Human Reproduction vol.8 no.1 pp.102–106, 1993

Effect of xenobiotics on quinone reductase activity in first trimester explants

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The placental protective enzyme quinone reductase (QR) has recently been reported to be induced by exposure to mercury, which is a toxic metal *in vitro* at term. In the present study we have examined the effect of three groups of xenobiotics—carcinogens, chemoprotectors and a natural antioxidant, ascorbic acid (vitamin C) on this enzyme activity in the first trimester placenta *in vitro*. Incubations with the carcinogen benzo[*a*]pyrene (BP) at 10–50 μM doses increased the enzyme activity at 6 h. At 24 h the effect of 10 μM BP was significant while that of 50 μM BP was not consistent. On the other hand the effect of 50 μM 3-methylcholanthrene at both time points was not significant. Ascorbic acid (5–25 μM) added for 24 h caused a 2- and 4-fold increase in the enzyme activity, respectively ($P < 0.005$). Exposure to a 25 μM concentration of different classes of chemoprotectors 2(3)-tert-butyl-4-hydroxyl-anisole (BHA), dicoumarol and Sudan I caused a 2.5- to 3.6-fold significant increase in the enzyme activity after 24 h ($P < 0.01$). Present data suggest that QR activity in the early placenta is responsive to a wide variety of xenobiotics *in vitro*. Vitamin C in concentrations usually consumed, exerted a potent effect on local QR activity *in vitro* which may protect pregnant women and their conceptus in an adverse environment.

Key words: enzyme/quinone reductase/xenobiotics

Introduction

The placental metabolic competence towards xenobiotics is well established. However, most studies dealing with this subject were carried out at term. We and others have reported that at term the placenta responds to exposure to a variety of xenobiotics including antihypertensive drugs, heavy metals such as mercury and carcinogens *in vitro*. This involved increases in the activity of a number of monoxygenases including aryl hydrocarbon hydroxylase (AHH), oestrogen hydroxylase (EH), epoxide hydrolase, and phase II enzymes, catechol-O-methyltransferase (COMT), quinone reductase (QR), glutathione-S transferase (GST) and diethylase (Juchau *et al.*, 1978, 1987; Awashti and Dao, 1981; Chao *et al.*, 1981; Barnea *et al.*, 1986a,b, 1988a,b; Barnea and Naftolin, 1987; Barnea and Kaplan, 1989; Barnea, 1991; Boadi *et al.*, 1991). In addition, there are reports showing that maternal cigarette smoking induces activity of various monoxygenases and phase II enzymes in placental tissue at term (Walsh *et al.*, 1969; Manchester and Jacoby, 1982; Pacifici and Rane, 1982; Pelkonen, 1984; Pelkonen and Pasanen, 1990).

We have recently reported that in the first trimester, xenobiotic activating enzymes EH and AHH are sensitive to exposure to xenobiotics *in vitro* (Barnea and Avigdor, 1990, 1991). In addition, we found that COMT, a major protective enzyme activity in explants was increased by these exposures (Barnea and Avigdor, 1990). However, until the present time, the activity of quinone reductase (QR) (EC 1.6.99.2, also named DT-diaphorase, menadione reductase, NAD(P)H dehydrogenase) an important xenobiotic anticarcinogenic/mutagenic enzyme in other systems (Benson *et al.*, 1980) was not studied in the first trimester. This enzyme has broad specificity for a variety of hydrophobic quinones. The toxicity of several xenobiotics like benzo[*a*]pyrene depends on their conversion to quinones. The protective function of QR is through conversion of quinones to stable hydroquinones thus preventing oxygen free radical formation and enabling conjugation. In the present study we have measured the activity of QR in the first trimester, and examined the effect of various groups of xenobiotics, carcinogens, chemoprotectors and vitamin C on the enzyme activity *in vitro*. We report here that the enzyme activity is increased *in vitro* among others, by exposure to the natural antioxidant, vitamin C.

Materials and methods

2,6-Dichlorophenolindophenol, dicumarol, 1-phenylazo-2 naphthol (Sudan 1), 2(3)-tert-butyl-4-hydroxyl-anisole (BHA), benzo[*a*]pyrene (BP), 3 methylcholanthrene (MC), Tris(hydroxy-methylaminomethane hydrochloride) and ascorbic acid (vitamin C) were obtained from Sigma, St Louis, MO, USA. Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose but without L-glutamine, was from Biological Industries (Beth Haemek), Israel. Nicotinamide adenine dinucleotide hydrogen, reduced form (NADH), and NADPH were obtained from US Biochemical.

Placental material

After obtaining appropriate consent, twelve placentas from women who did not take any medications or smoke were obtained after elective pregnancy termination at 7−11 weeks. Placentas were immediately placed on ice and separated from membranes and decidua using dissecting microscopy as previously reported (Barnea and Fakih, 1985; Barnea and Avigdor, 1990) and 150−200 mg explants were excised and rinsed copiously in cold sterile saline (0.9% NaCl) until free of blood. Tissues were rinsed in DMEM containing 1% antibiotic solution (penicillin 10 000 U, streptomycin 25 μg/ml and amphotericin B 50 μg/ml).

Explant cultures

Explants were cultured as previously described (Barnea and Fakih, 1985) with 2 ml DMEM plus 1% antibiotics or with vehicle only. For each compound tested per dose, or vehicle, six explants were used in each placenta.

Incubations were carried out for 6−24 h in a 95% air 5% CO_2 atmosphere at 37°C. At the end of the incubation period, dishes were placed on ice and explants rinsed with fresh media were homogenized with cold 0.25 M sucrose using a teflon homogenizer. After centrifugation at 800 g at 4°C for 10 min the supernatant was used for the enzyme and protein assays. The explants' viability was documented by progressive glucose consumption and a linear increase in human chorionic gonadotrophin (HCG) secretion during culture. An additional criterion for viability (Roth, 1981) was measurement of lactate dehydrogenase (LDH) by the method of Zimmerman and Weinstein (1956). In addition, we have recently shown that 50 μM BP causes a significant increase in HCG secretion in both static cultures as well as in superfusion where the spontaneous HCG pulsatility was significantly increased. However, the response to GnRH agonist was maintained which is an indication for maintained membrane integrity (Barnea and Kaplan, 1989; Barnea and Shurtz-Swirsky, 1992).

Enzyme assay for quinone reductase

The method for assaying QR activity in the placenta was recently reported by us (Boady *et al.*, 1991) and is based on the assay by Ernster (1967) with some modifications by Benson *et al.* (1980). The final assay mixture in a total volume of 3 ml contained 2800 μl of 25 mM Tris−HCl buffer (pH 7.4), 80 μM 2,6-dichlorophenolindophenol, 20 μM NADH and homogenate. The reaction mixture was incubated for 10 min at 25°C and the rate of reduction of 2,6-dichlorophenolindophenol was measured at 600 nm in a Milton-Roy Co. Spectronic 1201 Model spectrophometer. All values were corrected for the non-enzymatic reaction rates. Data obtained were calculated against a standard curve of 2,6-dichlorophenolindophenol reduced/mg protein/10 min. The reaction was linear from 0 to 10 min with protein concentration of 0.1 to 0.5 mg/ml; assays were run in duplicate. The specificity of QR activity was determined by incubations with various concentrations of dicoumarol. At 1 mM concentration, >90% inhibition of the enzyme activity was noted. Subsequently, samples which contained dicoumarol (1 mM) served as blanks. As reference we used a rat liver cytosolic fraction, prepared as described by Ernster (1967). The assay was run under saturating conditions with respect to substrates and cofactors. Under such zero-order kinetics the activity was directly proportional to enzyme concentrations. Protein measurements were carried out using the method of Lowry *et al.* (1951).

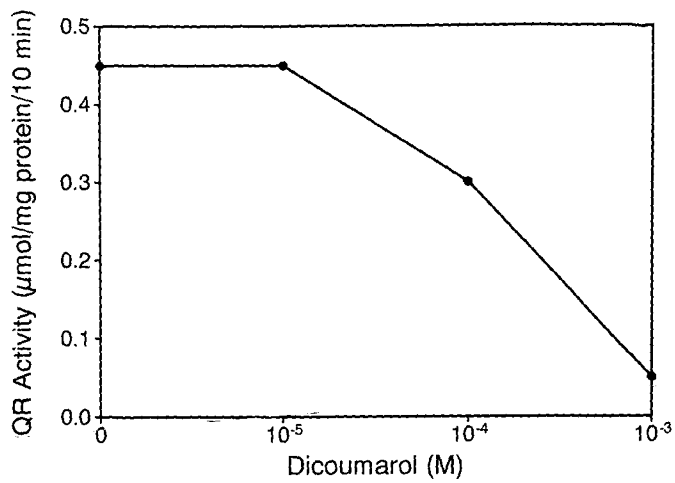

Fig. 1. Dose dependent inhibition of quinone reductase (QR) activity in placental homogenates following exposure to dicoumarol. Maximal inhibition of >90% was noted at 10^{-3} M concentration (P < 0.001).

Table I. Levels of quinone reductase activity mean ± SEM expressed as μmol/mg protein/10 min in the first trimester placenta and rat liver cytosol

Tissue	Mean + SEM	Range	*n*
Human placenta	0.35 ± 0.09	0.51−0.25	6
Rat liver	5.1 ± 0.4	4.7 −5.3	4

n = number of samples measured in duplicate.

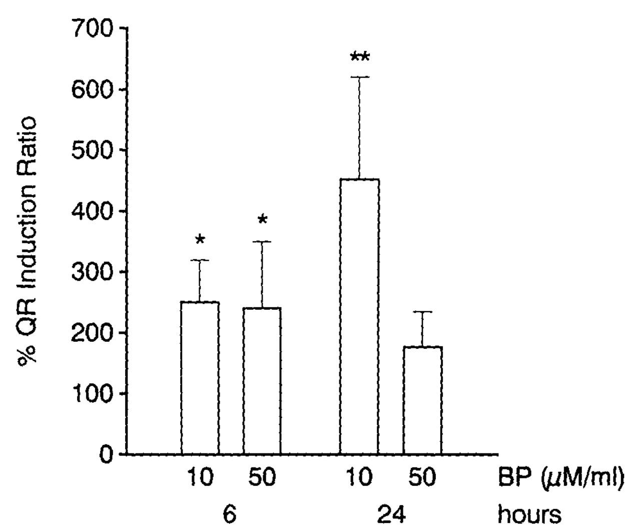

Fig. 2. Effect of 10−50 μM benzo[*a*]pyrene (BP) exposure for 6−24 h on quinone reductase (QR) activity in placental explants. The highest increase was noted with 10 μM at 24 h. The induction ratio measured specific QR activity using 2,6-dichlorophenolindophenol as substrate relative to dicoumarol. *P < 0.05, **P < 0.009.

Statistical analysis

Data in Figures 2−4 are expressed as mean ± SEM μmol QR/mg protein/10 min. One-way analysis of variance (ANOVA) and two-tailed Student's t-test were used whenever appropriate.

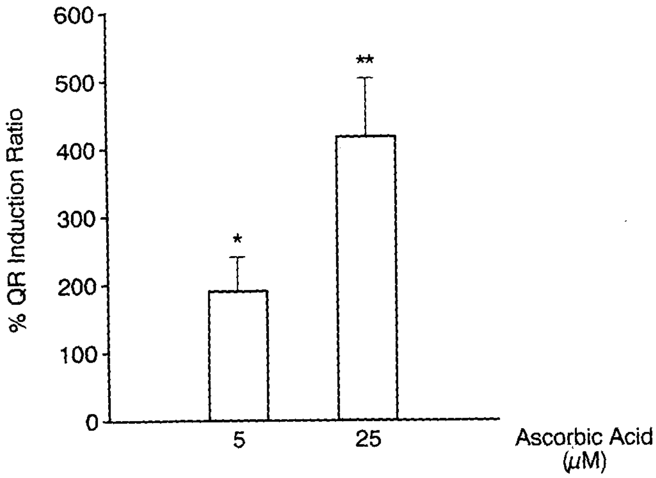

Fig. 3. Effect of 5–25 μM vitamin C added for 24 h to placental explants on quinone reductase (QR) activity. The highest increase by a factor of 4 was noted at 25 μM. *$P < 0.007$, **$P < 0.005$.

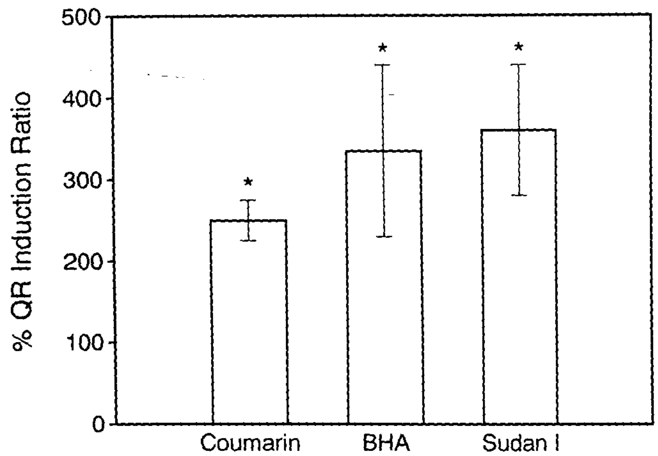

Fig. 4. Effect of 25 μM chemoprotectors added for 24 h to placental explants on quinone reductase (QR) activity. The most significant increase was noted with Sudan I. *$P < 0.01$.
BHA = 2(3)-tert-butyl-4-hydroxyl-anisole.

A *P*-value < 0.05 was considered statistically significant. The data in the figures are the results generated from three or more different placentas.

Results

Figure 1 shows that dicoumarol, a specific QR inhibitor, caused a dose dependent inhibition of the enzyme activity, an indication of enzyme specificity. Maximal inhibition was noted at 10^{-3} M ($P < 0.001$).

Table I shows that QR activity in the first trimester placental tissue was close to the micromolar range; however it was 15-fold lower compared to that in the rat liver cytosol.

Figure 2 shows that following 6 h of incubation there was a significant increase in the QR induction ratio at both 10 and 50 μM concentrations of BP ($P < 0.05$). At 24 h this increase with 10 μM BP became highly significant ($P < 0.009$). At 24 h, the effect of 50 μM BP was slightly lower than at 6 h; from the five placentas tested in three cases the increased value at 6 h was significant ($P < 0.05$).The effect of 50 μM MC at the two time points was not significant (data not shown).

Figure 3 shows that vitamin C caused a highly significant 4-fold increase in the enzyme activity after 24 h of incubation at 25 μM ($P < 0.005$). At 5 μM, a 2-fold increase was already present ($P < 0.007$). Figure 4 demonstrates the stimulatory effect of three chemoprotectors BHA, Sudan I and coumarin (25 μM) on QR activity; a 2.5- to 3.6-fold increase in the enzyme activity was noted after 24 h incubation ($P < 0.01$).

Discussion

In the present work we have extended our observations on QR activity in the placenta by showing that significant activity of the enzyme can be detected in the first trimester and that the enzyme *in vitro* responds to exposure to xenobiotics.

The activity of QR in the first trimester was close to the micromolar range. This is almost 30-fold higher compared to that of other xenobiotic enzymes which we have recently reported to be present in the first trimester placenta, i.e. AHH, COMT and EH all of which were in the 1–10 nanomolar range (Barnea and Avigdor, 1990, 1991). Also compared to our previous observations at term (Boadi *et al.*, 1991) the enzyme activity is 4- to 5-fold higher in the first trimester, which is a strong indication for the importance of this protective enzyme at this early and critical stage of pregnancy development.

We provide evidence that local QR is capable of responding to exposure to carcinogens *in vitro*. The effect was noted with BP but not MC. The increase obtained at the low BP concentration of 10 μM was up to 4-fold and time dependent. In high concentration (50 μM) BP could affect the local enzyme activity, but only at 24 h and not at 6 h, thus indicating that the effect was time dependent. This would suggest that local QR is capable of inactivating carcinogens/mutagens *in vitro*, providing evidence for the enzyme's protective role. The finding was significant, but expected, that the effect of chemoprotectors on AHH activity was mild (Barnea and Avigdor, 1991) while on QR activity it was pronounced. Such a preferential effect is advantageous since the balance is towards xenobiotic inactivation. It was of great interest to note that vitamin C caused a major increase in the enzyme activity at concentrations estimated to be those at which it is usually consumed (500–1000 mg/day). This suggests that this vitamin among others may protect against carcinogens/mutagens also in the placenta by increasing the activity of a major protective enzyme. Whether this is the case *in vivo* and whether such an effect could have a protective role for the pregnant women who are cigarette smokers or who are exposed to environmental toxins remain to be established. The antineoplastic effect of vitamin C was previously shown (Mirvish, 1981). Recent studies show that dietary imbalances cause *in vivo* an increase in mutagenic BP metabolites in the rat liver which covalently bind to DNA (Cassano *et al.*, 1987). Chemoprotectors are regarded as classical inducers of QR (however, they are not used currently as food additives) and we found that the three different classes tested increased the enzyme activity >3-fold but slightly less efficiently than vitamin C. A similar increase by exposure to classic chemoprotectors was also reported by us recently in the first trimester placenta, with COMT, a protective enzyme (Barnea and Avigdor, 1990); this activity was co-ordinated with that of a P-450 dependent enzyme, oestrogen hydroxylase *in vitro*.

The effect of carcinogens on QR activity was previously reported. Hommes *et al.* (1978) showed that the activity of hepatic QR increased markedly following the administration of BP to neonatal rats. MC had no effect on any phase I or phase II enzyme activity thus far tested by us which suggests that this carcinogen may not be activated in the early placenta. Our preliminary studies show that several other polycyclic aromatic hydrocarbons are activating QR activity *in vitro* (S.Avigdor *et al.*, unpublished observation). Previously, an increase in the activity of the QR enzyme in tissues was found following exposure of mice to a variety of dietary antioxidants. The highest increase with BHA fed animals was noted in the liver (Benson *et al.*, 1980). Others have reported that in-vivo exposure to antioxidants reduced BP induced carcinogenicity in rats by increasing QR and glutathione-S-transferase activity and reducing BP binding to DNA in various tissues (Hiroshe *et al.*, 1988). Caffeine was shown also to have antineoplastic effect in mice (Nomura, 1980). Other vitamins like vitamin A were shown to inhibit neoplastic transformation as well (Matheus-Roth, 1982). It was previously reported that at least one of the chemoprotectors tested, Sudan I (Batzinger *et al.*, 1978), acts through AHH competing for the Ah locus cytosolic receptor and the increase in activity is expressed only in mice of the strain which expresses this locus (Lubet *et al.*, 1983). Whether the mechanism is similar in the placenta remains to be shown, though our data on substantial AHH activity in the first trimester would support this possibility (Barnea and Avigdor, 1991).

The first trimester is the time when the conceptus is most sensitive to exposure to xenobiotics. Our recent work points to the significant metabolic potential of the young placenta suggesting that QR together with COMT and other protective mechanisms may have a role in limiting the consequences of the adverse environment in the first trimester.

Acknowledgements

Supported in part by a grant from the Israel Cancer Research Fund to E.R.B.

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Received on January 28, 1992; accepted on September 3, 1992