Pharmacological Correction of the Negative Effects of Acetylsalicylic Acid on the Energy Production System

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Experiments of outbred rats with modeled xenobiotic load with acetylsalicylic acid (250 mg/kg for 7 days) revealed inhibition of mitochondrial respiration rate in states of rest and active phosphorylation, inhibition of succinate-dependent oxidation pathway, and a decrease in energization of organelles in the heart. For correction of the observed changes in energy production, succinic acid was preventively administered in a dose of 50 mg/kg for 7 days, which abolished the negative metabolic shifts in myocardial mitochondria. Comparison of pharmacokinetics of acetylsalicylic acid and acetylsalicylic acid against the background of succinate treatment performed on rabbits revealed complete coincidence of the studied parameters, which attests to the possibility of prevention of mitochondrial dysregulations with this Krebs cycle intermediate.

Key Words: *pharmacokinetics; heart mitochondria; energy production system; succinic acid; acetylsalicylic acid*

Acetylsalicylic acid (ASA) is widely used as a disaggregation agent in complex therapy and prevention of thrombotic complications in various pathologies [1,3,5]. It is known that salicylates even in therapeutic doses produce a negative effect on the energy production system by inhibiting various metabolic pathways and increasing membrane permeability for hydrogen ions [9]. Some described cases of Reye syndrome development against the background of salicylate [10] can be explained by damage to mitochondria (MC) [2]. Experiments on the models of hypoxia, stress, and intoxications showed that succinic acid (SA) can abolish the negative metabolic shifts in the system of energy production of MC [6] and hence can be used for prevention and correction of ASA-induced disturbances.

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Here we studied the effects of ASA and its combination with SA on the MC energy production system and evaluated the effects of therapeutic administration of SA on pharmacokinetic parameters of ASA.

MATERIALS AND METHODS

Myocardial bioenergetics was studied on 30 outbred male rats (body weight 220-250 g) divided into 3 equal groups. Group 1 animals intragastrically received ASA in a dose of 250 mg/kg ($^1/_{10}$ LD_{50}) for 7 days. In group 2, SA (50 mg/kg intragastrically) was administered daily for 7 days against the background of daily treatment with 250 mg/kg ASA. Controls (group 3) received vehicle (1% starch gel).

Functional state of MC in heart homogenate was evaluated by polarography (LP-9 polarograph) using closed-type Clark electrode made in our laboratory. The isolation medium contained 3×10^{-1} M sucrose, 2×10^{-3} M tris-buffer, 1×10^{-2} M EDTA, 1 mg/ml BSA,

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1.2×10^{-1} M KCl (pH 7.2 at 0°C). The incubation medium contained 3×10^{-1} M sucrose, 1.2×10^{-1} M KCl, 5×10^{-3} M KH_2PO_4, 1×10^{-2} M HEPES, 1×10^{-3} M EDTA, (pH 7.2 at 26°C). We measured the rate of oxygen consumption by MC before (V_4), during (V_3), and after (V_3-V_4) a cycle of phosphorylation of 1×10^{-4} M added ADP and the time of phosphorylation. The method of measurement required the use of two types of substrates: flavin-dependent (succinate, 1×10^{-3} M) and NAD-dependent (malate and glutamate, 3×10^{-3} M each). SDH inhibitor malonate (2×10^{-3} M) and aminotransferase inhibitor aminooxyacetate (5×10^{-4} M) were used for evaluation of the contribution of endogenous SA into energy production of MC during oxidation of NAD-dependent substrates. We also calculated coefficients of respiration stimulation (V_3/V_4), respiratory control (V_3/V_3-V_4), and oxidation–phosphorylation coupling ADP/O reflecting metabolic control of substrate oxidation coupled to respiration.

Pharmacokinetics was studied on 10 outbred male rabbits weighing 2.5-2.7 g. The preparations suspended in 1% starch gel were administered intragastrically (single administration). The animals were divided into 2 groups. Group 1 rabbits received 125 mg/kg ASA (single therapeutic dose for humans) and group 2 animals received a mixture of ASA and SA (125 and 50 mg/kg, respectively). Since ASA is rapidly (within 10-15 min) hydrolyzed by esterases, blood concentration of ASA metabolite (salicylic acid) was measured. The blood (500 µl) was sampled from the marginal ear vein into heparinized tubes before and 0.5, 1, 4, 7, 8, and 9 h after treatment. Blood samples were mixed with 0.5 ml 5% NaCl and extracted with 2 ml diethyl ether for 10 min. The samples were centrifuged at 3000*g* for 10 min. Organic layer was quantitatively transferred into glass tubes and evaporated under nitrogen stream at 45°C. The dry residue was dissolved in 100 µl mobile phase and shaken for 10 min (300 shakes per min). An aliquot (50 µl) was used for chromatography. Analysis was performed by HPLC on a Milikhrom A-02 chromatograph.

The following pharmacokinetic parameters were analyzed: maximum concentration (C_{max}), the time of attaining maximum concentration (T_{max}), and area under the pharmacokinetic curve (AUC_{0-t}) reflecting the amount of preparation circulating in the blood over the period from its entry into the body to the decrease in its concentration to a minimum detectable level. The C_{max}/AUC_{0-t} ratio was calculated as a parameter reflecting the rate of preparation absorption; the mean values were also calculated.

RESULTS

Administration of 250 mg/kg ASA for 7 days reduced respiration rate in rat heart MC during utilization of endogenous substrates before, during, and after a cycle of ADP phosphorylation compared to the control group. The coefficient ADP/O increased under these conditions, which attests to inhibition of succinate- and NAD-dependent processes of energy production (Fig. 1). Similar changes were observed during utilization of exogenous substrates. For instance, the rate of oxygen consumption during succinate oxidation by myocardial MC before, during, and after a cycle of ADP phosphorylation decreased in parallel with a decrease in respiratory control and an insignificant increase in ADP/O coefficient (Fig. 1). In rats receiving ASA, the time of ADP phosphorylation during succinate oxidation by heart MC also increased. This attests to inhibition of succinate-dependent pathway of substrate oxidation and a decrease in energization of organelles (Fig. 1) [4].

The rate of phosphorylating respiration during utilization of NAD-dependent substrates by heart MC from animals receiving ASA also decreased, while the time of ADP phosphorylation increased compared to the control (Fig. 1). The decrease in respiration stimulation paralleled by an increase in ADP/O coefficient attests to kinetic character of energy deficiency in heart MC from rats treated with ASA [7]. At the same time, the inhibitory analysis with competitive SDH inhibitor malonate and aminotransferase inhibitor aminooxyacetate showed increased contribution of oxidation of endogenous SA into respiratory activity of organelles during oxidation of NAD-dependent substrates. This process has a compensatory nature and reflects peculiarities of metabolic regulation of Krebs cycle in ASA-modified MC, in particular activation of the rapid metabolic cluster [4].

Administration of SA in a dose of 50 mg/kg promoted correction of ASA-induced negative metabolic shifts in rat heart MC (Fig. 1). Thus, normalization of MC respiration rate in all metabolic states was observed during oxidation of endogenous substrates compared to the corresponding parameters in animals receiving ASA alone. The values of respiration stimulation and respiratory control increased, while ADP/O decreased under these conditions, which attested to recovery of succinate-dependent energy production processes.

During succinate oxidation by heart MC from rats receiving SA against the background of SA treatment, the rate of oxygen consumption before, during, and after ADP phosphorylation cycle and coefficients of respiration stimulation and respiratory control considerably increased compared to the corresponding parameter in animals receiving ASA alone (Fig. 1). This attests to activation of succinate-dependent pathway of substrate oxidation and oxidative phosphorylation under the effect of SA. The value of ADP/O coefficient in

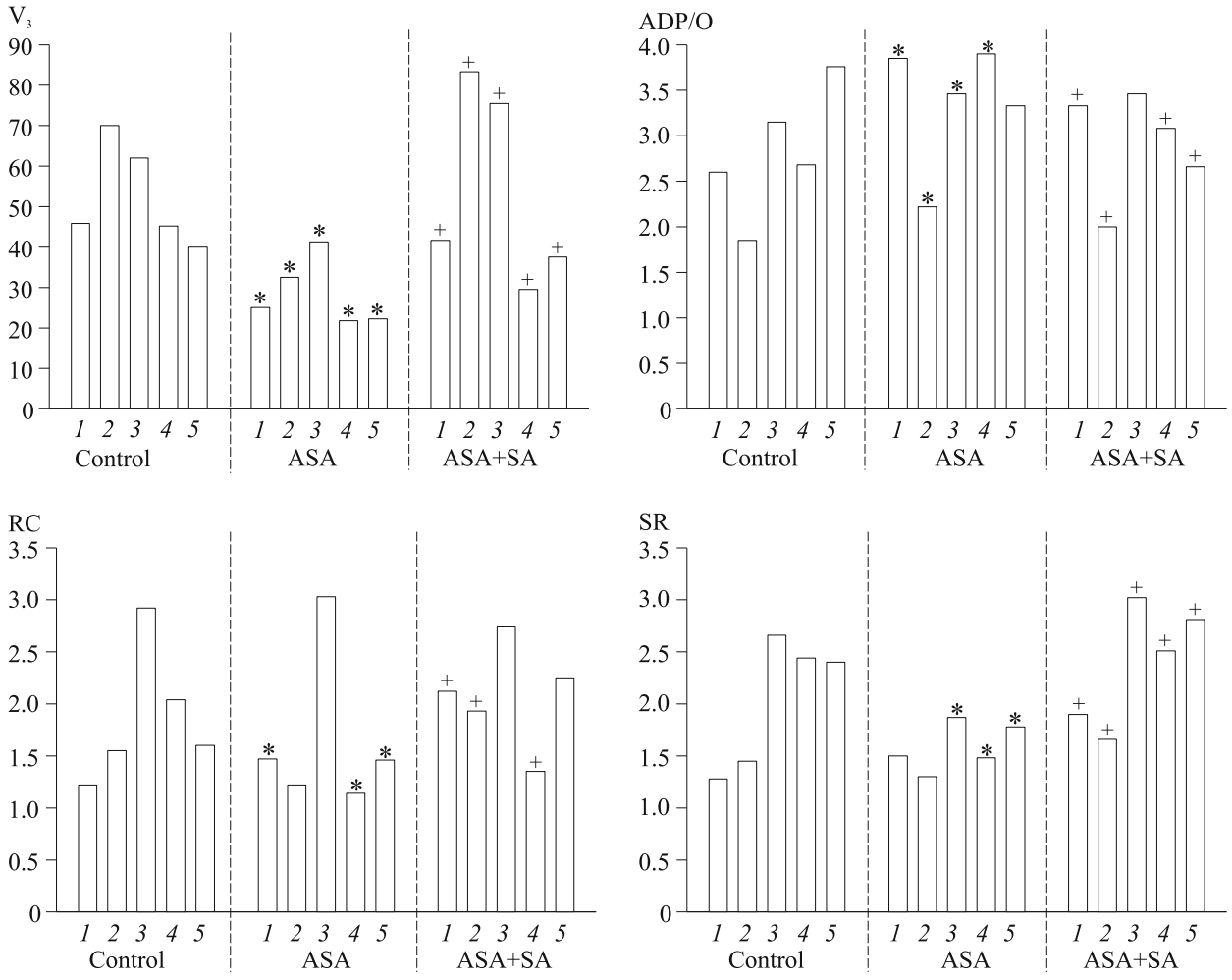

Fig. 1. Functional state of heart MC during ADP phosphorylation in rats treated with ASA alone and in combination with SA. Oxidation substrates: *1*) endogenous; *2*) succinate; *3*) malate+glutamate; *4*) malate+glutamate+malonate; *5*) malate+glutamate+aminooxyacetate. $p<0.05$ compared to: *control group, +animals receiving ASA alone.

these animals did not differ from the control level [4,8].

During oxidation of the mixture of NAD-dependent substrates by heart MC from rats receiving SA against the background of ASA treatment, the rate of phosphorylating respiration and stimulation of respiration considerably increased compared to those in rats receiving ASA alone (Fig. 1). This attests to normalization of NAD-dependent respiration and oxidative phosphorylation in SA-protected animals. Inhibitory analysis with malonate and aminooxyacetate showed that the rate of oxidation of endogenous SA considerably increased compared to that in animals receiving ASA alone, which attests to stimulation of the reactions of rapid metabolic cluster of MC.

Study of pharmacokinetics of ASA and ASA+SA mixture showed that the concentration of its metabolite salicylic acid peaked on minute 30 and attained 8 μg/ml. By the 9th hour, the concentration of salicylic acid in the blood of experimental animals dropped to its minimum: 1 μg/ml (Fig. 2).

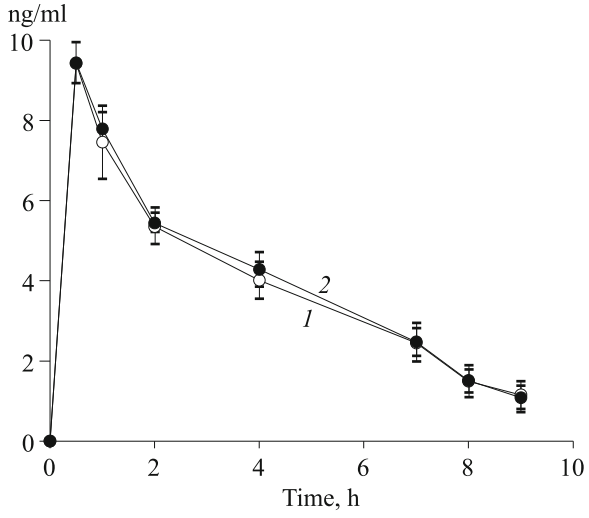

Fig. 2. Average dynamics of the concentration of salicylic acid in blood plasma of rabbits receiving ASA alone and in combination with SA. *1*) ASA, 125 mg/kg; *2*) ASA, 125 mg/kg+SA, 50 mg/kg.

Pharmacokinetics of salicylic acid was practically similar in animals receiving ASA alone and in combination with SA. The boundaries of confidence intervals for AUC_{0-t}, C_{max}, and C_{max}/AUC_{0-t} were 82-115.4, 89-131.9, and 96.6-125.8%, respectively, and remained within the permissible limits 80-125% for parameter AUC_{0-t} and 75-133% for parameters C_{max} and C_{max}/AUC_{0-t}. Thus, no significant differences by the rate and degree of absorption of ASA and the combination of ASA and SA were revealed.

Hence, preventive administration of SA promotes correction of ASA-induced negative metabolic shifts in rat heart MC due to normalization of succinate and NAD-dependent respiration and oxidative phosphorylation and can be used for reducing side effects of ASA. SA produced practically no negative effects on ASA pharmacokinetics and hence, no correction of treatment schedule is required.

REFERENCES

1. E. V. Adonina, O. Yu. Aristarchova, A. L. Vertkin, *et al., Russ. Med. Zh*., **17**, No. 8, 570-575 (2009).
2. A. I. Vengerovskii, V. A. Khazanov, M. S. Timofeev, *et al., Byull. Eksp. Biol. Med*., Suppl. 2, 89-91 (2003).
3. Yu. A. Karpov, *Russ. Med. Zh*., **16**, No. 11, 1554-1558 (2008).
4. M. N. Kondrashova, *Biokhimiya*, **56**, No. 3, 388-402 (1991).
5. R. G. Oganov and G. Ya. Maslennikova, *Kardiologiya*, No. 6, 4-8 (2000).
6. N. B. Smirnova and V. A. Khazanov, *Regulators of Energy Metabolism. Clinical and Pharmacological Aspects*, Ed. V. A. Khazanov [in Russian], Tomsk (2004), pp. 109-113.
7. V. A. Khazanov, *Pharmacology and Pharmacoeconomics of a New Class of Drugs, Regulators of Energy Metabolism* [in Russian], Tomsk (2003), pp. 13-14.
8. V. A. Khazanov, *Regulators of Energy Metabolism. Clinical and Pharmacological Aspects*, Ed. V. A. Khazanov [in Russian], Tomsk (2004), pp. 3-7.
9. J. Gutknecht, *Mol. Cell. Biol.*, **114**, Nos. 1-2, 3-8 (1992).
10. P. Jolliet and J. Widmann, *Lancet,* **335**, No. 8703, 1457 (1990).