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Proceeding Paper

Approaches to Reduce the Side Effects of Antibiotic Therapy in Premature Newborns †

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Abstract: Significant risk of side effects of drug therapy in newborns, especially in premature neonates, is associated with immaturity of a number of enzyme systems and biotransformation mechanisms and pharmacokinetic specificity. We consider the use of thiotriazoline), a drug licensed in Ukraine with proven hepatoprotective and cardioprotective activity and a high safety profile, for premature infants to reduce side effects of antibiotic therapy. The use of thiotriazoline in antibiotic therapy results in an increase in the concentration of eNOS in blood plasma and a decrease in alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase and bilirubin in blood plasma. These results provide experimental support for the use of thiotriazoline to reduce the side effects of azithromycin therapy in newborns.

Keywords: antibiotic therapy; side effects; premature newborns; thiotriazoline

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1. Introduction

Significant risk of side effects of drug therapy in newborns, especially in premature neonates, is associated with immaturity of a number of enzyme systems and biotransformation mechanisms and pharmacokinetic specificity [1–4]. First of all, it is associated with the immaturity of the microsomal mechanism of liver cells. Newborns have lower concentrations of cytochrome P450, NADPH oxidase, cytochrome C reductase, (uridine diphosphate)-glucuronyl transferase, as well as enzymatic and non-enzymatic modulators of the thiol–disulfide system [5,6]. Low activity of the antioxidant system and endogenous antioxidants enhances the ROS formation during biotransformation of antibiotics and increases their toxicity at concentrations safe for adults, resulting in increased hepatotoxicity. In the Zaporizhzhya region (Ukraine), in chronic pulmonary suppuration, transaminase levels increased in 17% of children treated with chloramphenicol and aminoglycosides, in 7.8% with semi-synthetic penicillins and in 5.3% with cephalosporins and macrolides, as well as in 34.5% of children receiving combined therapy [7]. This has made it necessary to reduce dosages, usually through infrequent administration of drugs, and to conduct laboratory monitoring not only of the hepatobiliary system, but also of the antioxidant glutathione system. Another important approach for premature infants in the first weeks of life when it comes to reducing antibiotic therapy side effects, including hepatotoxicity, is the administration of agents that modulate the activity of the glutathione system. The

difficulty in implementing this approach is the strict requirements for safety and proven efficiency for drugs in neonatal intensive care. We consider the use of thiotriazoline, a drug licensed in Ukraine with proven hepatoprotective and cardioprotective activity and a high safety profile.

2. Materials and Methods

This study was conducted on 30 newborn rat pups born from white Wistar rats. One-month-old rat pups were administered azithromycin at a dose of 15 mg/kg for 5 days. Ten animals received only azithromycin and ten animals also received thiotriazoline *w*/*w* at a dose of 50 mg/kg for 14 days with azithromycin. Ten pups received only physiological solution.

At the end of treatment (day 15), all animals were removed from the experiment under anesthesia (sodium thiopental, 40 mg/kg), and their blood was collected. Plasma was obtained through centrifugation (20 min at 1500 rpm) using an Eppendorf 5810 R centrifuge (Germany) with the addition of 0.129 mol/L sodium citrate in a 1:9 ratio to the blood. The resulting plasma was stored in 0.5 mL Eppendorf tubes at −40 °C in an NZ-280/75A freezer. Subsequently, the samples were thawed, and 0.2 mL was utilized for biochemical studies, while 0.1 mL was allocated for immunoenzymatic studies.

Biochemical methods were employed to determine markers such as C-reactive protein (immunoturbidimetric method with a kit from Cormay on the ACCENT-200 biochemical analyzer, Poland), D-dimer (kit from Vector-Best), and hepatic enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) (Cormay, on the ACCENT-200 biochemical analyzer, Poland). The thymol test was conducted using the turbodynamic method (NPP "Filisit-Diagnostics"). Endothelial NO synthase (eNOS) was determined using the enzyme immunoassay method (Cloud-Clone Corporation; Immunochem-2200 immunoassay analyzer, USA).

Statistical analysis of the results was carried out using standard software packages such as "STATISTICA® for Windows" (StatSoftInc., Hamburg, Germany, AXXR712D833214FAN5), "SPSS 16.0", and "Microsoft Office Excel 2016". The normality of distribution was assessed using the Shapiro–Wilk criterion, and data were presented as mean values. Student's criterion was employed to determine the significance of differences between means in cases of normal distribution, while the Mann–Whitney U-criterion was used for non-normally distributed or ordinal variables. Analysis of variance (ANOVA) was applied for normal distributions, and the Kruskal–Wallis test was used for distributions deviating from normal. A significance level of $p < 0.05$ (95%) was considered statistically significant for all types of analyses.

3. Results and Discussion

It was found that azithromycin administration leads to a decrease in eNOS activity, which may indicate the formation of endothelial dysfunction (Table 1).

Table 1. Concentration of eNOS in rat blood plasma (15 days from treatment onset).

Groups of Animals	eNOS, ng/mL
Intact rat pups (n = 10)	62.8 ± 4.7
Rat pups treated with azithromycin (n = 10)	41.3 ± 4.2 [1]
Rat pups treated with azithromycin + thiotriazoline (n = 10)	58.4 ± 4.7 *[1]

[1]—$p < 0.05$ in relation to the intact group; *—$p < 0.05$ in relation to the basic therapy.

Our study suggests that thiotriazoline has a protective effect on vascular endothelium, reducing endothelial dysfunction, which is one of the serious side effects of antibiotic therapy. Endothelial dysfunction contributes to the development of serious heart and vascular pathologies leading to heart attacks and strokes [8–10]. Alterations in the endothelium are associated with changes in the formation of NO, which is an unstable, short-lived radical and in high concentrations has a toxic effect for cells. Its binding and transportation are carried out via interaction with low-molecular thiol-containing compounds (glutathione,

cysteine, methionine), resulting in the formation of stable S-nitrosyl complexes. Excess NO in conditions of thiol compounds deficiency easily reacts with oxygen and superoxide radical and hydroxyl radical and forms a cytotoxic product–peroxynitrite, the toxic effect of which enhances the formation of endothelial dysfunction [11,12]. Preclinical study reports on thiotriazoline have shown that thiotriazoline increases the level of SH compounds, thereby increasing the bioavailability of NO. Thiotriazoline can also independently form nitrosothiol complexes with NO [13,14]. Binding of excess NO prevents its interactions with reactive oxygen species and the formation of cytotoxic peroxynitrite. Thiotriazoline increases endothelial cell density, increases the number of proliferating endothelial cells, and increases the expression of vasculoendothelial factor (VEGF) and endothelial nitric monoxide synthase (eNOS). In clinical studies, the endothelioprotective effect of combining thiotriazoline and arginine was demonstrated, based on the increase in NO bioavailability [15]. Previously, it was shown that thiotriazoline inhibits endothelial damage in the zone of alteration and promotes normalization of blood circulation in the inflammation zone, inducing the conversion of plasminogen into plasmin with subsequent enhancement of fibrinolysis [14–16].

One of the important components of the treatment of azithromycin is the development of adverse side effects, which is a disruption of the hepatobiliary system and the protective function of the liver (Table 2).

Table 2. Biochemical parameters of rat liver tissue (15 days from treatment onset).

Groups of Animals	ALT, U/L	AST, U/L	GGT, U/L	Thymol Test, Sh	Total Bilirubin, µmol/L
Intact rat pups (n = 10)	18.4 ± 1.5	9.2 ± 0.6	24.5 ± 2.5	1.2 ± 0.07	12.8 ± 1.5
Rat pups treated with azithromycin (n = 10)	65.5 ± 3.9 [1]	52.4 ± 2.5 [1]	84.7 ± 3.7	11.7 ± 2.1 [1]	22.9 ± 1.7 [1]
Rat pups treated with azithromycin + thiotriazoline (n = 10)	25.4 ± 2.7 *[1]	14.2 ± 1.1 *[1]	24.4 ± 2.3 *	0.7 ± 0.02 *[1]	10.4 ± 0.5 *[1]

[1]—$p < 0.05$ in relation to the intact group; *—$p < 0.05$ in relation to the basic therapy.

We observed an increase in liver enzymes in blood plasma and bilirubin content in rats. The rats were sedentary, anxious, they did not go to the lighted part of the cage. There was decreased exploratory activity in them. Based on previous research, thiotriazoline demonstrates hepatoprotective effects attributed to its antioxidant, membrane-protective, and mitoprotective properties. It sustains a sufficiently high level of energy and plastic processes in hepatic tissue by safeguarding enzymes involved in the tricarboxylic acid cycle and pentose-phosphate shunt in hepatocytes from oxidative damage [16]. The thiotriazoline molecule contains free SH groups that can bind and inactivate cytotoxic products of oxidative stress and xenobiotic metabolites [17,18]. Thiotriazoline has a protective effect on hepatocyte mitochondrial membranes in toxic hepatitis, as evidenced by the preservation of mitochondrial membrane potential and functional preservation of cyclosporin-A-dependent mitochondrial pore [14]. Thiotriazoline has membrane-stabilizing and membrane-protective effects, as it inhibits the processes of lipid peroxidation in the membranes of the endoplasmic reticulum of hepatocytes in toxic liver injury and normalizes the physicochemical parameters of the membrane structure [19,20]. The obtained biochemical results show the hepatoprotective effect of thiotriazoline [21,22]. Considering the various severe side effects associated with basic agents such as antibiotics; antiviral drugs; antiplatelet NSAIDs, which target the intricate links of metabolic pathways in cardiomyocytes; endothelial cells; hepatocytes, etc., incorporating thiotriazoline into comprehensive therapy may enhance the safety of the prescribed drug treatment [14,21].

4. Conclusions

The use of thiotriazoline in antibiotic therapy results in an increase in the concentration of eNOS in blood plasma and a decrease in alanine aminotransferase, aspartate

aminotransferase, gamma-glutamyltransferase and bilirubin in blood plasma. These data provide experimental support for the use of thiotriazoline to reduce the side effects of azithromycin therapy in newborns.

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