

Synthesis and Secretion of TNF α , Interleukins (1 β and 6) in Cocaine Users during Pregnancy in Humans: In Vitro and Ex Vivo Study

Balwant Ahluwalia (Corresponding author), Shakuntala Rajguru & Lalita Kaul
Department of Obstetrics & Gynecology, Community Health & Family Medicine
College of Medicine, Howard University, Washington D.C. 20060, USA
E-mail: bahluwalia@howard.edu

Received: July 23, 2010 Accepted: August 10, 2010 doi:10.5539/gjhs.v3n1p161

Abstract

Preterm parturition is often preceded by systemic infection and cytokines are recognized as the principle mediators of a variety of immunologic and pathophysiologic events in infection. The study was designed to examine whether cocaine affects synthesis and secretion of cytokines. Among the cytokines, tumor necrosis factor (TNF α) and Interleukins (IL-1 β and IL-6) were chosen because of their presumptive role in infection.

For in vitro study, blood was obtained from mothers who were drug free (control); lymphocytes were isolated and stimulated with lipopolysaccharides. The synthesis of TNF α , IL-1 β and IL-6 was examined in the presence of 2.1 μ M of cocaine. The data show that cocaine stimulated TNF α synthesis ($p < 0.01$) but had no effect on IL-1 β and IL-6 synthesis.

For in ex vivo study, cytokine levels were measured in fetal cord blood in cocaine users. The data were similar to that found in vitro study.

In cocaine users, cortisol blood level increased significantly in the fetal cord blood ($p < 0.01$) but not in the mother, however, in mothers with known infection (positive control), blood cortisol level increased significantly without similar effect in the fetus.

The babies born to cocaine users were significantly of low birth weights ($p < 0.05$) the incidence of pre-rupture of membranes (PPROM) increased significantly ($p < 0.001$) in cocaine users.

The data show that cocaine selectively increased TNF α synthesis and secretion. Higher cortisol level in the fetal cord blood suggests that in cocaine users fetus is more susceptible to infection than the mother.

Keywords: Cocaine, Pregnancy, TNF α , Infection, Humans

1. Introduction

It is estimated that 50 million people in the United States use cocaine on a regular basis and almost one million are women ranging in age from 18- 34 years (Das, 1994., Rizk *et al.*, 1996.,). Using multivariate logistic modeling, cocaine use was significant predictor of pre-maturity and infection in the newborn (Schiono., *et al.*, 1995). Animal data show that after in vivo administration of cocaine there was a decrease in lymphoid organ weights and the number of lymphocyte derived from these organs (Bagasra and Forman., 1989). Cocaine use during pregnancy has been linked to developmental toxicity including still birth (Bingol *et al.*, 1987), preterm delivery (Handler., *et al.*: 1991), and low infants birth weight (Chasoff., *et al* 1992)). and is associated with immune alterations in a wide variety of lymphocytes including natural killer cells (NK cells), helper T (Medina, *et al.*, 1993) Pregnancy is accompanied by specific changes in the lymphoid tissue suggesting that pregnant animals are more susceptibility to the affects of cocaine and thereby to the developing fetus (Ou *et al.*, 1989:Clark., *et al* 1994). Animal and human data clearly suggest that cocaine affects immune system (Watzel *et al.*, 1992: Pacifica *et al.*, 1993). There are several studies which report that cocaine suppressed cytokine synthesis especially the TNF α . however, it is difficult to draw conclusion from these data because of the lack of uniformity in the design of these studies, for example acute versus the chronic effects of cocaine, specie differences, variations in dose levels and assay protocols, route of administration and the time of blood sampling, with few exceptions almost all studies were done using animal models (Wetzal *et al.*;1992; Wang *et al.*; 1994; Pellegrino *et al.*, 2001) and none using human fetal model. We report in vitro and ex vivo effects of cocaine on

TNF α , interleukine 1- β and 1-6 synthesis and secretion during pregnancy using mother and the fetal blood as a source. These cytokines were chosen because of their presumptive role in infection. (Singh *et al.*, 1996).

2. Material and Methods

Phosphate buffered saline (PBS) RPMI 1640 medium (Whiteaker Biomedical Products, Whitaker, CA), Hank' balanced salt solution, glutamine, HEPES, and the antibiotics penicillin and streptomycin were purchased from Gibco (grand Island, NY); LPS (*Escherichia coli* 26:B6) and dexamethasone were purchased from Sigma Chemical (St.Louis, MO) and cocaine was obtained from National Institute of Drug Abuse. Ficol-hypaque was purchased from Pharmacia (Piscatway, NJ). All other chemicals were purchased from Fishers Scientific (Pittsburgh PA). All glass wares were sterilized prior to use. For cytokine immunoassay, commercial kits were used from Quantkine R&D system (Minneapolis, MN).

2.1 Quantikine commercial kit

The Quantikine commercial kit was used because of its precision compared with other commercial kits and because the same kit can be used at more than one occasion. Other investigators have also used this kit for analysis of cord blood samples (Strieter *et al.*,1990).

2.2 Selection of Subjects

The participants in the program were pregnant women who came to Howard University Hospital for delivery and consented to take part in the study. The protocol was approved by the Institutional review Board (IRB-98OBG-02). A resident physician explained the scope of the project and provides the participants with a questionnaire. The selection of the participants in the program was based on the following criteria:

Three groups were selected among the participants.

1. In group 1, subjects were chosen among those who were drug- free throughout pregnancy (licit and illicit) and especially avoided smoking during pregnancy. Singleton pregnancies without congenital malformations with gestational age between 36-40 weeks were included in the study.
2. In group 2, subjects were selected on the basis of positive cocaine test in their urine. No other drug except cocaine was detected in the urine in these subjects at the time of sampling. There were no obvious signs of infection or any known nutritional deficiencies at the time of delivery in subjects who participated in the study.
3. In group 3, participants in this group were diagnosed with infection.

All participants were African-American, of low socio-economics status between the ages of 20-25 years. Each subject's medical history and level of cocaine use were obtained prior to their participation by a questionnaire. The study was completed in two years period. The data presented are the averages of 16 subjects in each group. Each group (16 subjects) was then subdivided into four equal subgroups to determine intra group differences. Since intra group differences within the group were not significant the data from each subgroup were pooled into one group. The data presented here show the differences between three groups. All three groups were used for comparing blood cortisol in the mothers and fetus.

Blood sample from mothers and fetal cord blood were collected at the time of delivery from each subject.

2.3 Urine screening for cocaine

The method used for screening for cocaine was enzyme-mediated immunoassay technique (EMIT). The EMIT detects benzoylecgonine, a cocaine metabolite in the urine for 24-72h after use (Osterloh *et al.*; 1989., Hamilton *et al.*; 1977). The test has 85% accuracy.

2.4 Cord blood samples (*ex vivo* study)

Ten to fifteen milliliters of cord blood was obtained (drug free and cocaine users) immediately after transaction of the umbilical cord. Samples were collected aseptically from the umbilical cord while still attached to the placenta, and placed in 15 ml of endotoxin-free glass blood collection tubes containing EDTA (Becton Dickinson, Franklin Lakes, and NJ) as an anticoagulant. Blood was centrifuged at 1,500 xg for 10 min. Plasma was removed and frozen at -70c until assayed. Samples were used for measuring cytokines and cortisol levels. Simultaneously, 10-15 ml of blood was obtained from the mothers at the time of delivery, and plasma was removed and frozen at -70c. Extreme care was taken to avoid inter mixing of fetal and mother's blood at the time of sampling these sample were used for cortisol levels.

For the *in vitro* study, blood was obtained from subjects who were drug free (licit and illicit) throughout pregnancy and tested negative for cocaine in the urine (group 1).. Blood was obtained at the time of delivery and

blood lymphocytes were isolated immediately for in vitro study to examine cytokines with and without 2.1 μM cocaine.

2.5 Dose level of cocaine for in vitro study

Several factors were taken into account for determining dose levels of cocaine in the media in vitro study. With the reported value of cocaine in the range of 1-2.5 μM in regular cocaine users. We decided to use 2.1 μM for in vitro studies. This dose level is consistent with the plasma concentration of cocaine in cocaine users (0.71 mg/L). Stead and Moffat (1983) It is reported that concentration of cocaine in whole blood greater than 0.9 mg/L is associated with fatalities in fetus and adults. Preliminary studies were conducted using 1.00, 1.5, 2.1, 2.8 and 3.00 μM of cocaine; the controls were incubated with equivalent volume of media solution. Differences between 2.1 and 3.0 μM of cocaine were not significant in terms of cytokines production. Less than 1.5 μM of cocaine had no effect on the cytokine production. Based on this information, we used 2.1 μM of cocaine in all incubations.

2.6 In vitro incubation

Blood was diluted 1:1 with PBS, mixed gently, layered onto Ficoll-Hypaque (Pharmacia, Piscataway, NJ), and centrifuged at 600 $\times g$ for 30 min at 20 $^{\circ}\text{C}$. the layer containing Ficoll-Hypaque-isolated mononuclear cells were suspended in RPM 1640 (Whitaker Biochemical Products, Whitaker, CA), containing 1mM glutamine, 25mM HEPES, 100 units penicillin and 100 units streptomycin (Life Technologies, Grand Island, NY). Differential cell counts and viability test was performed using trypan blue exclusion method. An aliquot for each experiment (10^6 cells) was plated in 5 to 10 ml individual cultures in Leighton tubes (Belco Glass, Thomas scientific, and Swedesbro, NJ). Lipopolysaccharides (10ug/ml) stimulus was used as a primary challenge for cytokine bioactivity. Cocaine was added 2.1 μM . The tubes were gassed with 10% CO_2 and 90% air and tightly stoppered. The culture was incubated at 37 $^{\circ}\text{C}$ for 3h. Cocaine was monitored at the beginning and at the end of incubation in each experiment. Less than 10% of original level of cocaine in the media was lost during incubation. An aliquot was obtained for each assay for measuring cytokines. The procedure to obtain lymphocytes has been described elsewhere (Ahluwalia *et al.*, 2000)

2.7 Suppression of cytokine production by dexamethasone

To determine the efficacy of isolated lymphocytes for monitoring cytokine production, dexamethasone (DEX) was added in the media at a concentration of 10^{-6} M. cells (10^6) were incubated for 4 h in the presence of LPS (10 $\mu\text{g}/\text{ml}$) alone, LPS with cocaine or LPS with DEX (10^{-6} M). The dose level of DEX was decided on the basis of a published report (Kern *et al.*, 1988).

Dexamethasone (DEX) is known to have an inhibitory effect on cytokine production in isolated human monocytes (Lee *et al.*, 1988).

2.8 Immunoassay of cytokines

Samples were thawed at room temperature before incubation and assayed as a single batch. The assay is based on the quantitative sandwich enzyme immunoassay technique. The assay uses antibodies raised against recombinant human TNF α , IL-1 β , IL-6 and the inter assay and intra assay precision is less than 10%. The relative sensitivity of the assay is 0.09 pg/ml. The detail of the assay has been described elsewhere (Ahluwalia *et al.*, 2000)

2.9 Statistical methods

The data were analyzed by using one way analysis of variance in combination with the Student-Newman –Keuls test. A *p* value of less than 0.05 was considered statistically significant.

3. Results

Shown in Figure 1 is in vitro data, showing TNF α synthesis in the presence of cocaine in the isolated lymphocytes stimulated with LPS.

The data presented here show that LPS stimulated TNF α synthesis and dexamethasone suppressed the synthesis. When cocaine was added in the media together with LPS there was further increase in the level of TNF α by at least two and one half folds suggesting that cocaine caused increase in TNF α synthesis ($p < 0.001$). These data suggest that isolated lymphocytes responded to LPS stimulation and suppression by dexamethasone.

Shown in the same figure (see Figure 1) are the results of cytokine analysis in fetal cord blood in cocaine users during pregnancy (ex vivo). The data show that TNF α level increased significantly compared with controls while the levels of IL-1 β and IL-6 were not significantly affected.

Shown in Figure 2 are in vitro data for IL-1 β and IL-6. These values are not significantly different in cocaine treated and controls ($p>0.05$).

Shown in Figure 3 is the cortisol level in mother's blood and fetal cord blood in cocaine users. Cortisol level in the mother's blood was not affected; the increase in fetal blood cortisol level was significantly higher compared to drug free (control).

Shown in the same figure are the results of cortisol values in subjects who were identified to have infection (positive control). The data showed that cortisol level increased in mothers as expected while fetal blood showed no similar affect ($p<0.05$).

Shown on Table 1 is the gestation age of mothers and birth weight of newborn. Although the gestation age of cocaine user mothers was lower than controls the differences were not significant, however, the babies born to cocaine users were of low birth weights (2250 ± 495) compared to drug free (controls) (2678 ± 434). These differences were significant ($p<0.05$). Placental abruption was higher in cocaine users (3/16 compared to controls 1/16).

Differences in Apgar scores were not significant in either group ($p>0.05$).

4. Discussion

To interpret the data presented here some explanation is required. First, can the oral account of drug use by the participants be relied upon, second how much, how often and whether drugs other than cocaine were used by the participants in this study. This is an important issue because multiple uses of drugs would confound the results.

To answer these questions, it should be noted that subjects who participated in the study tested positive for cocaine only and none other drugs which confirmed their oral history of drug use. Further more, none of the subjects who denied using any drug during pregnancy tested positive for the drugs tested in the study. There still remains a question for how long and how much cocaine these subjects used during their pregnancy since urine toxicology data reveals only recent cocaine use (Ostrea *et al.*, 1992). Based on the results of the questionnaire the subjects used cocaine regularly (more than twice a week) and the amount corresponded to the level of cocaine usually used by street people. We could not be more specific regarding the level of cocaine used by the participants. We are aware that it is common among cocaine users to use drugs other than cocaine such as alcohol, methadone, heroine, amphetamines (Gillogley *et al.*; 1990 Frank *et al.*; 1988). Despite these limitations, we believe that data presented here merit serious discussion because based on the urine test it is clear that participants in the study used cocaine only and the effect of other drugs if any had a negligible effect on the results of this study.

It is well accepted that cocaine dependence increases the risk of infection and to combat infection the body immune system triggers a set of proinflammatory cytokines especially TNF α and interleukins 1 β and 6 (Van Dyke *et al.*, 1986; Ruiz *et al.*, 1994). Among the three cytokines TNF α received the most attention because of its role in various infections, endotoxins and cachectin (Beutler *et al.*, 1986) the controversy lays whether there is an increase or decrease of TNF α in cocaine users. Although several studies reported a decrease in blood level of TNF α following cocaine administration (Ou *et al.*; 1989., Pacifica *et al.*, 1993., Baldwin *et al.*, 1997, Baldwin *et al.*, 1998., Pellegrino and Bayer., 1998) others studies reported an increase in TNF α levels (McDuffie *et al.*; 1992., Gravette *et al.*, 1992., Fidel *et al.*, 1994). We report here that cocaine caused an increase in TNF α synthesis and secretion in vitro and in ex vivo studies. Since none of the studies reported previously dealt with human fetal model it is difficult to reconcile our data with the published data. Moreover compare to adults these parameters are further complicated in the fetus because of its size, slower metabolism and clearance rate. In addition, it is clear that in previous studies the role of gonadal hormones, hypothalamic-pituitary hormones (Mandrup *et al.*, 1995) growth hormone, prolactin IGF-1 and other neuroendocrine hormones have not taken into account in moderating the body's immune system (Mendelson *et al.*, 1989; Gala *et al.* 1991; Kelley *et al.*, 1992). Our data to be published (unpublished) elsewhere show that cocaine use during pregnancy decreased progesterone synthesis in the placenta, suggesting again that pregnancy hormones play a major role in regulating the body's immune system. Further more, add to this complex system is the suggestion that there is a diurnal variations in the secretion of cytokines (Redwine *et al.*, 2000) should be taken into account in the interpretation of the existing data. The interesting part of the study presented here show that in cocaine users there is a high level of cortisol, a known indicator of infection, in fetal blood but not in the mothers suggest that fetus is prone to infection in cocaine users.

The increase or decrease blood level of TNF α is hardly a sign of individual immunity however, many investigators in the past have taken that decrease in TNF α in cocaine users is a sign of decrease immunity and

vice versa however, it is also known that low TNF α level is beneficial to individual (Beutler *et al.*, 1985, Beutler *et al.*, 1986), on the other hand enhancement of immune response although sounding beneficial in fact may be equally detrimental. For example in HIV infection an increase in antibody response at the expense of cell mediated immunity may hamper resistance to viral infection (Redwine *et al.*, 2000).

TNF α initiates a wide variety of biological responses including apoptosis, regulation of cell death, regulation of transcription and inflammatory chemicals including toxins (Beutler *et al.*, 1985, Beutler and Cerami., 1986, Beutler *et al.* 1989)). On the surface it appears that proliferation and apoptosis seem in opposition to each other, however when analyzed individually it is apparent that one receptor can control both these events. The paradox regarding cytokines is that they can cause cell proliferation and apoptosis in the same organs (Kunkal *et al.*; 1986 Aggarwal and Natarajan 1996., Cohen and Cohen., 1996.) It is known that when TNF α is released in large quantities, the resulting metabolic derangement can be catastrophic leading to death of organism conversely, a low level of TNF is beneficial (Cohen and Cohen., 1996). Three separate factors apoptosis, the activation of NF-kB (a transcription factor that inhibit apoptosis), and activation of JNK (a protein kinase) that does not inhibits apoptosis-can be controlled by the same receptor (Kunkal *et al.*, 1986). Although increase or decrease in the level of cytokines suggest immune dysfunctions but it represents the same side of the coin in normal functioning of the system (Breen *et al.*, 1990). Blood levels of TNF α fluctuates and does not provide a measure of immunity. In situ factors such as cytokine synthesis and or/or release, cytokine receptor, and transmembranes signaling pathway leading to intracellular events, determine the overall biological response to exogenous agents (Cohen and Cohen 1996)... Cytokine over expression has been found in chronic diseases. Short term and long term studies in cocaine users may provide more detailed information regarding the role of these cytokines. It is also clear that in vivo and in vitro support the concept that cocaine increases apoptosis as likely mechanism mediating the thymic atrophy and damage thymocyte population. (Verma *et al.*, 1993). It is well known that TNF α and interleukin 1 are endogenous pyrogen and can activate not only monocyte/macrophages but also lymphocytes, endothelial cells, neutrophils. It is concluded that cocaine may alter the balance of cytokine production, and thereby adversely effects immune response and host defense (Van Voorhees *et al.*, 1996) Acute and chronic effects of cocaine in human and animal studies document that cocaine alters the function of natural killer (NK) cells. T cells, neutrophils and macrophages, and alters the ability of these cells to secrete immunoregulatory cytokines. (Miles *et al.*, 1990; Baldwin *et al.* 1998). The relationship between various cytokines examined in this study is that they are all inflammatory agents. TNF α and IL-1 β and IL-6 are biochemically and immunologically distinct protein, but biologically they are similar. IL-6 is a multifunctional cytokine (Van Dyke *et al.* 1986; DiFrancesco *et a.*, 1992 and 1994). Although data presented here in vitro and ex vivo showed no difference in the levels of IL-1 β and IL-6 more data need to be done to define the role of these cytokines in acute and chronic cocaine users.

The exact mechanism by which cocaine facilitates onset of disease remains to be determined however, it has been reported that cortisol is the mediator of cocaine affects (Sanyal *et al.* 1992., Stanulis *et al.*, 1997., Dudley *et al.*; 1999.). Acute administration of both cocaine and corticosterone produces an enhancement of the T-dependent antibody response to sheep erythrocytes and T helper cell. It is known that LPS toxicity can be reduced by administration of immunosuppressive glucocorticoids which inhibit the production of TNF and it has been reported that DHEA (dehyepiandrosetrone) protects from endotoxin and reduces TNF α production (Hinshaw *et al.*, 1982, Danenberg *et al.*, 1992.).

Higher cortisol level in the fetal blood in the absence of similar affect in the mother as presented here is interesting and needs further investigation. Using ultrasound markers it has been reported that fetal infection can be diagnosed even in the absence of any sign of infection in the mother (Bailao *et al.*, 2005). It is known that administration of cocaine mimics the stress response as measured by endocrine changes (DiPaolo *et al.* 1989). Stress causes release of cortisol and high level of cortisol is predictor of infection (Owiny *et al.*, 1991)

Our data (see table 1) showing significant increase in preterm births is in agreement with several studies. (Das., 1994), Preterm labor is the final common pathway after several potential insults to the uterus. The preterm labor syndrome may be precipitated by several different pathophysiologic events, including intra uterine infections and maternal stress (Handler *et al.* 1991; Chasnoff *et al.*; 1992).

Although our data are quite small on the incidence of pre- rupture of membranes in cocaine users. Our data are in agreement with published reports that premature rupture of membranes (PPROM) was higher in women who used cocaine alone or cocaine and opiates than drug free controls (Bingol *et al.*, 1987).

The decrease in weight of the developing fetus could be the result of vasoconstrictive effects of cocaine during the first trimester of pregnancy (Patel *et al.*, 1999). Direct exposure of fetus to cocaine as a result of

accumulation of the drug in the amniotic fluid via transport across the chorion-amnion has been reported by us Ahluwalia *et al.*, 1992).

References

- Aggarwal, BB., Natarajan K. (1996). Tumor necrosis factor during the last decade 1996, *Eur Cytokine Network* 7, 93-124.
- Ahluwalia B, Clark, JF, Westney LS, Smith, DM, James M, Rajguru, S. (1992). Amniotic fluid and umbilical artery levels of sex hormones and prostaglandins' in human cocaine users. *Reproductive Toxicology* 6, 57-62.
- Ahluwalia, B., Wesley, B., Olanrewaju, Adeyiga., Deborah, M smith., Anita Da-Silva and Shakuntala Rajguru. (2000). Alcohol modulates cytokine secretion and synthesis in human fetus an in vivo and in vitro study *Alcohol*, 21, 207-213.
- Bagasra O. Forman I. (1989). Functional analysis of lymphocytes subpopulation *Clin Experimental Immuno* 77, 289-293.
- Bailao, LA., Osborne, NG. Christina. M., Bonilla-Musoles F., Duarte, G and Sicchieri Bailao. (2005). Ultrasound markers of fetal infection Part 1 *Viral infections 2005 Ultrasound Quarterly* 21, (4) 295-308.
- Baldwin, GC. Tashkin, DP., Buckley, DM. Park, AN, Dubinet SM and Roth, MD. (1997). *Am J Respir Crit Care Med*. Nov., 156 (5) 1606-13.
- Baldwin GC., Roth MD., Tashkin DP. (1998). Acute and chronic effects of cocaine pn the immune system and possible link to AIDS. *J. Neuroimmunol March*, 15, 83 (1-2) 133-8.
- Beutler B., Milsark IW., Cerami AC. (1985). Passive immunization against cachectin/tumor necrosis factor protects mice against lethal effect of endotoxin, *Science*, 229, 869-871.
- Beutler B., Krochin N., Milsark IW., Leudke C., Cerami, A. (1986). Control of cachectin/tumor necrosis factor synthesis Mechanism of endotoxin resistance *Science*, 232, 977-980.
- Beutler R., Cerami AC. (1989). The biology of cachectin, TNF α : primary mediator of host response. *Ann Rev Immunol*, 6, 625-655.
- Bingol N., Fuchs M., Diaz V, Stone., RK., Gromisch DS. (1987). Teratogenicity of cocaine in humans *Pediatrics* 110, 93-96.
- Breen BC., Rezai AR., Nakajima K., Beall GN., Mitsuyasu RT., Hirano T., Koshimoto T., Martinez-Maza O. (1990). Infection with HIV associated with elevated IL-6 levels and production. *J. Immunol*, 144,480-484.
- Chasnoff, JJ.,Griffith DR., Frier C., Murray J. (1992). Cocaine /polydrug use I. pregnancy two years follow up *Pediatrics* 88, 284-289.
- Clark AG., Gil AL., Kendall MD. (1994). The effects of pregnancy on the mouse thymic epithelium. *Cell Tissue Research*, 275, 309-318.
- Cohen MC., and Cohen, S. (1996). Cytokine function a study in biologic diversity. *Is J Clin Pathol*, 105, 589-598?
- Danenberg, HD., Alpert G., Lustig S., Ben-Nathan D. (1992). Dehydroepiandrosterone protects mice from endotoxin toxicity and reduces tumor necrosis factor production. *Antimicrob Biol Agents and Chemotherapy*, 36, 10 2275-2279.
- Das G. (1994). Cocaine abuse and Reproduction *Inter. J. Clin Pharmac. and Therap*, 32 (1) 7-11.
- DiFrancesco P, Marini S, Pica F, Favalli C, Tubaro E, Garaci E. (1992). In vivo administration influences lymphokines production and humoral immune response. *Immunol Res*, 11, 74-79.
- DiFrancesco PR., Gaziano R., Lanzilli. G, Belogi I., Ravagnan G., Garaci E. (1994). Differential effects of short term or prolonged cocaine exposure on peripheral blood cells in mice. *Life Sciences*, 54, Suppl 2015-2020.
- DiPaolo T., Roullard C., Morissette M., Levesque D., Bedard PJ. (1989). Endocrine and neuroendocrine actions of cocaine *Can J. Physiol Pharmacol*, 67, 1177-1181.
- Dudley, DJ. (1999). Immunoendocrinology of preterm labor: the link between corticotrophin hormone and inflammation *Am Obstetric Gynecol*, 180-S2512-6.
- Fidel PI, Romero R, Wolf N, Cutright J, Ramirez M, Araneda H, Cotton DB. (1994). Systemic and local cytokine profiles in endotoxin induced preterm parturition in mice. *Am J.Obstet Gynecol*, 170, 1467-75.

- Frank, DA., Zuckerman, BS., Amaro, H., Aboagye, K., Bauchner, H., Cabral, H., Fried, L., Hingson, R., Kayne, H., Levenson, SM., Parker, S., Reece, H., and Vinci, R. (1988). Cocaine use during pregnancy prevalence and correlates. *Pediatrics*, 82, 888-895.
- Gala RR. (1991). Prolactin and growth hormone in the regulation of immune system. *Proc Soc Exp Biol Med*, 198, 513-527 1991.
- Gillogley, KM., Evans, AT., Hansen, RL., Samuels, SJ., and Batra, KK. (1990). The prenatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening. Is *J. Obstet, Gynecol*, 163:1535-1542?
- Gravette MG., Haluska. GJ., Edwards JL. (1992). Amniotic fluid (AF!) and preterm labor in rhesus macaques. *Am J. Obster Gyn*, 167, 290-294.
- Hamilton, HE. Wallace, JE., Shimek, EL., Land, P., Harris, SC., and Christenson, JG. (1977). Cocaine and benzoylecgonine excretion in humans. *J. Forensic Sci*, 22, 697-707.
- Handler A, Kistin N, Davis f, Ferre C. (1991). Cocaine use during pregnancy: Perinatal outcome 1991, *Am J. Epidemiology*, 133, 818-825.
- Hinshaw LB., Beller-Todd BK., Archer LT. (1982). Current management of septic shock patient's experimental basis for treatment. *Circ Shock* 9, 543-553.
- Kelley KW, Arkins S Li. (1992). YM Growth Hormone Prolactin and Insulin like growth actors New job for old players. *Brain Behav Immunity*, 6, 317-326.
- Kern, JA. Lamb, RJ., Reed, JC. Daniel, RP and Nowell, PC. (1988). Dexamethasone inhibition interleukin 1 beta production by human monocytes. Post transcriptional Mechanisms *J. clinical Investigat* 81, 237-244.
- Kunkel SL, Wiggins RC, Chensue SW Larrick J. (1986). Regulation of macrophage tumor necrosis factor by prostaglandins E2. *Biochim Biophys Res Comm* 137, 404-410.
- Lee, W. Oppenheim JJ and Matsushima, K. (1988). Analysis of the suppression IL-1 alpha and IL_beta production peripheral mononuclear adherent cells by a glucocorticoid hormone. *J. Immunol*, 140-1895-1902.
- McDuffie, RS. Sharma MP., Gibbs RS. (1992). Amniotic fluid tumor necrosis factor α and interleukin 1 in rabbit model of bacterially induced preterm pregnancy loss. *Am.J.Obstet Gynec*, 167, 1583-88.
- Mandrup-Poulsen T, Nerup J, Reimers JI, Pociot F, Andersen HU, Karlens A, Bjerre U and Bergholdt R. (1995). Cytokines and the endocrine system1. The immunoendocrine network. *The European J of Endocrinology*, 1`33, 660-71.
- Medina KL, Smithson G Kinkade. (1993). PW Suppression of B lymphopoeisis during normal pregnancy, *J. Exp Med*, 178, 1507-1515
- Mendelson JH Mello NK, Teoh SK, Ellingboe J Cohen J. (1989). Cocaine effects on pulsatile secretion of anterior pituitary gonadal and adrenal hormones. *J. Clin Endocrinol Metab*, 69, 1256-1260.
- Miles SA, Rezai AR, Salazar-Gonzalez JF, Vander Meyden M, Stevens RH, Logan DM, Mitsuyam RT, Taga T, Hirano T, Kishimoto T. (1990). AIDS Kaposi sarcoma derived cells produce and responds to interleukin- 6. *Proc Natl Acad Sci USA* 87, 4068-4072.
- Osterloh, JD. and Lee, BL. (1989). Urine drug screening in mothers and newborns. *Am J. Dis.Child*, 143, 791-793.
- Ostrea, EM, Brady, MJ. Parks, PM, Ansio, DC and Naluz, A. (1992). Drug screening of newborns by meconium analysis: a large prospective, epidemiologic study. *Pediatrics*, 89, 107-113.
- Ou, DW Shen, MI, Luo, YD. (1989). Effects of cocaine on the immune system of Balb/c mice. *Clin Immune.Immunopath*, 52, 305-312.
- Owing JR., Jones MP., Sadowsky D, Myers T, Massman A. Nathanieisz, PW. (1991). Cocaine in pregnancy effect of maternal administration on the maternal and fetal adrenal axis. *Am J. Obstet Gynecol*, Feb 164 (2) 658-663.
- Pacifica R., Dicarolo S., Bacosi A., Zuccaro P. (1993). Microphage function drugs of abuse treated mice. *Int J. Immunopharmac*, 15, 6, 711-16.
- Patel. TG. Laungani, Rg. Gross, EA., Dow-Edwards DL. (1999). Cocaine decreases uteroplacental blood flow in the rat. *Neurotoxicol*, 21, 559-65.
- Pellegrino T, Bayer BM. (1998). In vivo effects of cocaine on immune cell function. *J. Neuroimmunol*, (1-2) 139-47.

- Pellegrino TC, Dunn KL, Bayer. (2001). BM Mechanism of cocaine induced decreases in immune cell function. *Int. Immunopharmacol*, 1 (4) 665-75.
- Redwine, L., Hauger RL, Gillin JC., Irwin M. (2000). Effects of sleep and sleep deprivation on interleukin 6 growth hormone cortisone melatonin levels in humans. *J.Clin Endo Metab*, 85, 3597-3603.
- Rizk, B, R., Atterbuy, JL., and Groome, LJ. (1996). Reproduction Risks of Cocaine. *Human Reproduction Update*, 2, 1, 43-55.
- Ruiz P., Cleary T., Nassiri M., Steele B. (1994). Human T lymphocytes subpopulation and N K cell alteration in persons exposed to cocaine. *Clin Immunol Immunopathol*, 70 245-2150.
- Sanyal Z, Biro E, Panke B Telegdy G. (1992). The cocaine induced elevation of plasma corticosterone mediated by endogenous corticotrophin releasing factor, *Brain Research*, 28, 589 (1) 154-6.
- Schiono PH, Klebnoff MA, Nugent RP, Cotch MF, Wilkens DG. (1995). The impact of cocaine and marijuana use on low birth weight and preterm: a multicenter study. *Am J. Obster Gynecol*, 172, 19-27.
- Singh, B., Merchant, P, Walker, CR, Kryworuchko, M and Diaz-Mitoma, F. (1996). Interleukin-6 expression in cord blood of patients with clinical chorioamnionitis. *Pediatric Research*, 39, (6) 1 976-979.
- Stanulis ED., Matuka, RA Jordan, SD Rosecrans, JA Hoisapple, MP. (1997). Role of corticosterone in the enhancement of the antibody response after acute cocaine administration, *The Journal of Pharmacology and Experimental Therapeutics*, 280,284-291.
- Stead, AH., Moffet, AC. (1983). A collection therapeutic, toxic and fetal drug concentration in man. *Human Toxicol*, 3, 437-464.
- Strietet, RM, Remick DG, Ham JM Colletti LM Lynch JP and Kunkel SL. (1990). Tumor necrosis factor –alpha gene expression in human whole blood. *J. Leukocyte Biology*, 47, 366-370.
- Van Dyke C, Stesin A, Jones R, Chonthrapal A and Seaman, W. cocaine increases natural cell killer activity L. *Pharmacol Exp. Ther*, 77, 1387-1390.
- Van Voorhis Wc, Barnett IK, Sweeney YT, Kuo CC, Patton D. (1996). Analysis of lymphocytes phenotype and cytokine activity in the inflammatory infiltrates of the upper genital tract of female macaques infected with Chlamydia Trachomatil. *Infec Dis*, 174:647-650. *J. Neuroimmunol* 1998, Mar 15, 83, (1-2) 133-8.
- Verma BK, Fogarasi M, and Szabo G. (1993). Down regulation of tumor necrosis factor alpha activity by acute ethanol treatment in human peripheral blood monocyte. *J. Clin Immunol*, 13, 8-22.
- Wang, Y, Huang, Ds Watson RR. (1994). In vivo and in vitro cocaine modulation on production of cytokines in C57BL/6 mice. *Life Sciences*, 54, (6) 401-411.
- Wetzel B Chen G, Scuderi P, pirozhkov S. Watson RR. (1992). Cocaine induced suppression of interferon-gamma in leucocyte from young and old C57BL/6 mice. *Int. J.Immunopharmacol*, 14 96) 1125-1131.

Table 1.

Factors	Cocaine users	Control	pvalue
Maternal Age	25±4	26.3±7	NS
Birth Weight (g)	2250±495	2678±434	P<0.05
Gestation Age (weeks)	36.5±4	38.4±3	NS
Apgar Score	6.3±2.0	7.8±3.0	NS
Placental Abruption	3/16	1/16	P<0.05

NS=Non Significant

Significant p>0.05.

Newborn weighing less than 2500 gm is considered low birth weight. Gestation age of 37 weeks and above is considered normal, 34 weeks or below is considered pre-term. Although cocaine users had low gestation age but the data were not significant (p>0.05)

Apgar score of 7 and above is considered normal.

Although Apgar score of newborn to cocaine users were below the normal range (6.3±2) the data were not significant.

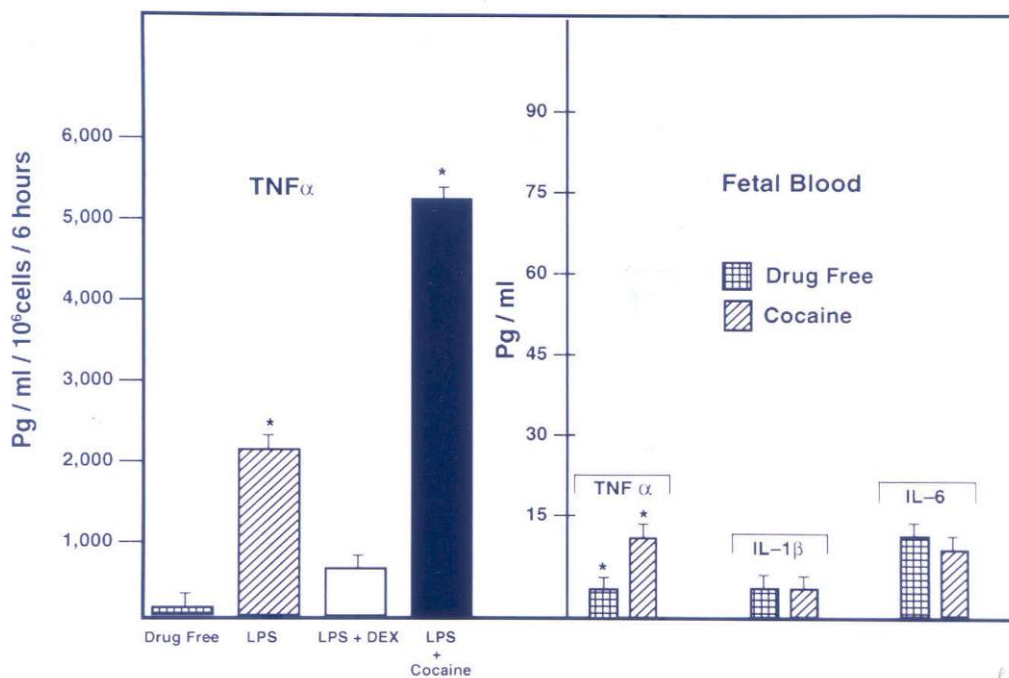


Figure 1. Showing the effect of cocaine (2.1μM) in vitro on TNFα synthesis in isolated lymphocytes from mothers who were drug free (licit and illicit) throughout pregnancy

Note LPS stimulated TNFα synthesis and dexamethasone suppressed LPS stimulated TNFα synthesis.

When cocaine was added in the media together with LPS there was further increase in the TNFα synthesis showing that cocaine increased the TNFα synthesis.

Showing in the same figure ex vivo study (left panel) is the effect of cocaine use during pregnancy on TNFα, Interleukin 1-β and Interleukin 1-6.

Note: As in vitro study, TNFα increased significantly (p<0.01) while Interleukin 1-β and Interleukin 1-6 synthesis were not effected in fetal blood.

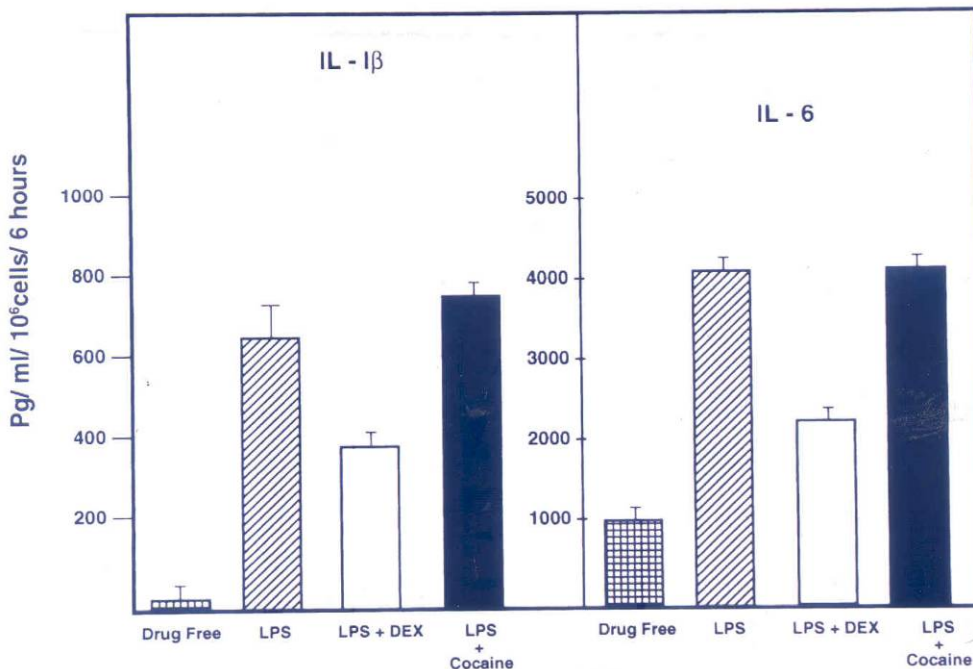


Figure 2. Showing the effect of in vitro study on IL-1β and IL-6 synthesis in the isolated lymphocytes stimulated with LPS

Note: No significant differences in the IL-1β and IL-6 synthesis in cocaine users and non drug users

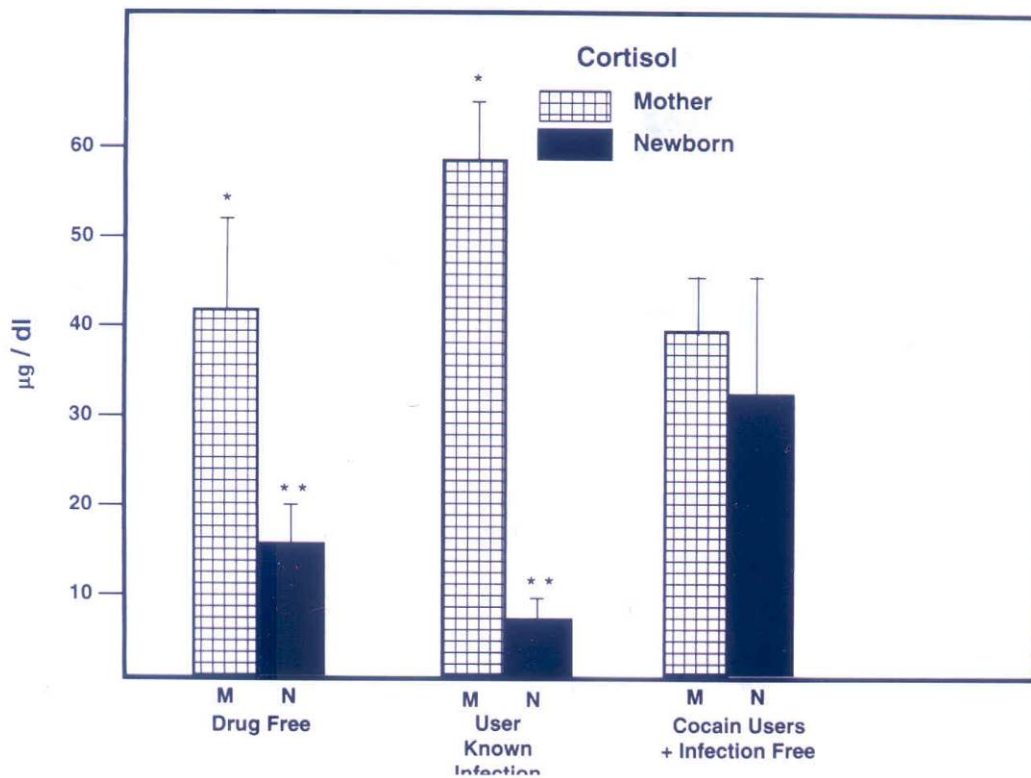


Figure 3. Showing blood cortisol levels in the mother and the fetus in cocaine users

Note: Although use of cocaine during pregnancy didn't affect blood cortisol level in the mother the fetal blood showed significant increase.

Shown in the same figure is the result of blood cortisol level in subjects who were diagnosed with infection. They served as positive control. Data clearly show that cortisol level increased in subjects who were known to have infection. These data imply that even though mother may not show any evidence of infection in cocaine users the fetus may still be infected.