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Paraquat inhibits progesterone synthesis in human placental mitochondria

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ABSTRACT

Introduction: Human placenta mitochondria produces huge amounts of progesterone necessary for maintaining the pregnancy. Lipid peroxidation in human placental mitochondria inhibits progesterone synthesis and that inhibition can be reversed by superoxide dismutase and other antioxidants. Paraquat (PQ) a highly toxic herbicide generates superoxide radical inside cells and induces lipid peroxidation. Hence, it is supposed to stimulate lipid peroxidation in human placental mitochondria and in consequence to inhibit a placental mitochondrial steroidogenesis.
Methods: Placentas were obtained from normal pregnancies. All experiments were done using isolated human placental mitochondria. Mitochondrial lipid peroxidation was determined as tiobarbituric acid reactive substances (TBARS). A conversion of cholesterol to pregnenolone or pregnenolone to progesterone was measured using radiolabeled steroids and thin layer chromatography.
Results: PQ enhanced the iron-dependent lipid peroxidation as also PQ heightened the inhibitory action of this process on progesterone synthesis in isolated human placental mitochondria. Paradoxically, a superoxide dismutase (SOD) reversed the inhibition of progesterone synthesis only minimally although it strongly inhibited PQ stimulated iron-dependent lipid peroxidation. When iron was absent, PQ stimulated only negligible lipid peroxidation but strongly inhibited progesterone synthesis. SOD had no effect on inhibition of progesterone synthesis by PQ. PQ strongly inhibited of the conversion of cholesterol to pregnenolone but had not got any influence on the enzymatic activity of mitochondrial 3β-hydroxysteroid dehydrogenase. PQ strongly decreased the efficiency of NADPH-dependent cytochrome P450 reduction as well as it promoted the rapid oxidation of the pre-reduced mitochondrial cytochrome P450. However PQ has not inhibited combined activity of adrenodoxin reductase and adrenodoxin.
Discussion: We conclude that the most important reason of the inhibition of progesterone synthesis by PQ is the escape of electrons from cytochrome P450scc to that compound what leads to cytochrome oxidation and, in consequence the inhibition of the reaction catalyzed by it. The action of PQ described here should be considered as potentially harmful for pregnancy and fetal development.

1. Introduction

Paraquat (PQ) is a highly toxic herbicide and the intoxication of humans by this compound often leads to death or serious multi-organ failure. Most cases of the PQ poisoning are related to farming and have accidental nature, although suicide cases have also been noted. If a pregnant woman is a victim of such poisoning, apart from the typical effects of poisoning, the metabolism of fetus and placenta can be affected as well [1,2]. That should be expected because PQ concentration in fetal blood is 4–6 times higher than in mother blood [1,2].

Human placenta produces large quantities of progesterone – necessary to maintain pregnancy [3]. This production is localized in the inner mitochondrial membrane and consist of two consecutive steps [4,5] first of which is the conversion of cholesterol to pregnenolone that is catalyzed by cholesterol desmolase (cytochrome $P450_{scc}$, CYP11A) whereas the second is the conversion of

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pregnenolone to progesterone catalyzed by 3β-hydroxysteroid dehydrogenase/$\Delta^{5,4}$-isomerase (3β-HSD).

It has been previously shown that iron- and NADPH-dependent lipid peroxidation inhibits progesterone synthesis in human placental mitochondria (HPMit) which are very susceptible to lipid peroxidation [6–9]. It is also well documented that PQ can be easily reduced by a one-electron process forming in consequence superoxide radical [10,11]that promotes lipid peroxidation and the injury of cells [12,13]. These premises have persuaded us to study the hypothesis according to which PQ can inhibit human placental steroidogenesis in mitochondria and this possibility is the main aim of the study presented here.

2. Materials and Methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), $FeCl_2$, glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase, NADPH, paraquat (PQ), superoxide dismutase (SOD), thiobarbituric acid (TBA) were purchased from Sigma-Aldrich. [4-^{14}C]cholesterol and [4-^{14}C] pregnenolone were obtained from Radiochemical Centre (Amersham, England). [^{3}H]progesterone and [^{3}H]pregnenolone were obtained from PerkinElmer (Boston, USA). Silica Gel G and Rhodamine 6G were purchased from Merck. All other materials were of the highest analytical grade available from POCh (Gliwice, Poland).

2.2. Preparation of placental mitochondria

Placentas were obtained immediately after the delivery from healthy women at the Department of Obstetrics and Gynecology, Medical University of Gdańsk. Human term placental mitochondria (HPMit) were prepared as described previously [6]. Mitochondrial protein was determined by the biuret method in 0.25% sodium deoxycholate using bovine serum albumin as the standard.

2.3. Progesterone and pregnenolone synthesis determination

The incubation was carried out for 30 min at 37 °C under air with constant shaking in 2.5 ml medium containing 0.1 M Tris-HCl buffer (pH 7.4), 5 mg of mitochondrial protein, either 0.5 μCi [4-^{14}C] cholesterol or 0.5 μCi [4-^{14}C]pregnenolone with 40 μg of cold pregnenolone and NADPH-generating system consists of 0.5 mM NADPH, 3 mM G-6-P and 5 units of G-6-P dehydrogenase.

After the incubation the each sample was divided into two parts. The volume of 0.5 ml was used for TBARS determination as described below. The remaining volume of 2 ml of mixture was transferred to conical tubes containing known amounts of [^{3}H] progesterone and [^{3}H]pregnenolone which were used to check recovery and non-radioactive progesterone and pregnenolone (2 mg of each steroid) as unlabeled carrier. The contents of tubes were extracted twice with 10 ml of chloroform-diethyl ether (1:5, v/v). The organic and aqueous phases were separated by centrifugation and the organic phase was evaporated to dryness. The dry residue was subjected to thin-layer chromatography on Silica Gel G impregnated with Rhodamine 6G. On developing the chromatogram in (I) methylene chloride-diethyl ether (5:2, v/v), three fractions were obtained: progesterone, pregnenolone, and cholesterol. Progesterone and pregnenolone fractions were further purified by thin-layer chromatography in the following systems: (II) benzene-ethanol (9:1, v/v), (III) benzene-ethyl acetate (3:2, v/v) and (IV) methylene chloride-methanol (98:2, v/v). Purification was continued to a constant ^{14}C/^{3}H ratio in the end products.

The radioactivity of isolated steroid was measured using a Beckman LS 6000 IC liquid scintillation spectrometer. The total incorporation of ^{14}C into [^{14}C]progesterone was found on the basis of tritium present in the final product of purification procedure.

2.4. Lipid peroxidation assay

When the incubation was finished 0.5 ml of incubation mixture was transferred and used to determination of lipid peroxidation which was later estimated from the amount of thiobarbituric acid-reactive substances (TBARS) formed as previously described [14]. The values for TBARS are expressed in nmol per mg mitochondrial protein using a molar absorption coefficient of 1.56×10^5 M^{-1} at 535 nm [15].

2.5. Determination of combined activity of adrenodoxin and adrenodoxin reductase in human placental mitochondria

Combined activity of adrenodoxin and adrenodoxin reductase was determined as NADPH-dependent cytochrome c reduction by measuring the absorbance increase at 550 nM in the presence of rotenone and potassium cyanide. The reaction mixture (2.0 ml final volume) contained 0.1 mM Tris-HCl pH 7.4, 0.5 mM NADPH, 3 mM G-6-P, 5 units of G-6-P dehydrogenase, 10 μM rotenone, 0.5 mM potassium cyanide, 50 μM cytochrome c and 1.0 mg mitochondrial protein. The reaction was initiated by addition of NADPH to the sample cuvette. The initial rate of cytochrome c reduction was monitored for every sample and the value of $21 \cdot 10^3$ M^{-1} cm^{-1} for the molar absorption coefficient of reduced cytochrome c was assumed.

2.6. Cytochrome P450 determination

The total mitochondrial cytochrome P450 was determined spectrophotometrically by the modification of the method described by Omura and Sato [16] using a molar absorption coefficient of $100 \times 10^3 \times cm^{-1}$ for the difference in absorbance between 450 and 490 nm. Mitochondrial suspension was bubbled with CO for 1 min after which 2.5 ml of the suspension was transferred to the sample cuvette and the base line was determined. After determination of the base line sodium dithionite was added to the same cuvette and next spectrum was determined once again.

The reduction and oxidation of cytochrome P450 by NADPH in HPMit was determined in a mixture consisting of 10 mM phosphate buffer, 0.5 mM NADPH, 5 unit of G-6-P dehydrogenase, 3 mM G-6-P and 2.5 mg protein/ml (about 0.15 nmol cytochrome P450/mg mitochondrial protein). All the samples were saturated by CO and then the reaction was initiated by the addition of $NADP^+$ to the sample cuvette (time "0"). The changes in absorbance differences between 450 and 490 were recorded across for the time given in figures.

2.7. Statistical analysis

The data collected were analyzed by one-way Analysis of Variance (ANOVA), and Tukey's post-hoc test was used for further determination of the significance of differences. Statistical significance was considered significant at $p < 0.05$. The data are expressed as means of all values ± standard error of mean (±S.E.M.) and N represents the number of experiments, which were performed in triplicate.