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EFFECTS OF NITRIC OXIDE SYNTASE INHIBITOR ON EXERCISE-MEDIATED PRO- AND ANTI-OXIDATIVE BALANCE IN RAT BLOOD PLASMA

WPŁYW INHIBITORA SYNTAZY TLENKU AZOTU NA WYSIŁKOWO MEDIOWANY BALANS PRO- I ANTY-OKSYDACYJNY W OSOCZU KRWI SZCZURA

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Studies showed that nitric oxide synthase (NOS) inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), enhanced anti-oxidative shifts in the blood plasma of rats subjected to exhaustive running exercise. A type of running training (continuous endurance and intermittent) before the exhaustive exercise was found to differentiate L-NAME-induced effects in rats.

Key words: nitric oxide synthase (NOS), L-NAME, lipid peroxidation, total anti-oxidant status (TAS), Trainings and exhaustive running exercises, rats
Słowa kluczowe: syntaza tlenku azotu (NOS), L-NAME, peroksydacja lipidów, całkowity status antyoksydacyjny (TAS), treningi i wysiłki biegowe do wyczerpania, szczury

INTRODUCTION

Nitric oxide (NO) is believed to be a key mediator of physiological functions that range from beneficial effects, including vasoregulation, neural signaling or immune defences to detrimental one, such as lipid peroxidation [6,11]. More recently, the agent (NO) from the amino acid L-arginine was elucidated to have anti-oxidant properties, and it plays a key role in decreasing the enhanced level of lipid peroxidation in cells [15]. Although it was also shown to nitrate polyunsaturated fatty acids to form nitro-derivatives, which brakes lipid peroxidation processes in these cellular components [15], the NO radical was recognized to promote pro-oxidant shifts due to formation of peroxynitric anion ($ONOO^-$), a product of reaction between NO and superoxide anion [6].

Over the last years, a well-recognized benefit of regular and moderate running exercises, including the reduced risk of lipid peroxidation has been identified in experimental animals

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and human subjects [3,4,8,19]. Because of the distinctive characteristics in moderate and exhaustive running exercises due to pro- and anti-oxidative properties [1], the major goal of the study was to elucidate a potent role of different type of running exercise trainings, *e.g.* continuous endurance and intermittent, on lipid peroxidation processes and total anti-oxidation balance in the blood plasma of exhaustive exercised rats. Since inhibition of nitric oxide synthase (NOS) was previously shown to reduce capacities to perform running exercises, but it also mitigated lipid peroxidation in the heart, liver, *soleus* as well as the red part of *gastrocnemius* muscle in rats [3], a potent role of NO from the endogenous origin due to NOS inhibition was also studied in trained animals.

MATERIAL AND METHODS

Male *Wistar* rats were used in the studies, and they were fed with Murigran chow (Motycz, Poland), and water *ad libitum*. The animals were randomly divided into four experimental groups and they were acclimatized for one week before the respective training. In the group 1, the animals were left untrained. In the groups 2 and 3, the rats were subjected to continuous endurance training models on a treadmill at speed intensity of 10 and 22 m/min (°15), respectively. The animals were daily trained for 60 minutes (5 day/week) for 3 weeks. In the group 4, the rats were subjected to intermittent training model using a treadmill (30 m/min; °15), and they were trained for 60 minutes *per* day (10 x 2 minutes following 4 minutes of rest) for 3 weeks (5 days/week). On the last day of the training period, the half of randomly selected rats in each groups (n = 8) were treated *per os* with either a single dose of *N*ω-nitro-L-arginine methyl ester, L-NAME (30 mg/kg m.c.) or normal saline (control), and they were subjected to exhaustive running exercise on a treadmill (22 m/min; °15) two hours later. The animals were killed by cervical dislocation at 3 hours post-exhaustive exercise, and further used for blood plasma analysis. Thiobarbituric acid reactive substances (TBARS), as a biomarker of lipid peroxidation and the total antioxidant status (TAS) of rat blood plasma were assayed to calculate the TBARS/TAS ratio (TB/TA), a novel indicator and/or biomarker of the pro-oxidation and anti-oxidation balance in both rest and exercised animals [3,5]. Briefly, thiobarbituric acid test was performed using rat blood plasma, which was added to 8.1% SDS. Thereafter, 20% glacial acetic acid and 2-thiobarbituric acid (v/v) were added to the reaction mixture. To start the reaction, the samples were heated with a spectral pure ***n***-butanol and centrifuged (4000,0 x g) for 10 min at 4°C. All butanol extracts were measured spectrophotometrically at 532 nm. Standard samples contained 1,1,3,3-tetraethoxypropane instead of blood plasma [5]. The total anti-oxidant status (TAS) of rat blood plasma was determined using the RandoxÔ assay kit (TAS, RandoxÔ, 1992, pp. 1-6, Randox Laboratories Ltd., Antrim, UK). In this procedure, the azo-compound, ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $ABTS^+$. The cation had a relatively stable blue-green colour, which it was measured spectrophotometrically at 600 nm. Anti-oxidants in the added blood sample caused suppression of this colour production to a degree, which was proportional to their concentration. In the present assay, TMCA (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard [5].

The statistical significance of the differences was determined by using *Student's t*-test for comparison between the repeated analysis of variance (ANOVA) in two groups and *Dunnet's* tests for multiple comparison where appropriate. Differences were considered significant when probability (p) values were less than 0,05.

RESULTS AND DISCUSSION

Pretreatment of animals with L-NAME decreased the level of lipid peroxidation, expressed as TBARS in untrained rats (group 1), resulting also with unchanged TAS levels in these animals (Fig. 1). Since the TB/TA ratio was composed of these two parameters (TBARS, TAS), the decreased TB/TA ratio, as a biomarker of pro-oxidation and anti-oxidation balances was identified in the blood plasma of untrained and exhaustive exercised animals (Fig. 2). As shown in figure 2, a similar effect of the NOS inhibitor (L-NAME) was also observed in animals subjected to intermittent training model (group 4). In contrast to the untrained and exhaustive exercised rats, the L-NAME agent was found to abolish both TBARS and TAS levels (Fig. 1), resulting further in decreasing the TB/TA ratio in the intermittent type of training performed before the exhaustive exercise (Fig. 2). It should be also noted that the selected speed of continuous endurance training model at 10 m/min was found to failure L-NAME-mediated effects, but it was not observed in exhaustive exercised animals due to the higher intensity of continuous endurance exercise, arranging at 22 m/min, respectively (Fig. 1). As shown in figure 1, the continuous endurance training at speed intensity (22 m/min) mitigated both TBARS and TAS levels in animals pretreated with L-NAME. Interestingly, both types of the continuous endurance exercises did not change the TB/TA ratio in animals pretreated with L-NAME, as compared with those of the saline-treated groups (Fig. 2).

Results of the present studies clearly evidenced an association between the applied model of running training and the observed level of NOS-related pro-oxidation and anti-oxidation balance in the blood plasma of animals pretreated with or without L-NAME (Figs. 1,

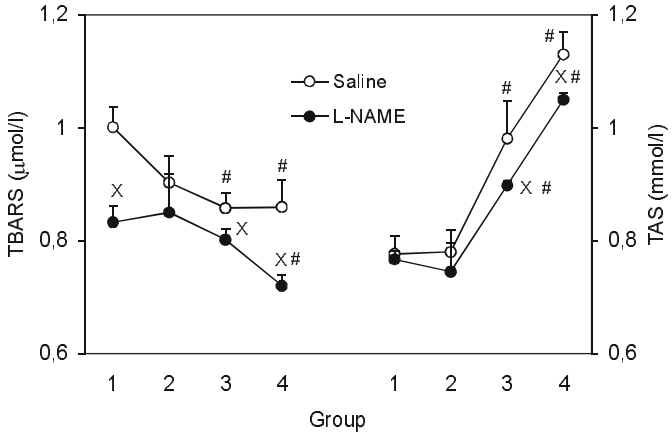

Fig. 1. Effects of nitric oxide synthase inhibitor (L-NAME) on tiobarbituric acid reactive substances (TBARS) and total anti-oxidant status (TAS) in the blood plasma of exhaustive exercised rats.
Values are mean ± SEM, n = 8, x $p<0,05$ *vs.* respective (untrained or trained) saline, # $p<0,05$ *vs.* respective (untrained) saline or L-NAME; 1 – untrained, 2 and 3 – continuous endurance training at 10 and 22 m/min, respectively, 4 – intermittent training at 30 m/min (see material and methods for details).

2). These results were found in accordance with those previously reported by *Niess* et al. [12] and *Roberts* et al. [14], who also noted that physical exercise induced the inducible form of nitric oxide synthase (iNOS) and NO production in both blood leucocytes and

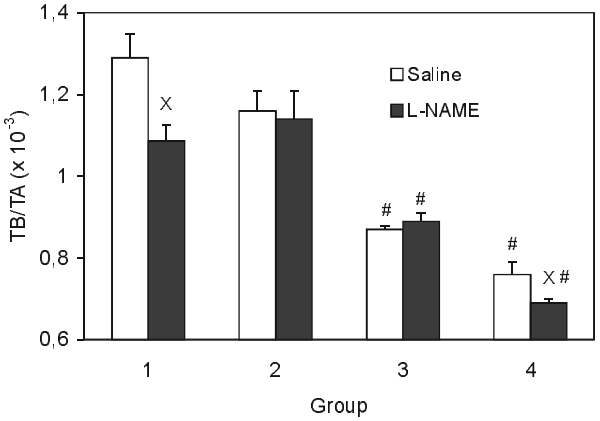

Fig. 2. Effects of nitric oxide synthase inhibitor (L-NAME) on pro- and anti-oxidative balance (TB/TA) in the blood plasma of exhaustive exercised rats.
See figure 1 for abbreviations and comments. TAS levels were recalculated for mmol/l to express the TB/TA ratio as TBARS (mmol/l)/TAS (mmol/l).

skeletal muscle, as well. Since proinflamatory mediators mainly induce the iNOS enzyme in cellular components, it was recognized as a risk factor in peroxynitrite ($ONOO^-$) elevation due to exhaustive exercise in both animals and humans [6,12,14]. More recently, chronic exercise increased both inducible and endothelial nitric oxide synthase gene expression in endothelial cells of murine aorta, and it also increased NO production, plausibly *via* iNOS expression in exercised animals [18]. It should be noted that the quantitative assessment of nitric oxide (NO) in rat skeletal muscle and plasma after exercise showed the highest concentrations of cytosolic nitrate ion (NO_3^-), further suggesting that inorganic nitrate was a final break-down product of NO oxidation in exercised animals [13].

In the present studies, the intermittent running training of animals and the continuous endurance trainings at the higher regiment of speed intensity (22 m/min) were found to mitigate the TB/TA ratio in both saline- and L-NAME-treated animals, as compared to untrained controls (Fig. 2). To data, the applied trainings were also shown to elevate TAS levels in animals, but the intermittent model of running exercise was only noted to mitigate lipid peroxidation in both saline and L-NAME-dosed rats (Fig. 1). No such results were observed for the continuous endurance exercise of animals at the lower intensity (10 m/min), respectively (Fig. 1). In our experiments, the training intensity was a critical element of the applied type of continuous endurance training, affecting the measured level of pro- and anti-oxidant balance in animals. As shown in figure 1, it differentiated the continuous endurance models of running exercise, favoring the higher intensity (22 m/min), as a more effective in increasing the antioxidant potential of the blood plasma of L-NAME-treated animals. Since the agent (L-NAME) is well-known as a selective inhibitor of nitric oxide synthase (NOS), especially the inducible (iNOS) and endothelial (eNOS) isoforms in ro-

dents, a novel mechanism of the modulation of pro-oxidant and anti-oxidant balance was elucidated in L-NAME-treated and exercised animals. More recently, *Maeda* et al. [10] showed that plasma NO concentration due to eNOS expression was significantly increased after exercise training in humans and that elevated plasma NO levels were lasted to four weeks post-exercise [10]. It should be noted that *Kingwell* et al. [9] also reported that four weeks of cycle training increased plasma NO in humans. To data, *Sessa* and associates reported that exercise training increased expression of mRNA NOS in dogs [16], and that was also observed by others, who noted that endurance exercise trainings affected NOS gene expressions in porcine coronary resistance arteries [17].

The biochemical mechanism by which prolonged exercise trainings enhance NO production and/or anti-oxidative shifts is still far to complete. It seems plausible that exercise trainings up to 3-4 weeks, or even more, are enable to induce a large number of physiological responses, including circulating hormones and/or local autacoids, which may exert an influence on NO production [11]. Based on results from our present experiments, there is further support for nitric oxide, showing that the increased anti-oxidant shifts, as induced by prolonged exercise and L-NAME, may affect both endogenous NO, and the $ONOO^-$ anion, origin from the reaction between NO and superoxide anion ($O_2^{\cdot-}$), resulting in changing pro-oxidation and anti-oxidation balances in animals (Figs. 1, 2). In accordance, endurance trainings have been previously shown to increase superoxide dismutase (SOD) activity in skeletal muscle [7], and SOD gene expression (mRNA SOD) also was postulated to play a regulatory role in anti-oxidant adaptation processes to exercise-induced oxidative stress in endurance-exercised rats [7]. This was also confirmed in our recently published results, which provided more details on the pivotal role of SOD enzyme and GSH-linked anti-oxidation processes in exhaustive forced and NAME/L-arginine pretreated animals [2].

In summary, studies showed that inhibition of nitric oxide synthase (NOS) with L-NAME changed the pro- and anti-oxidative balance of the blood plasma towards anti-oxidative directions (TB/TA), elucidating a key role of nitric oxide (NO) in exhaustive exercised rats. Intermittent running trainings before the exhaustive exercise enhanced L-NAME-induced effects on TB/TA in animals, but it was not observed in both continuous endurance trainings.

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Summary

Rats were subjected to different running trainings on a treadmill for 3 weeks, including continuous endurance and intermittent exercises at speed intensity of 10, 22 and 30 m/min (°15), respectively. On the last day of the training period, the animals were dosed with *N*w-nitro-L-arginine methyl ester, L-NAME (30 mg/kg b.w.), and they were further subjected to exhaustive running exercise (22 m/min; °15). Studies showed that inhibition of nitric oxide synthase (NOS) with L-NAME mitigated pro- and anti-oxidative (TB/TA) balance in the blood plasma of rats subjected to exhaustive exercise. Intermittent training before the exhaustive exercise enhanced L-NAME-induced effects on TB/TA levels in rats, but it was not observed in continuous endurance trainings

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Streszczenie

Szczury poddawano różnym treningom biegowym na bieżni przez okres 3 tygodni, w tym wysiłkom ciągłym o charakterze wytrzymałościowym i interwałowym przy szybkościach 10, 22 i 30 m/min (°15). Ostatniego dnia okresu treningowego, zwierzęta otrzymywały ester metylowy *N*w-nitro-L-argininy, L-NAME (30 mg/kg m.c.), po czym były one poddawane wysiłkowi biegowemu do wyczerpania (22 m/min; °15). Badania wykazały, że inhibicja syntazy tlenku azotu (NOS) za pomocą L-NAME obniżała równowagę pro- i anty-oksydacyjną (TB/TA) w osoczu krwi szczurów poddawanych wysiłkowi do wyczerpania. Interwałowy trening, który przeprowadzano przed wysiłkiem do wyczerpania zwiększał efekty indukowane przez L-NAME na poziom TB/TA u szczurów, czego nie odnotowano po zastosowania wysiłków ciągłych o charakterze wytrzymałościowym.

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