab program (Bio-Rad, USA).

Statistical Data Processing

The significance of differences between studied groups was determined using the STATISTICA 12 software package. The statistical significance of differences was assessed using the nonparametric Wilcoxon-Mann-Whitney test.

Differences in values between groups were considered as statistically significant at $p<0.05$.

Gene expression data are expressed as the mean of several independent experiments ± standard deviation (M ± SD). Relative protein abundance data are presented as boxplots showing medians (Me), upper and lower quartiles [Q1–Q3].

RESULTS

The Effects of CP and BABC on the Expression of cytochrome P450 and PPARA Genes in the Liver

It was found that CP and BABC influenced the expression of some genes of the CYP 450 superfamily in the liver (Fig. 3). Administration of CP caused a 2.4-fold increase in the expression of *CYP 1A2* ($p=0.02$) as compared to the control group. A separate administration of BABC insignificant influenced the expression of any of the studied *CYP 450* genes. At the same time, after sequential administration of BABC and CP, a significant (3.2-fold) increase ($p=0.005$) in the expression of the *CYP 2B10* gene was noted as compared to the control group.

After administration of CP, an increase in *PPARA* expression was observed ($p=0.045$) compared to the control group. However, no significant differences were found in the expression levels of the *PPARA* and *CYP 17A1* genes after the administration of the combination of CP+BABC (Fig. 4). Administration of BABC only to animals did not affect the mRNA level of these genes.

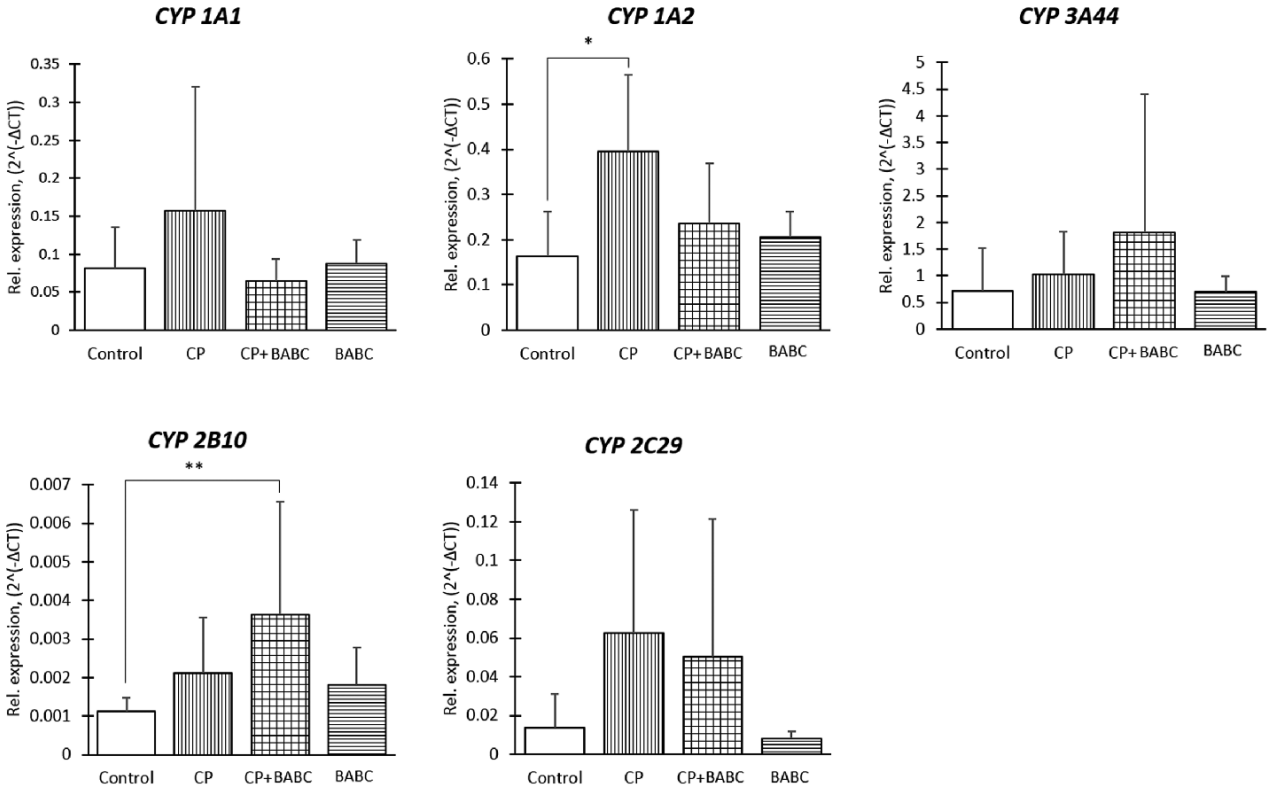

Figure 3. Relative expression ($2^{-\Delta CT}$) of genes encoding enzymes of the CYP superfamily in the liver of animals treated with CP and BABC (n=6). * – $p<0.05$, ** – $p<0.01$.

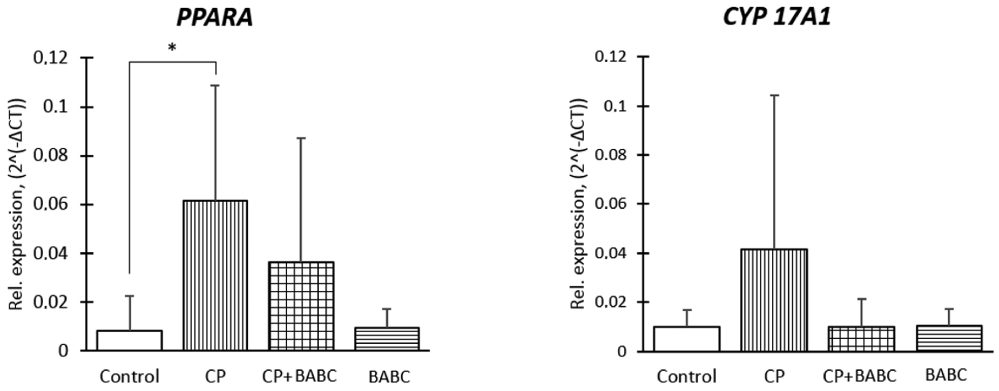

Figure 4. Relative expression of the PPARA and CYP 17A1 genes ($2^{-\Delta CT}$) in the liver of animals after treatment with CP and BABC (n=6). * – significant differences between groups.

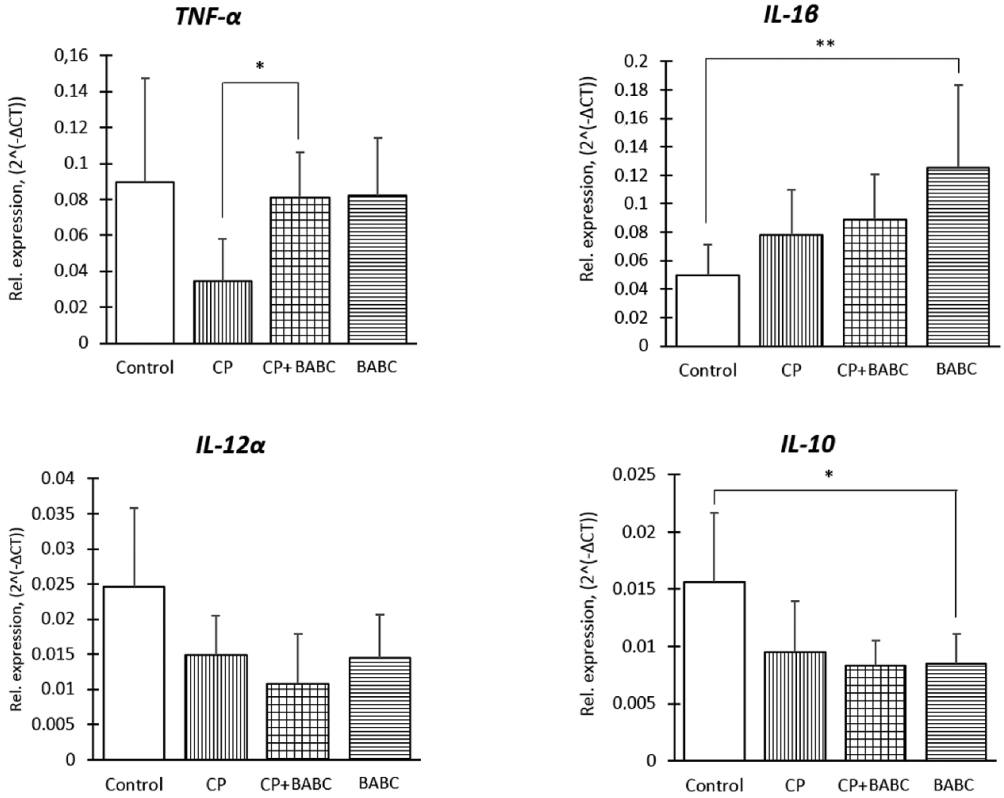

Figure 5. Relative expression of cytokine genes ($2^{-\Delta CT}$) in the liver of animals treated with CP and BABC (n=6). * – $p<0.05$, ** – $p<0.01$.

The Effect of CP and BABC on the Expression of Cytokine Genes in the Liver

To assess the protein content and the effect of CP and BABC on the expression of genes involved in the immune response, the following comparison between the groups of animals were made: CP/CP+BABC, Control/BABC. An assessment of the effect of the studied substances on the expression of cytokine genes has shown that in animals of the CP group there was a slight increase in the expression of the *IL-1β* gene, as well as a decrease in the expression of the *TNF-α*, *IL-12α*, and *IL-10* genes (Fig. 5). Presumably, these changes are due to the influence of DMSO administered the day before to animals of this group.

CP administration after BABC caused a 2.4-fold increase in the mRNA level of the pro-inflammatory *TNF-α* gene as compared with the CP group (p=0.022). At the same time, BABC administration to intact animals was accompanied by a 2.5-fold increase in the *IL-1β* expression of (p=0.008) and a 1.8-fold decrease in the expression of *IL-10* (p=0.031) as compared to the control group (Fig. 5).

The Effect of CP and BABC on the Relative Content of the Studied Liver Proteins

Administration of CP and BABC did not have a significant effect on the content of GST-π (Fig. 6). At the same time, BABC administration resulted in a 1.6-fold increase in the content of NF-κB p65

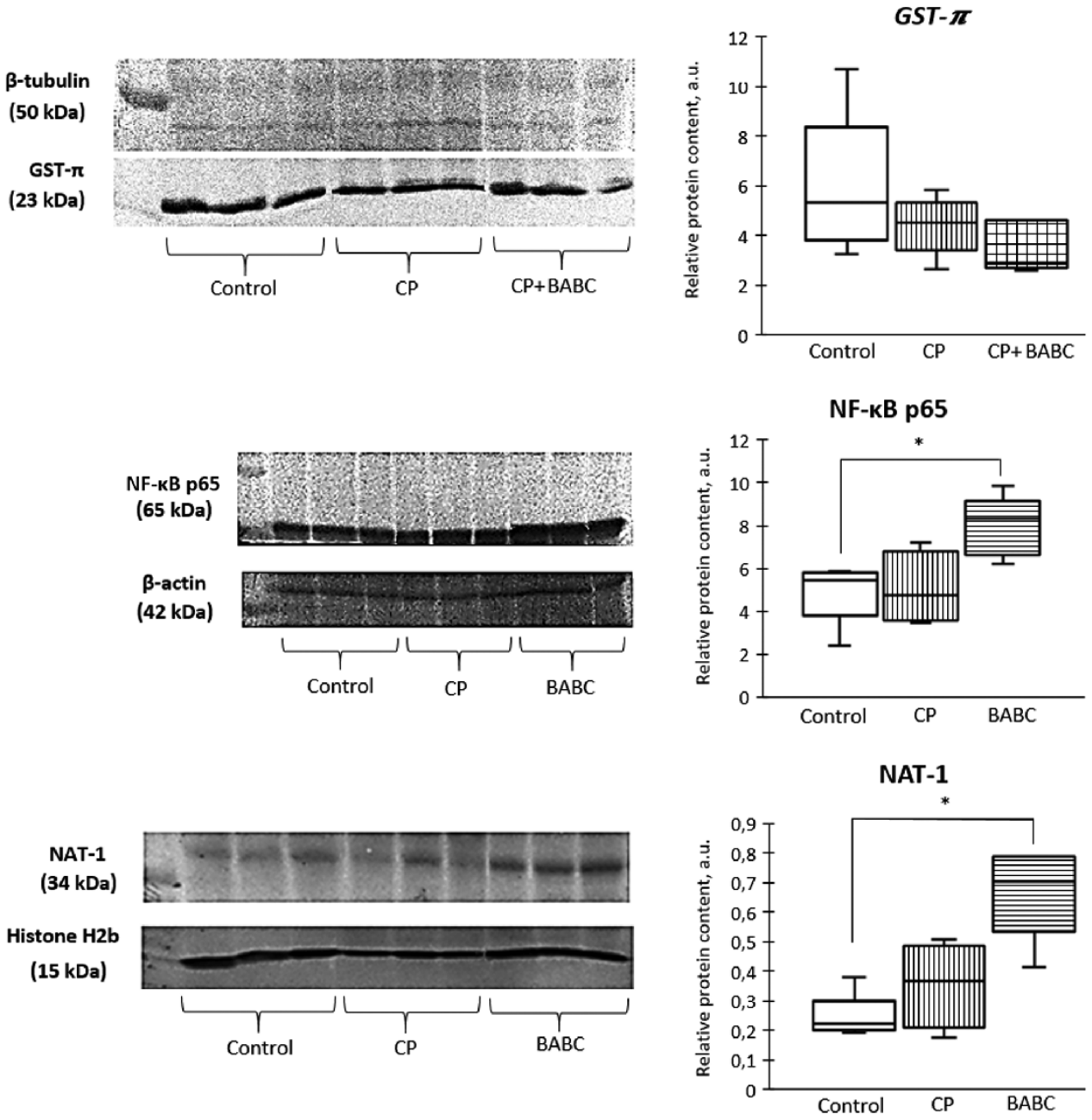

Figure 6. Relative protein content (a.u.) in the liver of animals treated with CP and BABC (n=6). * – significant differences between groups.

(Control/BABC, p=0.012) and a 2.7-fold increase in the content of NAT-1 (Control/BABC, p=0.0122). No significant increase was observed in the relative amount of the studied proteins in the CP group compared to the control group.

DISCUSSION

CP administration causes an increase in the expression of *CYP 450* genes [4]. However, in this study, the increase in the expression of the gene for the main metabolizer of CP (CYP 2B10) remained just at the trend level. Good evidence exists in the literature on gender differences in the *CYP 2B10* expression: in male mice its expression is lower than in females [24]. However, five days after a single administration of CP the *CYP 2B10* expression in animals of the CP+BABC group were significantly higher than in the control group. This indicates the influence of BABC on the expression of the *CYP 2B10* gene and its possible impact on CP metabolism. If the increase in the activity of this enzyme is confirmed, it can be argued that BABC helps to accelerate CP metabolism and to reach a more rapid onset of the therapeutic effect. At the same time, BABC does not affect the production of the GST-π enzyme, which is involved in the reduction of acrolein, a CP metabolite. Therefore, after

administering BABC together with CP, one should not expect an increase in the above-mentioned side effects associated with acrolein accumulation in the body.

In the past few years, evidence has accumulated regarding the role of PPAR-α agonists in stimulating apoptosis of breast cancer cells, as well as suppressing neoangiogenesis through inhibition of HIF-α and VEGF in tumor cells. Some of these agonists have already been introduced into chemotherapeutic regimens [25]. Increased expression of *PPARA* induced by CP may indicate additional ways of realizing its antitumor effect. On the other hand, it may reflect the reaction of organ tissues to CP administration, which causes liver damage and promotes oxidative stress.

In the CP group, an unexpected decrease in the expression of TNF-α, a slight increase in the expression of *IL-1β*, and no changes in the production of NF-κB p65 in the liver of experimental animals compared to animals of the control group were noted. It is known that administration of high doses of CP leads to a several-fold increase in the expression of the above genes and protein synthesis [26, 27]. In this case, the decrease in the expression of inflammatory mediators can be explained by the influence of DMSO administered to mice one day before CP. DMSO has been shown to suppress the production of TNF-α and NF-κB [28] and has a hepatoprotective effect [29].

BABC administration promoted an increase in the expression of genes for pro-inflammatory cytokines and the p65 subunit of NF-κB, as well as a slight decrease in the expression of the anti-inflammatory IL-10 gene, which indicates a clear immune-activating effect of this substance. This expression profile may play a beneficial role in some diseases. For example, it has been shown that increased expression of TNF-α and NF-κB in the tumor microenvironment of hepatocellular carcinoma (HCC) is associated with a better clinical prognosis in patients with this diagnosis [30, 31]. In turn, IL-1 can effectively inhibit hepatitis C virus (HCV) RNA replication and viral protein expression, having antiviral activity. Thus, increasing its expression in the liver may play an important role in viral clearance [32].

CONCLUSIONS

1) The use of CP in combination with BABC promotes an increase in the expression of mRNA of the *CYP 2B10* gene in the mouse liver, which indicates the influence of BABC on the expression of this gene, and presumably affects CP metabolism.

2) BABC increases the relative content of NAT-1 in the liver, which indicates the role of NAT-1 in the elimination of the test substance.

3) BABC administration caused an increase in the expression of pro-inflammatory cytokine genes (*IL-1β, TNF*-α) and increased production of the p65 subunit of NF-κB. In case of absent toxicity, its use may be reasonable in viral liver diseases (HCV) and HCC.

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COMPLIANCE WITH ETHICAL STANDARDS

The animals were kept in accordance with the interstate standard GOST 33216-2014 and the European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes. During the experiment, the animals were kept under standard vivarium conditions under artificial illumination (12:12 light/dark cycle, in plastic cages with fine wood shavings, and received standard granulated chow (PK 120-3) and water *ad libitum*. The publication of the material was approved by the Biomedical Ethics Committee at the FRC FTM (No. 2 at the meeting dated January 10, 2024).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ГЕПАТОТРОПНАЯ АКТИВНОСТЬ СОЕДИНЕНИЯ БЕТУЛОНОВОЙ КИСЛОТЫ

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На модели циклофосфамид (ЦФ)-индуцированной иммуносупрессии у лабораторных мышей линии C57BL/6 исследовали гепатотропные эффекты конъюгата бетулоновой кислоты с 9-(4-метилпиперазин-1-илметил)-2-(1,2,3-триазолил) ореозелоном (Соед). Определяли экспрессию генов цитохромов (*CYP 1A1*, *CYP 1A2*, *CYP 3A44*, *CYP 2B10*, *CYP 2C29*, *CYP 17A1*), *PPARA* и цитокинов (*TNF-α*, *IL-1β*, *IL-12α*, *IL-10*), а также относительный уровень белков NF-κB p65, GST-π и NAT-1 в печени животных. Введение животным Соед и ЦФ вызывало достоверное увеличение экспрессии гена *CYP 2B10* в 3,2 раза по сравнению с контрольной группой на шестые сутки после введения. После введения Соед и ЦФ было отмечено повышение уровня мРНК гена провоспалительного TNF-α в 2,4 раза по сравнению с группой животных, получавших ЦФ. Введение исследуемого Соед интактным животным способствовало увеличению экспрессии *IL-1β* в 2,5 раза и снижению экспрессии *IL-10* в 1,8 раза по сравнению с контрольной группой. Повышение экспрессии генов провоспалительных цитокинов в печени животных, получавших Соед, сопровождалось увеличением содержания NF-κB p65 в 1,6 раза, а также увеличением относительного количества белка NAT-1 в 2,7 раза по сравнению с образцами печени контрольных животных.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: бетулоновая кислота; фурокумарины; печень; белки; цитохромы; цитокины

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