MICROSOMAL LIPID PEROXIDATION IN HUMAN PREGNANT UTERUS AND PLACENTA

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SUMMARY

A study was made of the microsomal lipid peroxidation of the pregnant human uterus and placenta. It was found that the lipid peroxidation of the microsomal fraction of the uterus is specific for prostaglandin formation: the lipid peroxidation was enhanced by arachidonic acid, and inhibited by antiprostaglandins. Accordingly, it is suitable as a screening test for the pharmacological examination of antiprostaglandin effects. The lipid peroxidation in the placenta is not specific. In both tissues examined the lipid peroxidation is linked to ascorbic acid.

INTRODUCTION

On the basis of the most recent findings it is accepted that the first step in the biosynthesis of the prostaglandins /PGs/ is the transformation of the precursor fatty acid /arachidonic acid/ to endoperoxides by cyclo-oxygenase /1-3/. In fact, this oxidative cyclization is none other than lipid peroxidation /Fig. 1./

In the cases of some animal tissues /sheep seminal vesicle, rabbit renal medulla, lung/, where the PG

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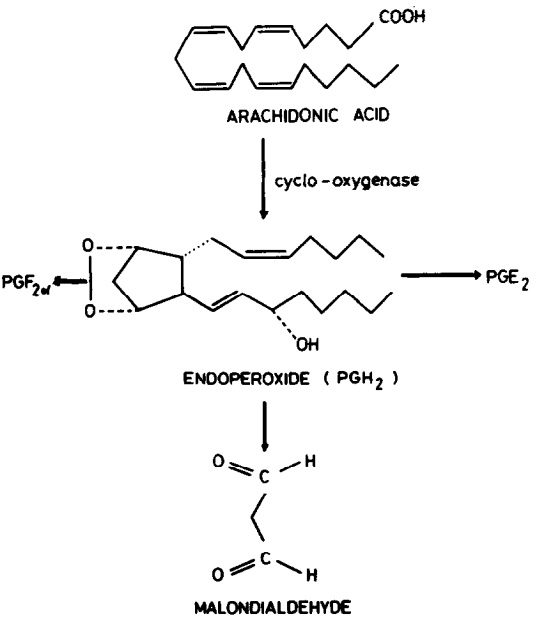

Fig. 1. Biosynthesis of prostaglandins and malendialdehyde from precursor acid.

synthesis is intensive, it has been proved that the lipid peroxidation is specific for PG formation: thus, PGs are formed from the lipid peroxides produced /endoperoxide isomerase, endoperoxide reductas/, while linear hydroperoxides are formed in the tissues with low PG synthetase contents /4-7/. All these lipid peroxides undergo reaction with malondialdehyde and can be determined quantitatively with thiobarbituric acid spectrophotometrically /4, 8/.

Two conditions must be satisfied for the specificity of the lipid peroxidation in a tissue to be proved:

/1/ with increasing concentration of arachidonic acid, as PG substrate, the lipid peroxidation must increase;

/2/ the antiprostaglandins must inhibit the lipid peroxidation in a dose-dependent manner /7/.

It is known that the PGs in the amniotic fluid increase significantly in pregnancy and during labour /9/.

No data are available on the in vitro PG biosynthesis of the microsomal fraction of the pregnant human uterus or placenta. In this paper an attempt is made to investigate the intensity of microsomal lipid peroxidation in the pregnant human uterus and the placenta, to see how specific this is for PG formation.

MATERIAL AND METHOD

Uterus and placenta tissue samples were obtained at the time of Caesarean sections. Exciped myometrium strips /ca. 600-800 mg/ were cut from region of uterine corpus immediately adjacent to the low transverse incision and stored at -30 °C until processing /max. 24 hr/. The tissue samples were homogenized at 0 °C in 10 volumes of TRIS-HCl buffer /pH 7.8/. The homogenizate was centrifuged for 10 min at 10,000xg, and the supernatant was recentrifuged for 60 min at 105,000xg. The sediment /microsomal fraction/ was resuspended in TRIS-HCl buffer, and 2 ml of the suspension /2.0-2.5 mg microsomal protein/ was used for the incubation examinations. The protein contetnts of the individual samples were determined by the method of Lowry et al. /10/. The 2 ml incubation mixture contained: 0-200 µmole arachidonic acid, 850 µmole ascorbic acid, 30 µmole Fe^{2+}.

After the incubation period /10 min/ the reaction was stopped with 1 ml 20 % TCA. The mixture was centrifuged for 5 min at 10,000Xg, and the supernatant was decanted off and boiled for 10 min on a 100 °C water-bath with 1 ml 0.67 % thiobarbituric acid. The red colour developing was measured spectrophotometrically in a 1 cm cell at 532 nm. A sample inactived for 5 min at 100 °C prior to the incubation was used as control. The malondialdehyde /MDA/ concentrations of the unknown samples were read off a calibration curve recorded with malondialdehyde-tetraethyl-acetal /K and K Lab. Inc., Plainview, USA./ /molar extinction coefficient 1.32×10^5/. The enzymatic activity is given in units of nmole MDA formed/mg protein/10 min.

Table I. Effect of cofactors on the microsomal lipid peroxidase activities.

Cofactors	Lipid peroxidation MDA formation nmole/mg protein/10 min.	
	Uterus	Placenta
None n = 5	3,0±0,17	4,9±0,26
Ascorbic acid 850 µmole n = 5	9,2±0,3	16,5±0,5
Ascorbic acid 850 µmole + Fe^{3+} 30 µmole n = 5	9,7±0,41	16,9±0,52
Ascorbic acid 850 µmole + Fe^{2} 30 µmole n = 5	26,4±0,6	21,8±0,47

RESULTS

In the system employed, the enzyme reaction at 37 ^{o}C under aerobic conditions is linear during the first 10 min in both the uterus and the placenta /Fig. 2./.

Table I. illustrates the effects of ascorbic acid, Fe^{2+} and Fe^{3+} on the microsomal lipid peroxidase activities of the uterus and the placenta. Ascorbic acid increased the enzymatic activity by approximately

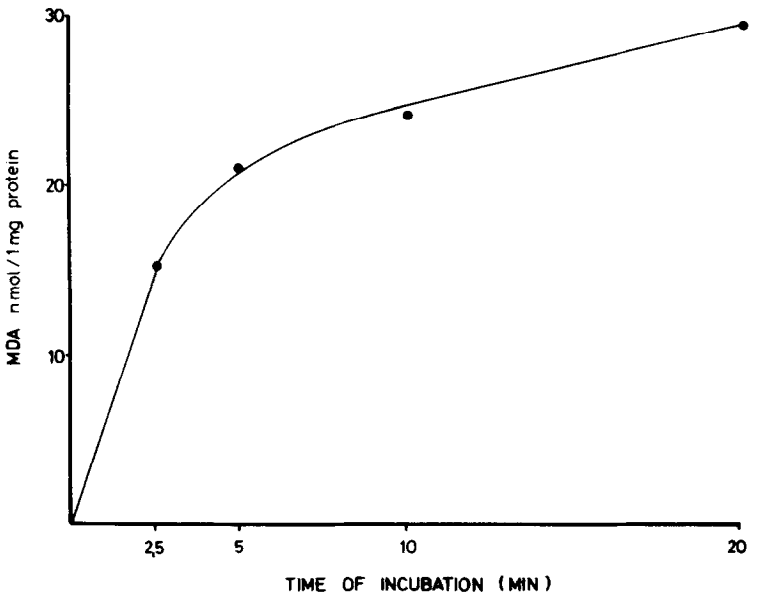

Fig. 2. Lipid peroxidase activity of microsomal fraction of human uterus, as a function of the incubation time.
Microsomal protein: 2.5 mg; arachidonic acid: 100 umole; cofactors: ascorbic acid: 850 umole, Fe^{2+}:30 umole

3-4-fold. The enzyme-induction effect of Fe^{3+} could not be detected, whereas Fe^{2+} markedly elevated the activity of the lipid peroxidase in the presence of ascorbic acid.

Figure 3. depicts the change in the activity of the lipid peroxidase as a function of increasing concentration of substrate /arachidonic acid/. The lipid peroxidation of the microsomal fraction of the uterus increases in proportion to the increase in the concentration of the arachidonic acid. No change occurs in the case of the placenta.

The inhibitory effect of the PG synthetase inhibitors /PGSIs/ on the lipid peroxidation could be demonstrated

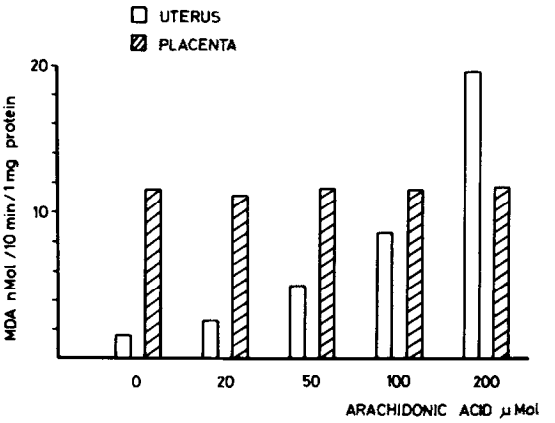

Fig. 3. Effect of arachidonic acid on lipid peroxidase activities of microsomal fractions of human uterus and placenta.
Microsomal protein: 2.2 mg /uterus/; 2.0 mg /placenta/. Incubation:10 min at 37 °C.

only in the myometrium. Arachidonic acid was added in increasing concentration to the microsomal fraction of the uterine corpus, and identical quantities of Naproxen^{R} /Syntex/ and Indomethacin^{R} /Chinoin/ /1 mmole each/ were added to every individual incubation vessel. During PG synthetase inhibition too, the correlation between the arachidonic acid concentration and the enzymatic activity is that to be expected on the basis of Michaelis kinetic: the double reciprocal plot is linear /Fig. 4/. The inhibitory effect of Naproxen is about half that of Indomethacin.

DISCUSSION

On the basic of the results it may be said that the pregnant human uterus possesses lipid peroxidase activity specific for PG formation, since with ara-

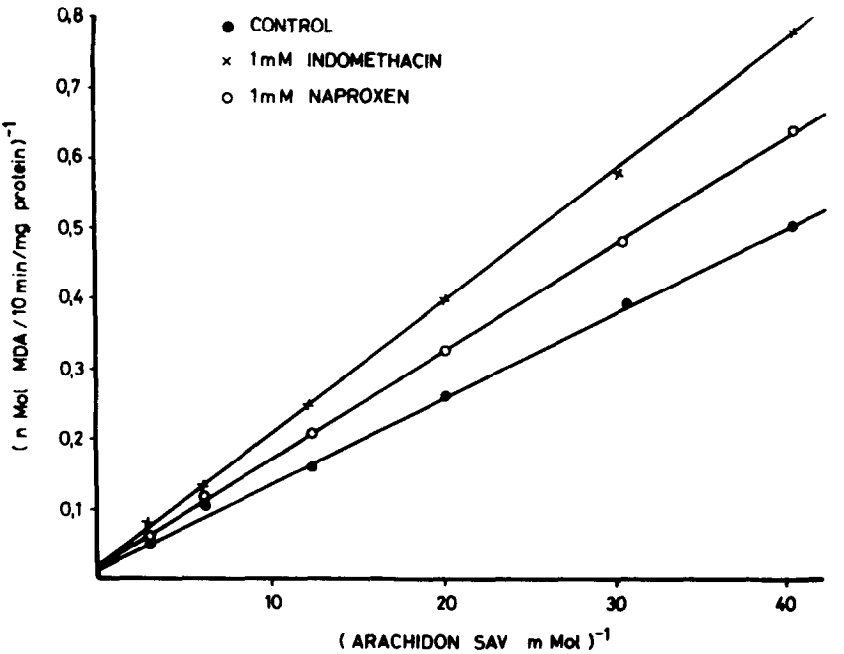

Fig. 4. Double reciprocal plot of MDA formations as a function of the arachidonic acid concentration in the presence and in the absence of inhibitors. Incubation: 10 min at 37 °C.

chidonic acid the enzymatic activity can be enhanced and with PGSIs it can be inhibited. In the placenta the lipid peroxidation is not specific. This suggests that there is no PG biosynthesis in the human placenta. This is also supported by the negative results of ^{14}C-arachidonic acid - PG incorporation examinations. However, the presence of the enzyme in the placenta is important as regards the inactivation /hydroxylation/ of drugs, as demonstrated earlier by Ernster and Nordenbrand /11/ in the rat liver.

Hochstein et al. /12/ pointed out that the presence of Fe^{2+} or Fe^{3+} is essential for the enzymatically induced peroxidation of the microsomal lipids.

However, since an inducing effect could be detected only with Fe^{2+} in the presence of ascorbic acid, on the basis of the rat liver experiments of Ernster and Nordenbrand /11/ it may be stated that the human uterus and placenta microsomal lipid peroxidation is typically a reaction linked to the ascorbic acid.

Since the lipid peroxidase activity of the human uterus can be inhibited with PGSIs this provides a possibility for the antiprostaglandin effect of newly synthetized compounds to be examined under in vitro conditions. There is rarely a possibility for this in the experimental /laboratory/ phase of drug research.

We consider the method elaborated to be suitable for the screening examination of a large number of potentially antiprostaglandin compounds.

Naproxen^{R} /Syntex/, studied in the present work, is the first drug with an antiprostaglandin effect that can be employed for the treatment of the danger of spontaneous abortion in gynaecology without any appreciable risk of side-effects. The results of the clinical-pharmacological examinations currently in progress are well supported by the demonstrated mechanism of molecular action.

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REFERENCES

1. Hamberg, M. and Samuelsson, B. /1973/ Proc. Natl. Acad. Sci. U.S.A. 70, 899.

2. Hamberg, M. Svensson, J., Wakabayashi, T., and Samuelsson, B. /1974/ Proc. Natl. Acad. Sci. U.S.A. 71, 345.

3. Nugteren, D.H. and Hazelhof, E. /1973/ Biochem. Biophys. Acta 326, 448.

4. Sih, C.J. and Takegushi, C.A. /1973/ Prostaglandins Vol. 1. /Ed. Romwell/ p. 83. Plenum Press, New York, London.

5. Flower, R.J., Cheung, H.S. and Cushman, D.W. /1973/ Prostaglandins 4, 325.

6. Robak, J., Dembinska-Kiec, A. and Gryglewski, R. /1975/ Biochem. Pharmac. 24, 2057.

7. Robak, J. and Sobanska, B. /1976/ Biochem. Pharmac. 25, 2233.

8. Hamberg, M. and Samuelsson, B. /1967/ J. Biol. Chem. 242, 5336.

9. Dray, F. and Frydman, R. /1976/ Am. J. Obstet. and Gynec. 126, 13.

10. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. /1951/ J. Biol. Chem. 193, 265.

11. Ernster, L. and Nordenbrand, K. /1967/ Methods in Enzymology Vol. 10. p. 574.

12. Hochstein, P., Nordenbrand, K. and Ernster, L. /1964/ Biochem. Biophys. Res. Commun. 14. 323.