Advances in Brief

O\textsuperscript{6}-Methylguanine DNA Adduct Formation and Modulation by Ethanol in Placenta and Fetal Tissues after Exposure of Pregnant Patas Monkeys to N-Nitrosodimethylamine


Placenta and Fetal Tissues after Exposure of Pregnant Patas Monkeys to N-Nitrosodimethylamine


Abstract

Perinatal nitrosamine exposures may contribute to childhood cancer risk. To test primate fetal susceptibility to formation of cancer initiation-related DNA adducts from nitrosamines, pregnant patas monkeys were given 1.0 0r 0.1 mg/kg N-nitrosodimethylamine. Appreciable levels of the promutagenic O\textsuperscript{6}-methylguanine adduct occurred in placental and fetal liver DNA after both doses and were lower but detectable in other fetal tissues after the higher dose. Coadministered ethanol (1.6 g/kg) reduced adducts in placenta and fetal liver by one-half and increased levels in other fetal tissues to the same degree. Thus, primate placenta and fetal tissues have a significant, ethanol-modulated capacity to activate N-nitrosodimethylamine, supporting implication of nitrosamines in human perinatal carcinogenesis and of alcohol as a modulating factor.

Materials and Methods

Eight pregnant colony-reared female Erythrocebus patas monkeys (6–8 kg; 7–16 years old) were housed in an American Association for Accreditation of Laboratory Animal Care-accredited facility and fed a commercial primate diet, supplemented with a vitamin mixture and fresh fruit. The time of conception was determined by intrarectal digital palpation of the enlarged uterus. On day 147 ± 2 days of gestation, four pregnant mothers were treated i.g. with 1.0 mg/kg and four with 0.1 mg/kg of an aqueous solution of NDMA. For ethanol treatments, two mothers from each treatment group received 8 ml/kg of a 20% solution (1.6 g/kg) of ethanol i.g. 45 min before administration of NDMA, a treatment which has been shown to cause complete suppression of 1 mg/kg NDMA clearance for at least 6 h (10). Administration of 10 mg/kg ketamine hydrochloride and 0.04 mg/kg atropine sulfate i.m. was given to induce anesthesia, a cesarean section was started 90 min later, and the fetus was removed approximately 2 h after NDMA treatment. Ethanol blood was obtained by cardiac puncture. Blood and amniotic fluid cells were obtained by centrifugation at 2500 rpm. Fetal tissues and placenta were chilled on ice during dissection. A piece of maternal liver was removed by biopsy. DNA was extracted, lyophilized, and stored at −80°C until O\textsuperscript{6}-meG levels were measured in DNA by a sensitive competition-repair assay as described previously (11). The level of detection with this assay was 0.08 μmol/molG; no adducts were found in control tissues. Because generally good agreement was obtained between duplicate monkeys receiving the same treatment, no additional animals were utilized, following policy to carry out surgery on as few nonhuman primates as possible. The data were analyzed statistically by using the one-way ANOVA.

Results

Exposure of pregnant monkeys to 1.0 mg/kg NDMA led to the formation of O\textsuperscript{6}-meG adduct in all tissues examined (Fig. 1). The highest levels were in maternal liver (average, 27.1 μmol O\textsuperscript{6}-meG adduct/molG), but amounts in placenta (average, 5.31 μmol/molG) and fetal liver (average, 2.08 μmol/molG) were 10–20% of those in maternal liver and higher than in maternal blood cells (0.82 μmol/molG; Fig. 1A). Adducts were relatively high in amniotic fluid cells (0.75 μmol/molG) and lower in the other fetal tissues: fetal spleen (0.43 μmol/molG), heart (0.35 μmol/molG), and adrenals (0.36 μmol/molG) were similar, followed by the fetal testes (0.27 μmol/molG), brain cerebellum (0.24 μmol/molG), skin (0.19 μmol/molG), cord blood (0.19 μmol/molG), fetal venous blood (0.13 μmol/molG), female genital tract (0.13 μmol/molG), lung (0.10 μmol/molG), and kidney (0.09 μmol/molG) (Fig. 1B).

1 To whom request for reprints should be addressed, at National Cancer Institute, Building 538, Room 205E, Frederick, MD 21702.
2 The abbreviations used are: NDMA, N-nitrosodimethylamine; O\textsuperscript{6}-meG, O\textsuperscript{6}-methylguanine.
Os-meG DNA ADDUCTS IN MONKEY PLACENTAS AND FETUSES

Fig. 1. Oα-meG adduct levels in tissues of pregnant patas monkey liver and fetal tissues after exposure to 1.0 mg/kg NDMA, with and without ethanol. Four pregnant patas monkeys received 1.0 mg/kg NDMA i.g., and cesarean section was performed 90 min later. Two of these monkeys received 1.0 g/kg of ethanol i.g. 45 min before NDMA (●). A, tissues with the highest levels of adducts. Differences between NDMA and NDMA + ethanol adduct levels approached significance in maternal blood (P = 0.056). B, adduct levels in other fetal tissues, amniotic fluid, and cord blood. Differences related to ethanol treatment were significant in adrenal (P = 0.035, data for right and left adrenals combined), kidney (P = 0.03, right and left kidneys combined), brain (P = 0.036, cerebellum plus cortex combined), and combined gonads (P = 0.045) and were near significant in the heart (P = 0.07) and lung (P = 0.07); skin and spleen (P = 0.1).

At the lower dose of 0.1 mg/kg NDMA (Fig. 2), adducts in maternal liver (2.08 μmol/molG), placenta (0.42 μmol/molG), fetal liver (0.17 μmol/molG), and amniotic fluid cells (0.08 μmol/molG) were slightly <10-fold of those detected in these tissues after 1 mg/kg NDMA. The adducts in maternal blood cells, on the other hand (0.24), were nearly 30% of those after 1 mg/kg. Adducts were not detected in the other tissues at this dose.

When ethanol was given before the NDMA, little effect was seen on adduct levels in maternal liver after either dose (Figs. 1A and 2A). Levels in maternal blood increased about 50% with each NDMA dose. In placenta and fetal liver, coexposure to ethanol resulted in a 50% decrease in DNA adducts after both NDMA doses, compared with NDMA alone. This was also true for amniotic fluid cells after 1 mg/kg NDMA (Fig. 1B) and probably after 0.1 mg/kg because adducts were not detected when ethanol was given before this dose (Fig. 2B). By contrast, all of the other fetal tissues exhibited an increase in adducts related to ethanol treatment along with the 1 mg/kg NDMA dose, 1.5–2.5-fold on average, with the largest effect in gonads, where there was a 3–5-fold increase. With the 0.1 mg/kg dose, cotreatment with ethanol resulted in the occurrence of measurable DNA adduct in the heart and spleen of one of the two fetuses (Fig. 2B).

Tests for significance by ANOVA, although obviously compromised because of the small number of monkeys used, showed the effect of ethanol to be at or near statistical significance in most comparisons (see figure legends).

Discussion

The presence of the Oα-meG adduct in the monkey placentas and fetal tissues, at levels higher than in maternal blood for placenta and fetal liver, indicates that these tissues do have the inherent capacity to bioactivate the carcinogen NDMA. Conclusions reached about the
transplacental ineffectiveness of NDMA in rodents may not pertain to the human situation because of major biochemical and physiological differences; the primate model is better suited for such studies. The relatively high levels of $\text{O}^\text{6}-\text{meG}$ in placenta are consistent with the presence of this adduct in human placentas reported previously (12) and may reflect in part the 2E1 expression detected recently in term human placentas (13).

The ability of human fetal tissues to metabolize nitrosamines has been little studied. Fetal stomach and esophageal explant cultures activated NDMA extensively, but no activity toward NDMA was found in the two esophageal preparations tested (14). Our results show that a primate fetus at term experiences DNA adduct formation in all tissues after maternal treatment with NDMA. The wide variation among tissues suggests local activation, rather than delivery of some stabilized intermediate via the circulatory system. P450 2E1 expression is evidently lacking in the human fetus, at least in the liver; a recent comprehensive survey found expression of only isoforms 2C, 2D6, 3A4, and 3A7 (6). One of the latter isoforms may provide the metabolism of NDMA. P450 3A7 is a major P-450 isoform in human fetal liver and also has NDMA demethylase activity (8). An isoform immunoreactive with anti-3A has been detected in patas fetal liver and placenta (9).

Coexposure to ethanol with NDMA was tested because our recent findings with both mice and monkeys have confirmed a major toxicokinetic effect of ethanol, due to suppression of hepatic clearance of NDMA, with greatly increased exposure of downstream targets [an idea originally promulgated by Swann et al. (15)]. There is a corresponding increase in $\text{O}^\text{6}-\text{meG}$ in these targets and in the mouse in numbers of lung tumors (Refs. in 16). We hypothesized that the feto-placental unit would be a similar target downstream of the maternal liver and would show overall a 7-10-fold increase in adducts, as determined previously for the uterus in nonpregnant monkeys. This prediction was not fulfilled; a more complex result was obtained. Adduct levels decreased to one-half in the placenta, fetal
liver, and amniotic fluid cells with ethanol treatment; this may reflect inhibition of activation of NDMA in these tissues by ethanol. However, within the fetal unit the toxicokinetic hypothesis was well supported; adduct levels were increased approximately 2-fold by ethanol exposure in all fetal tissues downstream of fetal liver, as adducts in these livers decreased by 50%. Thus, it appeared that placental and fetal liver metabolism dominated the kinetics and a 50% reduction in their metabolism of NDMA doubled the exposure of the other fetal tissues.

We further considered whether failure of maternal liver metabolism to dominate the overall kinetics could be related to NDMA dose. During pregnancy, 2E1 levels are known to decrease in rodent liver (17). The 1 mg/kg NDMA dose used, although not saturating in nonpregnant livers, could have become saturating for a lower pregnant 2E1 level in maternal liver. However, adducts formed after 0.1 mg/kg NDMA were close to 6–8% of adducts after 1 mg/kg in most tissues for which a comparison could be made (maternal and fetal liver and placenta), and the relative ethanol effects were the same. An interesting exception was maternal blood, for which adducts at the lower dose, with and without ethanol, were 27–29% of those at the higher dose.

Some comments may be offered regarding the implications of these findings for biomarker studies: (a) the high level of adducts in the cells of amniotic fluid and the suppressive effect of ethanol suggest that these cells are derived mainly from placenta and that they could be used as a measure of methylation damage during pregnancy; and (b) in situations where modulating agents such as ethanol or similar solvents are interplaying with nitrosamine exposure, changes in methylation damage in placental DNA may correlate positively with that in fetal liver DNA but negatively with that in other fetal tissues. This is in contrast to the situation for benzo[a]pyrene, where levels of DNA adduct in placas placenta correlated positively with those in various fetal tissues (18). For NDMA, our study results indicate that adducts in cord blood DNA would appear to give a conservative, positively correlated estimate of the trends in adduct levels in most fetal tissues.

In conclusion, our observations lend support to human epidemiological findings that link parental exposure to foods and other substances containing nitrosamines or nitrosatable substrates to childhood tumors (3, 4). They also indicate that alcohol use should be studied as a modulating factor, which might decrease risk for hepatoblastomas, but increase risk for neoplasms in other tissues (16). In the only animal model study available, ethanol, given together with the tobacco-specific nitrosamine 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone, potentiated the transplacental tumorigenic effect in the pancreas of fetal hamsters (19). Parental alcohol use has been suggested to increase risk of childhood acute nonlymphocytic leukemia (20) and infant leukemia of several types (21). Our results predict that, at least with regard to nitrosamines, alcohol will have mainly an enhancing effect, which in epidemiological approaches will be more likely to be detected by multivariate analysis. It is of interest that one investigation of common childhood brain tumors, as related to nitrosamine exposure, found an association for beer only (22), where obviously ethanol would be co-consumed with any contaminating nitrosamines. Multivariate analysis for interactive effects, e.g., maternal drinking along with smoking and exposure to nitrosamine-generating food or products, should receive more emphasis. The recent demonstration of a synergism between maternal irradiation and smoking on the risk of leukemia in their children (23) underscores the need for this type of multifactator analysis.

Acknowledgments

The skills of Dr. Richard Bradbury in primate facility management, Dr. Thomas Moskal in surgery, Steven Harbaugh in the treatment of monkeys, and Charles Riggs in statistical analysis are greatly appreciated.

References


6020
O\textsuperscript{6}-Methylguanine DNA Adduct Formation and Modulation by Ethanol in Placenta and Fetal Tissues after Exposure of Pregnant Patas Monkeys to N-Nitrosodimethylamine

Saranjit K. Chhabra, Vassilis L. Souliotis, Jeffrey W. Harbaugh, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/24/6017

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/55/24/6017. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.