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Pharmacological Correction of the Negative Effect of Acetylsalicylic Acid on the Energy-Generating System

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Abstract

The present paper demonstrates the effect of ASA and its combination with SUC on the energy-producing system of rat heart mitochondria as well as an assessment of SUC preventive application effect on ASA pharmacokinetic parameters. Experiments conducted on outbred male albino rats (200-250 g) on a model of a xenobiotic load induced by seven days of intragastric injections of acetylsalicylic acid at a dose of 250 mg/kg have shown inhibition of the oxygen consumption rates in the heart mitochondria as well as a limitation of the succinate-dependent substrate oxidation pathways and a decrease in the mitochondria ATP/ADP coefficient. Succinic acid (50 mg/kg for 7 days) was injected as a preventive medication to correct the mitochondrial bioenergetics revealed. A comparative research of the pharmacokinetics of acetylsalicylic acid and acetylsalicylic acid against the background of succinic acid performed on the model of rabbits has shown total similarity in the parameters analyzed. This fact demonstrates the possibility of prevention of mitochondrial dysfunction using the intermediate Krebs cycle. SUC as preventive medication promotes the elimination of ASA-induced negative metabolic shifts in the rat heart mitochondria by normalizing the succinate- and NAD-dependent respiration, oxidative phosphorylation, and therefore, it finds good use in the correction of ASA-induced negative side-effects of an energy-generating system. IJBM 2012; 2(1):58-61.
Key words: *mitochondria, pharmacokinetics, bioenergetics, succinic acid.*

Introduction

Acetylsalicylic acid (ASA) as antiplatelet drug is widely used in complex therapy particularly in the prevention of thrombotic complications in different diseases [1-3]. Salicylates are known to have a negative effect on the energy-generating system even at therapeutic doses, inhibiting the various metabolic pathways and increasing the membrane permeability for H^+ ions [4, 5]. The onset of Reye's syndrome was noted when patients took salicylates, as described in the literature [6]. The mechanism of the syndrome mentioned above is associated with mitochondrial malfunctions [7]. Interestingly, succinic acid (SUC) on the model of hypoxia, stress and intoxication is also known to promote the elimination of negative metabolic shifts in the mitochondrial energy-generating system [8, 9] and, therefore, SUC can be used for the prevention and correction of ASA-induced disturbances.

In this connection, the present paper demonstrates the effect of ASA and ASA in combination with SUC on the energy-producing system of rat heart mitochondria. It also assesses the preventive application effect of SUC on ASA pharmacokinetic parameters.

Methods

All animal experiments described in this article have been conducted in accordance with the EC Directive

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86/609/EEC and the results have been arranged according to the Uniform Requirements for manuscripts submitted to Biomedical Journals.

Bioenergetics experiments

Experiments performed on the myocardial bioenergetics research were done on 30 outbred male albino rats (200-250 g). ASA was injected at a single daily dose of 250 mg/kg (1/10 LD50) intragastrically to the Group 1animals for 7 days. Animals of Group 2 received SUC at a single daily dose of 50 mg/kg intragastrically in parallel with ASA (250 mg/kg). Controls received an equivalent volume of the solvent (1% starch gel).

The functional state of the mitochondria in the rat heart homogenate was evaluated using polarography (Polarograph LP-9, Czech Republic) with the closed Clark -type oxygen electrode connected to a recorder in a thermostated water-jacketed sealed 1-ml glass chamber with constant magnetic stirring, at 25°C [10]. The heart mitochondria isolation medium contained sucrose 0.3·M; TRIS buffer 20 mM; EDTA 1mM; BSA 1 mg/ml; KCl 120 mM (pH=7.2; t=0°C). The heart mitochondria incubation medium contained sucrose 300·mM; KCl 120 mM; KH_2PO_4 5 mM; Hepes 10mM; EDTA 1 mM (pH 7.2, t=26°C). The rates of O_2 consumption were calculated in the metabolic states before (V4p), during (V3), and after (V4o) phosphorylation of 1×10^{-4} M ADP. Phosphorylation time was also measured.

The method of evaluation required the application of two types of substrates, namely: flavine-dependent (succinate 1×10^{-3} M) and NAD-dependent (malate and glutamate, 3×10^{-3} M each). To reveal the contribution of endogenous SUC oxidation to the energy-generating system of the mitochondria in the oxidation of NAD-dependent substrates, the experiment was conducted in the presence of the SDH inhibitor malonate 2×10^{-3} M or aminotransferase inhibitor – aminooxyacetate 5×10^{-4} M. The coefficients of respiration stimulation (RS=V3/V4p), respiratory control (RC=V3/V4o), and oxidation-phosphorylation coupling (ADP/O) were calculated reflecting the metabolic control of respiration-coupled oxidation of substrates.

Pharmacokinetics experiments

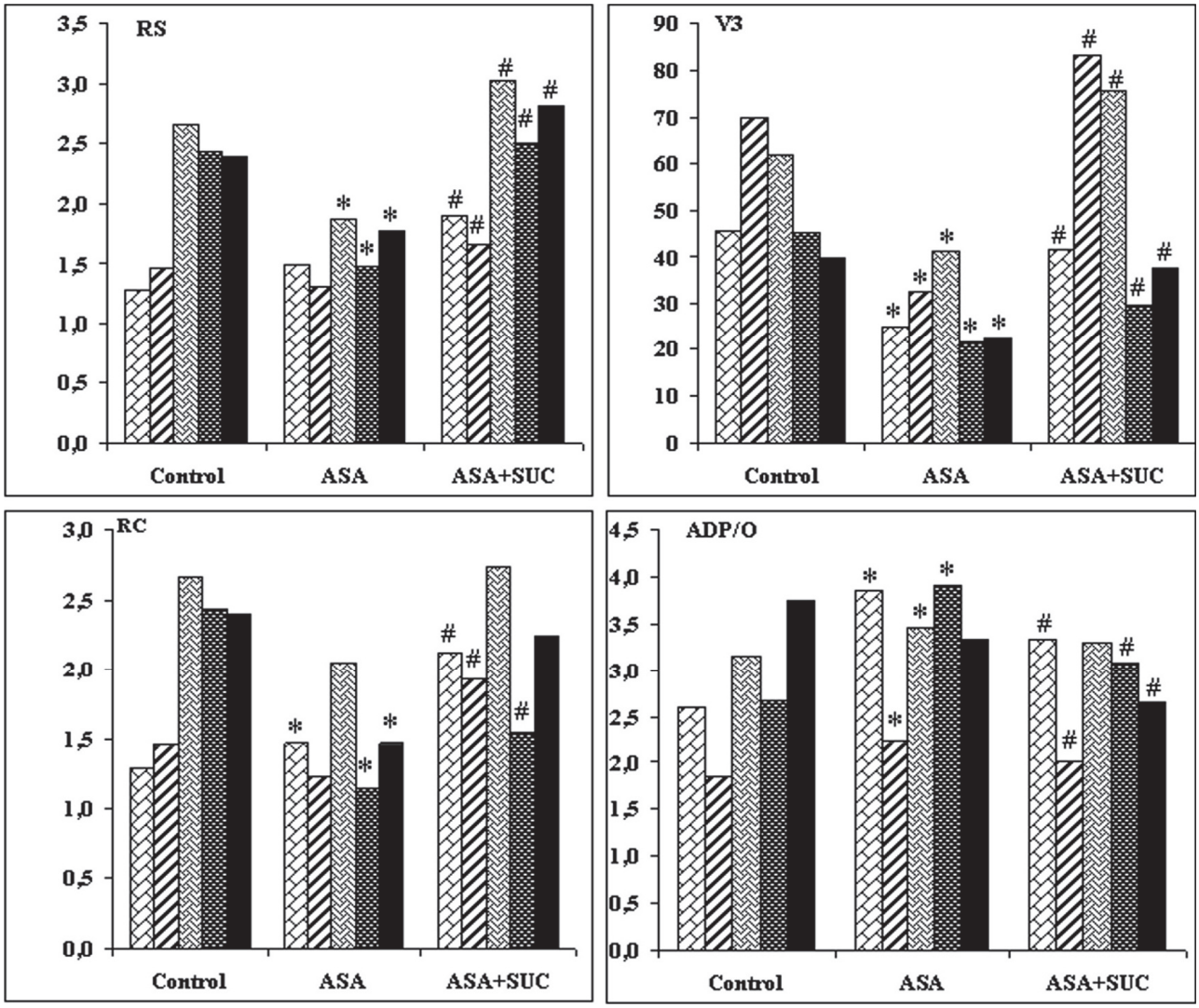
Pharmacokinetics experiments were conducted on 10 outbred male rabbits between 2.5 and 2.7 kg. Drugs were injected intragastrically at a single daily dose of suspension in 1% starch gel: ASA at a dose of 125 mg/kg, ASA and SUC mixture at a dose of 125 mg/kg and 50 mg/kg, respectively. ASA dose corresponds to a single therapeutic dose for a human being. Next the animals were segregated into two groups. ASA was injected into the animals of Group 1, while the ASA and SUC mixture was administered to the animals of Group 2. ASA was quickly hydrolyzed under the influence of esterases (within 10-15 minutes); therefore, we determined the concentration of ASA metabolite in the blood, i.e. salicylic acid. First, 500 μL of blood was drawn from the auricular vein and placed into plastic heparinized tubes before drug administration and in 0.5; 1; 4; 7; 8; and 9 hours after drug injection.. Next 0.5 mL of 5% NaCl solution and 2 ml of diethyl ether were added to the tubes for 10 minutes and extracted. The samples were then centrifuged at 3000 g for 10 minutes. The same quantity of the organic layer was transferred into glass evaporation tubes and vaporized by nitrogen flow at 45°C. Then 100 μL of the mobile phase was added to a solid residue of each sample and thoroughly mixed using a shaker for 10 minutes, at 100 shakes per minute. An aliquot of 50 μL was applied for chromatography. Analysis was done using the HPLC method (chromatograph «Millichrome A-02», Russia). The following pharmacokinetic parameters were determined, namely: maximum concentration (C_{max}); time required to reach maximum concentration (T_{max}); area under the pharmacokinetic curve (AUC0-t) reflecting an amount of the drug circulating in the blood from its penetration into the organism to the moment of minimum determined concentration. The C_{max}/AUC0-t ratio was calculated to determine the rate of drug absorption. Mean values (Mean), geometrical mean values (G_{mean}), standard deviation values (SD), lower and upper limits of 90% confidence interval were also calculated.

Results and discussion

Significantly, ASA injected to laboratory animals intragastrically at a single daily dose of 250 mg/kg for 7 days have been experimentally shown to produce inhibition of mitochondrial respiration rates in rat heart during the process of utilization of the endogenous substrate before, during and after ADP phosphorylation cycle (V4p, V3, V4o, respectively) when compared with the control group. An increase in the ADP/O coefficient was observed, in our opinion, to show the inhibition of the succinate-dependent and NAD-dependent energy-generating processes (Fig. 1). Similar changes were also observed in case of the oxidation of the exogenous substrates. Therefore, we noted the drop in the oxygen consumption rates during the period of succinate oxidation in myocardial mitochondria before, during and after ADP Phosphorylation, accompanied by a decrease in the RC value and a slight increase in the ADP/O coefficient (Fig. 1). During the oxidation of succinate in the myocardial mitochondria of rats which received the ASA, an increase in the ADP phosphorylation time was observed. This indicates a limitation of the substrate oxidation pathway and mitochondrial ATP/ADP coefficient [11] (Fig. 1).

During NAD-dependent substrate oxidation in the myocardial mitochondria of rats which received ASA, a decrease in the respiration rate was observed accompanied by an increase in the ADP phosphorylation time when compared with the control (Fig. 1). A drop in the RS value accompanied by an increase in the ADP/O coefficient indicates the kinetic nature of the heart mitochondrial energy deficiency in rats which had received the ASA [12]. Simultaneously, the inhibition test using the SDH competitive inhibitor malonate and aminotransferase inhibitor aminooxyacetate showed that the ASA induced an increase in the endogenous SUC oxidation which contributed to the mitochondrial respiratory activity during NAD-dependent substrate oxidation. This process is probably compensatory in character and reflects the peculiarity of the metabolic regulation of the Krebs cycle in the ASA-altered mitochondrial condition and specifically, the activation of a fast metabolic cluster [11].

Figure 1.
The functional state of rat heart mitochondria during ADP phosphorylation on injecting ASA and ASA with SUC combination.



Notes: *Oxidation substrates: – endogenous; – succinate; – malate with glutamate; – malate with glutamate and malonate; – malate with glutamate and aminooxyacetate.*
** – significant differences compared to controls when $p<0.05$;*
 # – significant differences compared to animals having received ASA when $p<0.05$.

SUC administration at a single daily dose of 50 mg/kg was found to facilitate the elimination of ASA-induced negative metabolic shifts in rat heart mitochondria (Fig. 1). Normalization of the respiratory rates in all the mitochondrial metabolic states was noted during the endogenous substrate oxidation relative to similar indices in animals which received only ASA. In that case, the RS and RC values were observed to increase, while the ADP/O value decreased that point to recovery of succinate-dependent energy-generating processes.

On SUC oxidation the myocardial mitochondria of rats which received SUC against the background of ASA, were observed to show a significant increase in the oxygen consumption rates before, during and after the ADP phosphorylation cycle, as well as an increase in the RS and RC coefficients when compared with those of the animals which received the ASA (Fig. 1). It testifies to an SUC-induced activation of the succinate-dependent substrate oxidation pathway and oxidative phosphorylation. Significantly, the ADP/O indices in this group of animals were similar to those of the controls [11, 13].

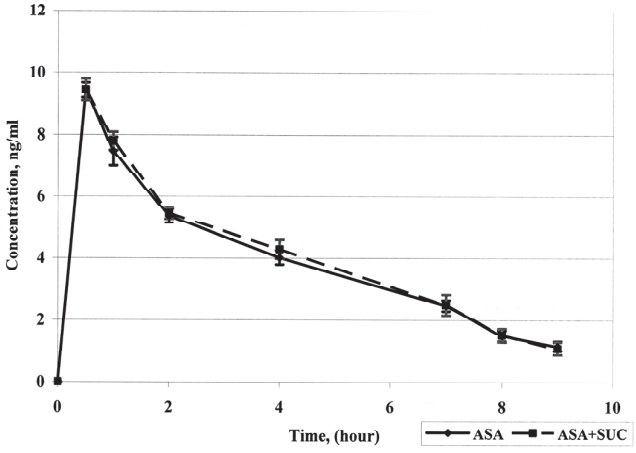
During the NAD-dependent substrate mixture oxidation in the myocardial mitochondria of rats which received SUC against the background of ASA administration, a significant increase in the oxygen consumption rates and RS value were noted when compared with the group of animals which received ASA (Fig. 1). This implies a normalization of the NAD-dependent respiration and oxidative phosphorylation in animals under SUC protection. The inhibition test using the SDH competitive inhibitor malonate and aminotransferase inhibitor aminooxyacetate has revealed a

considerable increase in the endogenous SUC oxidation relative to the group of animals which received the ASA that confirms stimulation of the responses of the mitochondrial fast metabolic cluster.

On studying the pharmacokinetics of ASA and ASA with SUC combination that concentration of salicylic acid (metabolite of SUC) in rabbits blood plasma was found to reach a maximum (8 μg/ml) in 30 minutes of the experiment . The concentration of salicylic acid in rabbits blood plasma dropped to a minimal detectable level (1 μg/ml) by the ninth hour of the experiment (Fig. 2).

Figure 2.
The dynamics of salicylic acid concentration averaged in the rabbit blood plasma on injecting ASA alone or in combination with SUC.

No statistic differences were noted either in the



pharmacokinetic parameters of salicylic acid after injecting only ASA or in the ASA and SUC combination. The limits of the estimated confidence intervals for AUC0-t, C_{max} and C_{max}/AUC0-t parameters constituted 82-115.4%, 89-131.9% and 96.6-125.8%, respectively, and were within the permissible limits of 80-125% for the AUC0-t parameter and 75-133% for the C_{max} and C_{max}/AUC0-t parameters. Thus, it has been concluded that ASA and ASA with SUC combination have the same bioequivalence.

As a result, the preventive effect of SUC was revealed to promote the elimination of ASA-induced negative metabolic shifts in the rat heart mitochondria due to normalization of the succinate- and NAD-dependent respiration, oxidative Phosphorylation. Therefore, it can be used to correct the ASA-induced negative side-effects of the energy-generating system. Incidentally, SUC has no pronounced effect on ASA pharmacokinetics and, therefore, there is no need to correct further administration of the drug.

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