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THE EFFECT OF THE TRIMER OF 16,16-DIMETHYL-15-KETO-PGB$_1$ ON METABOLIC AND FUNCTIONAL POST-ISCHEMIC RECOVERY OF THE HEART

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Abstract. The anti-ischemic effect of the trimer of 16,16-dimethyl-15-keto-PGB$_1$ (Trimer) and the prospects of its application in medicine were estimated. The functional parameters of an isolated rat heart and main cellular respiratory parameters were studied using the Langendorff perfusion method and micromethods for the determination of mitochondrial respiration without separating them from tissue. The Trimer (concentration in perfusion solution 0.5 μg/ml) does not influence the physiological parameters of an isolated rat heart either before or after the ischemic arrest. Nevertheless, this Trimer has a statistically reliable beneficial effect on mitochondria. Such functions of mitochondrial respiration as ADP stimulated and creatine stimulated respiration were maintained remarkably better in the hearts which were perfused with the Trimer containing solution. Better maintenance of the mitochondrial function may influence the recovery during a long post-ischemic period and improve the quality of life. Therefore, the application of this Trimer in the anti-ischemic protection of myocard is reasonable, but the protection would be more efficient if the Trimer was combined with the agents which guarantee the protection of the heart from irreversible injuries.

Key words: prostaglandin oligomer, ischemia, anti-ischemic protection, mitochondria, respiration, perfusion.

INTRODUCTION

In spite of intensive investigation, effective protection of the heart during ischemia and post-ischemic reperfusion has remained a typical problem. The problem is important both in practical and theoretical aspects: in case of intraoperational cardioplegia, especially during long-lasting

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surgery, the heart is not totally protected (Julian et al., 1989; Kempsford & Hearse, 1989; Laster et al., 1989; Hearse, 1990). Also, in clinical cardiology there is a need to protect the heart against reperfusional damage in the case of patients with myocardial infarction receiving thrombolytic therapy (Rosenkranz et al., 1986). The means expected to protect the heart against the loss of function associated with an ischemic episode include those which maintain the intracellular availability of ATP. Prostaglandin oligomers synthesized from 16,16-dimethyl-15-keto-PGB_1 have a number of beneficial effects concerning intracellular Ca^{2+} and oxidative phosphorylation in mitochondria. They have been shown to maintain oxidative phosphorylation during the hypotonic degradation in aged mitochondria (Polis et al., 1979; Devlin et al., 1986) and stimulate the release of Ca^{2+} from mitochondrial pool in isolated hepatocytes (Uribe et al., 1987a, 1987b). Later the inhibition of phospholipase A_2 has been found (Franson & Rosenthal, 1989). It has been reported that in vivo prostaglandin oligomers exhibit the protection of animals following the cardiac (Angelakos et al., 1980) and cerebral ischemia (Kolata & Polis, 1980; Von Lubitz & Redmond, 1989) and hypoxia (Polis & Cope, 1983).

In this paper, the anti-ischemic effect of the trimer of 16,16-dimethyl-15-keto-PGB_1 (further Trimer) and the prospects of its application in medicine are reported. The functional parameters of isolated rat heart and main cellular respiratory parameters were studied by using the Langendorff perfusion method and micromethods for determination of mitochondrial respiration without separating them from tissue.

MATERIALS AND METHODS

Synthesis and separation of the Trimer are presented in our earlier papers (Martin & Lille, 1989; Martin et al., 1991).

Perfusion of isolated hearts by the Langendorff method

The hearts were excised from Wistar rats with body weight 300–400 g and perfused with standard Krebs–Henseleit buffer without *orto*-phosphate. The main cardioplegic solution (solution of St. Thomas hospital (Kempsford & Hearse, 1989)) contained 10 mM $NaHCO_3$, 90 mM NaCl, 16 mM KCl, 16 mM $MgCl_2$, 1.2 mM $CaCl_2$, pH 7.8. The perfusion solutions were aerated with carbogen (95% O_2 / 5% CO_2). The hearts were transferred into a 20 mm NMR tube of Bruker SHR-200 Spectrometer and immersed totally into the perfusion solution. The hearts were perfused with normal Krebs–Henseleit buffer during 20–30 min. Then the perfusion was switched to the cardioplegic solution. The perfusion was allowed to continue for 5 more minutes after the heart was stopped, and then the ischemic arrest was initiated by switching off the pump. After 30–35 min of ischemic arrest the heart was reperfused with cardioplegic solution for 5 min followed by standard Krebs–Henseleit buffer during 30–50 min with simultaneous registration of ^{31}P NMR spectra as described by Saks et

al. (1989). The Trimer was added to the cardioplegic solution 0.5 μg/ml which was used prior ischemic arrest.

Determination of cellular respiration parameters

Medium A: 0.1 μM Ca^{2+}, 9.5 mM $MgCl_2$, 20 mM imidazole, 0.5 mM DTT, 20 mM taurine, 5.25 mM ATP, 15 mM PCr, 49 mM K-MES, 50 μg/ml saponin, pH 7.0. Medium B: 0.1 μM Ca^{2+}, 4 mM $MgCl_2$, 20 mM imidazole, 3 mM KH_2PO_4, 5 mM glutamate, 2 mM malate, 0.5 mM DTT, 20 mM taurine, 100 mM K-MES, 2 mg/ml BSA, pH 7.0.

Bundles of fibres 0.3–0.4 mm in diameter and 5–7 mm in length were separated from the endocardial surface of the left ventricle of the perfused and/or reperfused Wistar rat heart. The fibres were incubated in the medium A for 20 min followed by washing off the saponin in medium B during 10 min. All procedures were carried out at +4 °C and with intensive stirring. The extent of skinning was tested by measurement of lactic dehydrogenase in preparations as described earlier (Saks et al., 1989). The protein content in the preparations was determined after homogenization. Seven to eight bundles of chemically skinned fibres were transferred into the oxygraph cell equipped with a stirrer and charged with 3 ml of medium B. The oxygen consumption was measured using a Clark electrode and YSI oxygraph at 22 °C. The concentration of dissolved oxygen in the solution was considered to be 460 ngatoms ml^{-1}. All results were statistically analysed using Student's *t*-test.

Degradation of rat liver mitochondria

The isolation of rat liver mitochondria and determination of respiratory control index of isolated mitochondria, and the effects of the Trimer on the mitochondrial respiration parameters were performed by the method described by Kreutter & Devlin (1983) and Martin et al. (1986). The degradation medium contained 100 mM KCl, 20 mM HEPES buffer pH 7.4, 8 mM K-glutamate, 4 mM K-malate, 6 mM $MgCl_2$, 10 mM KH_2PO_4, and 2 mg/ml of mitochondrial protein at 30 °C. The Trimer was added before the initiation of the degradation by the addition of mitochondria. Then 0.5 ml of the degradation medium was transferred to the aliquot of 0.3 M sucrose, 20 mM HEPES buffer pH 7.4 and 0.6 mM EGTA at appropriate times. After a 1 min incubation 0.3 μmoles of ADP was added. The phosphorylation was terminated after 4 min by rapid centrifugation of the reaction medium and a sample of 30 μl was separated from the supernatant layer for the chromatographic analysis of AMP, ADP, and ATP. HPLC analysis of AMP, ADP, and ATP was performed by ion-pairing chromatography on the Zorbax ODS column with 5 mM tetrabutylammonium phosphate, 20 mM phosphate buffer pH 6.2, and 15% acetonitrile as an eluent system at 254 nm. The LKB 2220 Recording Integrator was used for the integration of peaks. The ATP/ADP ratio of intact mitochondria (degradation time 0 min) after the state of phosphorylation was determined to be in a range 16–30 depending on the quality of isolation and handling of mitochondria. The RCI values 6–7 and respiration of 70 ngatoms O_2 mg^{-1} min^{-1} in the state of phosphorylation were determined within one hour after the isolation.

RESULTS AND DISCUSSION

As Table 1 shows the Trimer does not influence the physiological parameters of an isolated rat heart either before or after the ischemic arrest: the recovery of the contractile function of the heart was almost equal in the control and in the experiment with the Trimer.

Table 1

Recovery of the functionality of the heart during reperfusion*

Reperfusion time, min	Control	Trimer, 0.5μg/ml
Prior ischemia	508±22	539±16
5	27±12	27±10
10	73±27	63±24
20	158±43	147±40
30	191±52	208±48
40	236±48	237±50
50	262±63	294±55

* The values are the products of pressure and frequency.

Table 2

The effect of the Trimer on ischemic damage of mitochondrial respiration in skinned fibres of rat heart (n = 8)

Experimental conditions	V_0	V_{adp}	V_{Cr}	V_{ADP}	$V_{ADP} - V_0$	V_{ADP}/V_0	%Cr
Control	8.4±0.9	17.4±1.8	27.8±2.5	43.6±2.4	35.2±2.2	5.14±0.41	60.0±4.4
35 min ischemia without Trimer	10.6±1.9	17.9±3.3	22.4±5.8	37.0±7.0	26.4±6.7	3.5±0.8	23.7±9.4
35 min ischemia with Trimer	13.0±2.1	22.4±4.1	30.4±5.6	44.7±6.0	31.7±6.1	3.5±0.7	35.5±6.3
P	<0.5	<0.5	<0.5	<0.5	NR	NR	0.05

V_0, respiration rate without nucleotides; V_{adp}, in the presence of 60 μM ADP; V_{ADP}, in the presence of 1 mM ADP; V_{Cr}, after addition of 60 μM creatine; %Cr, stimulation of respiration by creatine, %; NR, difference statistically not reliable.

Registration of ^{31}P NMR spectra showed that the Trimer had no effect on the tissue content of ATP and phosphocreatine. Nevertheless, a detailed investigation of energetic parameters of the heart applying micromethods

for the determination of cellular respiration indicated that the Trimer had a statistically reliable effect on mitochondria. Such functions of mitochondrial respiration as ADP stimulated and creatine stimulated respiration were maintained remarkably better in the hearts that were perfused with a Trimer containing solution (Table 2). Our recent investigations have demonstrated that in skinned fibres of the heart muscle 1 mM ADP is necessary for maximum activation of the system because of diffusion problems of ADP itself (Saks et al., 1991). The maximum stimulation of respiration, $V_{ADP} - V_0$, does not change much during 35 min ischemic arrest, which is an indication of the stability of the respiratory chain. The percentage of stimulation of respiration caused by creatine (%Cr) is a parameter that characterizes functional coupling between mitochondrial creatine kinase and oxidative phosphorylation, and the efficiency of aerobic synthesis of creatine phosphate. Its value decreased approximately three times in the absence of the Trimer and considerably less in the presence of the Trimer. This means that a high structural organization of the mitochondrial membrane complex that includes creatine kinase was maintained.

Table 3

The effect of the Trimer on the ATP/ADP ratio during the thermal degradation of isolated rat liver mitochondria

Degradation time, min	ATP/ADP	
	Control	Trimer, 3.11 nmol/mg
0	>15	>15
10	>15	>15
15	11.9	>15
20	3.3	>15
25	1.4	>15
30	1.0	11.3
40	0.75	1.7
50	–	0.9

– value not measured.

Preincubation of isolated rat liver mitochondria in the absence of phosphate acceptor at 30°C (thermal degradation) leads to the time-dependent decrease of the ATP/ADP ratio down to 0.75. The ATP/ADP ratio 1.4 was obtained after 25 min degradation (Table 3). The presence of the Trimer in the degradation medium prolongs the mitochondrial degradation time. For example, 45 min incubation was needed to decrease the ATP/ADP ratio to the same level.

However, a bell-shaped relationship was observed between the ATP/ADP ratio and the concentration of the Trimer in the degradation medium with the maximum at 3.3 nmol/mg mitochondrial protein at the 25 min

degradation (Fig. 1). This fact is consistent with the results of Kreutter & Devlin (1983) and Martin et al. (1986), which show the inhibition and uncoupling of oxidative phosphorylation by the oligomers of 15-keto-PGB_1 (PGB_x) at higher concentrations. Our results on the mitochondrial respiration at Steady State 4 (Fig. 2) suggest that the Trimer caused an increased State 4 respiration and consequently induced the uncoupling of phosphorylation at the concentration of 6–12 nmol/mg as well. Apparently, at higher Trimer concentrations the protective effect of the Trimer during the degradation was suppressed by the uncoupling. An optimum amount of the Trimer (3 nmol/mg) is needed for the demonstration of its beneficial effect in protecting the mitochondria from degradation.

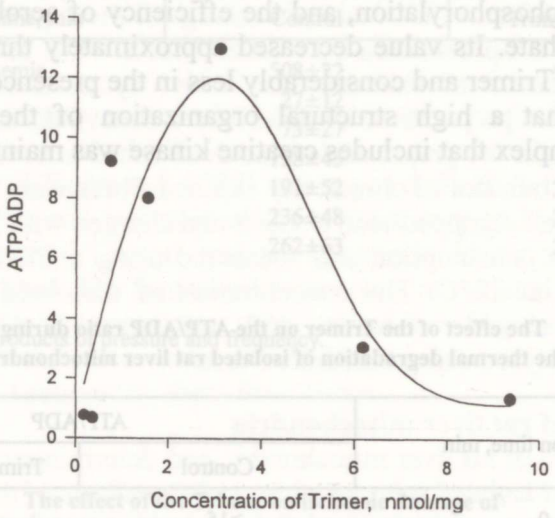

Fig. 1. The ATP/ADP ratio of 25 min degraded mitochondria by the 16,16-dimethyl-15-keto-PGB_1 trimer.

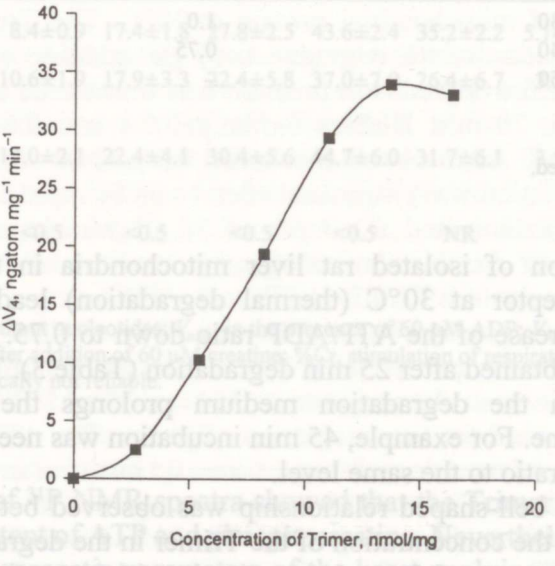

Fig. 2. Stimulation of mitochondrial State 4 respiration (ΔV_4) by the 16,16-dimethyl-15-keto-PGB_1 trimer.

The ischemic damage of cardiomyocytes has been investigated for a long time in many laboratories and at the present time a number of mechanisms of this complicated process are known. One of the key mechanisms is the breaking of the integrity of membrane structures, mainly of the sarcolemmal membrane, irreversible damage of which results in the release of intracellular enzymes into the blood plasma, in functional damages, and finally in the death of the cell. Additionally, a number of intracellular mechanisms result in reversible injuries expressed in a temporary decrease of the contractile function, which restores slowly during the reperfusion (Table 1). The myocard that has temporarily lost its contractile function has been called "stunned myocard" (Laster et al., 1989; Bolli, 1990; Lee & Allen, 1991; Triana et al., 1991).

It is evident that the Trimer has no influence on the processes responsible for irreversible damage of the cells during ischemia: the rate of damage and the recovery of the function in its presence do not change. Nevertheless, better maintenance of the mitochondrial function may influence the recovery during a long post-ischemic period and improve the quality of life. Therefore, the application of the Trimer in the anti-ischemic protection of myocard is reasonable, but the protection would be more efficient if the Trimer was combined with the agents that guarantee the protection of the heart from irreversible injuries.

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16,16-DIMETÜÜL-15-KETO-PGB_1 TRIMEERI MÕJU SÜDAME ISHEEMIAJÄRGSELE METABOOLSELE JA FUNKTSIONAALSELE TAASTUMISELE

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On antud hinnang 16,16-dimetüül-15-keto-PGB_1 trimeeri isheemiavastase toime ja meditsiinilise rakendatavuse kohta. Isoleeritud rotisüdame funktsionaalseid parameetreid uuriti Langendorffi perfusioonimeetodil, rakulise hingamise parameetreid aga mitokondrite hingamise kaudu. Viimane määrati mikromeetodi abil mitokondreid kudedest eraldamata. Ehkki 16,16-dimetüül-15-keto-PGB_1 trimeer (kontsentratsioon perfusiooni-

lahuses 0,5 μg/ml) ei mõjutanud isoleeritud rotisüdame füsioloogilisi parameetreid ei enne ega pärast esilekutsutud isheemiat, oli tal statistiliselt usaldusväärne kasulik toime mitokondritele. Selline mitokondritele iseloomulik funktsioon nagu ADP ja kreatiini poolt esile kutsutud hingamine oli säilinud märkimisväärselt paremini nendel südametel, mida perfuseeriti 16,16-dimetüül-15-keto-PGB_1 trimeeri sisaldava lahusega. Mitokondrite parem funktsioneerimine võib mõjutada isheemiajärgset taastumisperioodi ja parandada selle kaudu elu kvaliteeti. Nii on 16,16-dimetüül-15-keto-PGB_1 trimeeri kasutamine müokardi kaitsva agendina põhjendatud, kuid kaitsev toime oleks veelgi efektiivsem, kui seda kasutada kombineeritult agentidega, mis hoiavad ära pöördumatud kahjustused südamelihases.