

Cigarette Smoking and Hormone Balance in Women: New Evidence in the Anti-Smoking Campaign

Murray D. Meek, M.Sc. (OT3)

Abstract

Of the 400,000 deaths attributable to smoking among women in developed countries in 1995, 21% were due to lung cancer, 41% to cardiovascular diseases, primarily coronary heart disease and stroke, and 18% to chronic obstructive pulmonary disease. One problem physicians face in counseling younger female patients to stop smoking is that the long term health effects are simply too far away to worry about right now. However, new evidence is coming to light that cigarette smoking disrupts the endocrine system – an effect that is much more immediate than cancer or heart disease. Cigarette smoke has complex estrogenic and anti-estrogenic effects mediated by the estrogen and aryl hydrocarbon receptor respectively. These effects, in turn, have consequences for progesterone receptor up- and down-regulation. In addition, cigarette smoke inhibits the synthesis of progesterone. This has important implications for menstruation, hirsutism, hoarseness, pregnancy, and the influence of hormone dependent cancers. Given the increasing importance of smoking as a public health issue, ongoing efforts in the anti-smoking campaign should include education regarding these more acute endocrine problems.

Introduction

Cigarette smoke is an aerosol composed of volatile agents in the vapor phase and semivolatiles in the particulate phase. Approximately 400-500 individual gaseous compounds are contained in the vapor phase, including ammonia, hydrogen cyanide, toluene, benzene, hydrogen sulfide, and formaldehyde. More than 3500 compounds have been identified in the particulate phase, including naphthalene, pyrenes, phenols, benzofurans, aniline, toluenes, N-nitrosoamines, and the addictive substance, nicotine. Additionally, the particulate phase contains inorganic compounds including hydrazine, arsenic, nickel, chromium, cadmium, lead, and polonium.¹ This list of known chemicals is incomplete because the identities of flavor additives are trade secrets.

Researchers have known for a long time that cigarette smoking is associated with lung cancer and certain other cancers,

and have identified certain compounds in cigarette smoke as carcinogens. For example, oral cancer has been shown to develop with 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) treatment in rodents.¹ More recently, scientists have identified some of the molecular effects of cigarette smoke including effects on gene transcription and enzymes that affect hormone balance.

Epidemiology – Cigarette Smoke is an Endocrine Disruptor

Cigarette smoke is an endocrine disruptor because it disrupts the normal homeostatic mechanisms that control hormone levels in the body. This became evident in epidemiological studies that showed that smoking exerts an important anti-estrogenic effect in women. For example, smoking reduces the risk of endometrial cancer, an estrogen responsive cancer, by as much as 50 percent.²⁻⁵ The reduction in risk is greatest in women who are multiparous, obese, or not using estrogen replacement therapy. Among postmenopausal women, current smokers showed the greatest reduction in risk (relative risk = 0.4). Former smokers, including those who had recently stopped, were less affected (relative risk = 0.8), suggesting that smoking is protective.⁶

Other studies have shown that women who smoke experience menopause 2-5 years earlier than non-smoking women.⁷⁻⁸ Menopause normally occurs with cessation of estrogen production by the ovaries, however, anti-estrogens are thought to elicit the same physiological effect by mimicking reduced estrogen effectiveness.⁹ Rosenberg *et al.* have shown that women who smoke exhibit a significantly greater frequency of irregular bleeding during their menstrual cycle. Age at menarche was not affected but hoarseness and hirsutism (both conditions mediated by an elevated testosterone/estrogen ratio) were significantly increased in female smokers who smoked 10 or more cigarettes per day.⁷ Epidemiological data have also linked smoking to increased rates of osteoporosis, a condition associated with decreased serum estrogen levels.⁵

Anti-estrogenic Mechanisms of Cigarette Smoke

The mechanisms of the anti-estrogenic effect of smoking are unclear but several hypotheses have been proposed. Catabolic inactivation of endogenous estrogen has been proposed as a mechanism by which smoking could elicit an anti-estrogenic effect.¹¹ This hypothesis is based on the observation that the rate of 2-hydroxylation of 17 β -estradiol (E2) was significantly increased in premenopausal female smokers who smoked at least 15 cigarettes per day compared to non-smokers. Biologically, 2-hydroxyestrogens are significantly less potent estrogen receptor (ER) agonists than E2 and are rapidly cleared from the circulation.^{12,13} Thus, E2 hydroxylation effectively terminates the peripheral activity of estradiol.

In humans, CYP1A1 and 1A2 are the primary enzymes catalyzing the 2-hydroxylation of estradiol.¹⁴ The regulation of CYP1A1 and 1A2 gene activation occurs via an aryl hydrocarbon receptor (Ah receptor)-mediated process.^{15,16} Mechanistically, ligand binding to the Ah receptor and release of heat shock protein 90 allows heterodimerization of the ligand bound Ah receptor with the aryl hydrocarbon receptor nuclear translocator (ARNT) protein. The activated receptor-ligand complex translocates from the cytoplasm to the nucleus where it can interact with DNA response elements termed dioxin or xenobiotic response elements (DRE or XRE respectively), in the vicinity of target genes resulting in gene expression.¹⁵

Direct experimental evidence indicates that cigarette smoke binds to and activates the Ah receptor.^{17,18} Further, the activated receptor is able to stimulate CYP1A1/1A2, the enzymes responsible for the catabolic inactivation by estrogen by 2-hydroxylation.^{17,18} The Ah receptor complex formation assay was performed by incubating guinea pig liver cytosol with cigarette smoke and a radiolabeled dioxin response element (DRE) oligonucleotide.^{17,18} The resultant products were resolved on SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and visualized by autoradiography. The analysis showed an inducible protein-DNA complex that became absent if no DRE was present or was competitively inhibited by incubation with an excess of unlabeled mutated DRE sequence.^{17,18}

Further evidence that cigarette smoke can bind to and functionally activate the Ah receptor came from reporter gene studies. In this series of experiments, induction of Ah receptor-regulated luciferase reporter gene activity in liver cells occurred in a dose-dependent and saturable manner.¹⁷ Cigarette smoke exhibits approximately 50 percent of the potency of the prototypical Ah receptor ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In contrast, there was no luciferase activity in variant liver cells that do not contain either the Ah receptor or the ARNT protein.¹⁷ This demonstrates the requirement for a functional Ah receptor and ARNT protein for DRE-regulated luciferase expression.

Finally, when liver cells were exposed to cigarette smoke, CYP1A1/1A2 enzyme activity increased in a dose-dependent

and saturable manner.¹⁷ The maximal activity was approximately 50 percent of that by TCDD.¹⁷ The lack of any detectable increase in CYP1A1/1A2 enzyme activity in Ah receptor or ARNT deficient variant cells is consistent with the requirement of these proteins to activate CYP1A1/1A2 enzyme activity.^{19,20}

Estrogenic Effects of Cigarette Smoke and Involvement of the Estrogen Receptor

The role of the ER is another mechanism by which smoking could affect estrogen levels. The proposed mechanism of action of the ER involves ligand binding to the inactive oligomer complexed to several heat shock proteins.²¹ Upon ligand binding, the complex dissociates to allow dimerization of receptor monomers.²² The dimerized complex then binds to palindromic DNA binding sites termed estrogen response elements in the vicinity of estrogen responsive genes to elicit transcriptional activation.²³

Ligand binding experiments using the hydroxyapatite method²⁴ have demonstrated that cigarette smoke binds to the human ER in a concentration-dependent and saturable manner. Using a firefly luciferase reporter gene system that can only be activated by the agonist-occupied human estrogen receptor, cigarette smoke was able to induce luciferase activity in human breast cancer cells transfected with the reporter gene components.¹⁷ Taken together, this evidence indicates that cigarette smoke binds to the estrogen receptor, and activates it functionally to induce gene transcription of target genes. It is reasonable to suppose that estrogen-responsive genes would be activated *in vivo*; this has been confirmed experimentally in rodents through the measurement of uterine wet weight.²⁵ An alternate, and possibly very sensitive assay would involve the assessment of specific estrogen-responsive genes. The lactoferrin gene would be a reasonable candidate for *in vivo* studies because it contains estrogen responsive elements in the upstream region and has been shown to be estrogen receptor responsive.²⁶ It is expressed in rat uterus making it a convenient *in vivo* model to assess estrogen action.

Experimental evidence showing that cigarette smoke is able to bind to and activate the ER, thus inducing transcription, is unexpected given the obvious anti-estrogenic effects of smoking. Considering the pleiotropic effects of smoking and the sensitive mechanisms of hormone regulation however, this finding may be physiologically significant. Experiments suggest that cigarette smoke might elicit an anti-estrogenic effect through estrogen receptor down-regulation, via the Ah receptor. Polyaromatic hydrocarbon congeners which bind to the Ah receptor, including benzo[a]pyrene, benz[a]anthracene, and 7,12-dimethylbenz[a]anthracene caused a decrease in nuclear ER levels.²⁷ Thus, it is conceivable that the estrogenic and anti-estrogenic pathways are indirectly competing against one another and that the observed anti-estrogenic effect of smoking is actually the net result of this competition. It is equally conceivable that the ER pathway may be exerting subtle

estrogenic effects that make it difficult to detect an association between cause and effect. For example, several studies have shown a weak to moderate link between smoking and breast cancer²⁸⁻³¹ while other studies show no link.³²⁻³⁵ This apparent contradiction would be consistent with the idea that cigarette smoke exerts a subtle estrogenic effect in females.

Alternatively, others have proposed a temporal relationship between cigarette smoke and its estrogen agonist/antagonistic effects. The evidence for this is as follows. Cigarette smoke is estrogenic in young female rats (3 week old) because it increases uterine wet weight.²⁵ By comparison, in 3-month old animals, cigarette smoke inhibited the ability of estrogen to induce progesterone receptors in uterine tissue.²⁵ The study did not address the effect of cigarette smoke on uterine wet weight in the older animals as would have been both appropriate and expected. Induction of the progesterone receptors occurs via the ER, which is permissive for progesterone activity. This suggests a window of opportunity in which cigarette smoke may act as an estrogen and a later window when it acts as an anti-estrogen.

Involvement of the Progesterone Receptor

It is also possible that compounds present in cigarette smoke may disrupt the effects of progesterone by preventing the up-regulation of progesterone receptors. This might occur by two distinct mechanisms. First, the Ah receptor mediated *anti-estrogenic* effect could biologically inactivate estradiol levels directly through 2-hydroxylation.¹¹ Second, polycyclic aromatic hydrocarbons in cigarette smoke may cause a decrease in estrogen receptor levels as shown *in vitro*.³⁶ Both of these mechanisms would lead to the same biological effect – decreased estrogen activity leading to decreased number of progesterone activity through decreased progesterone receptors. These mechanisms may also contribute to the fact that cigarette smoking disrupts menses by increasing the frequency of spot bleeding and altering the normal menstrual frequency. Conversely, the estrogenic activity of cigarette smoke should up-regulate progesterone receptors which may enhance progesterone activity if there is sufficient progesterone present. However cigarette smoke inhibits progesterone synthesis, so the effect may be blunted.³⁷

Pregnancy and Smoking

Smoking during pregnancy is a serious public health problem with adverse effects for both mother and baby including increased infant mortality, spontaneous abortion, pre-term delivery, and low birth weight.³⁸⁻⁴⁰ Most smokers do not stop smoking during their pregnancy. According to one self-reporting study of women in Nova Scotia, the prevalence of smoking before pregnancy is about 30%, which declines to about 25% at delivery.⁴¹ The same study showed that the amount smoked decreased slightly but significantly from a mean of 15.3 cigarettes/day before pregnancy to 12.5 cigarettes/day at delivery.

Smoking during pregnancy is associated with an increased incidence of spontaneous abortion,⁷ thought to be due to corpus luteum insufficiency. This hypothesis is supported by experimental evidence demonstrating that incubation of human granulosa cells with cotinine, anabasine, nicotine (all components of cigarette smoke), or with an aqueous extract of cigarette smoke resulted in inhibition of progesterone synthesis.⁴²

Another study showed that nicotine augmented estradiol secretion and inhibited progesterone secretion by human granulosa cells in a dose-dependent manner.⁴³ Nicotine had little effect on other steroid secretion. If granulosa cells were stimulated with luteinizing hormone, nicotine suppressed estradiol secretion, with no change in inhibited progesterone secretion. The results suggest that cigarette smoking specifically affects the control mechanisms of intraovarian processes that are responsible for normal luteal function.⁴³ However, studies that examine isolated effects of a single compound are not reflective of the *in vivo* situation. Cigarette smoke is a complex mixture of aerosols and semivolatiles that contains endocrine-disrupting chemicals. Furthermore, binding to plasma proteins may sequester these chemicals. The half-lives of these chemicals in humans are unknown in most instances and effects on liver detoxification enzymes have not yet been identified. Further studies on human granulosa cells are required to assess the effect of whole cigarette smoke on progesterone secretion and progesterone receptor levels.

Conclusions

Although we have long known that smoking causes lung cancer, new evidence is coming to light that cigarette smoking disrupts the endocrine system. Cigarette smoke has complex estrogenic and anti-estrogenic effects mediated by the estrogen and Ah receptor respectively. These effects, in turn, have consequences for progesterone receptor up- and down-regulation. In addition, cigarette smoke inhibits the synthesis of progesterone. However, research into the effects of cigarette smoke on estrogen and progesterone action is still in its infancy. Given the increasing importance of smoking as a public health issue, research in this area is expected to intensify. Although we know that cigarette smoking causes lung cancer, the cancer typically does not present until several decades later. Therefore, the patient does not perceive the health consequences of their actions until later in life. The endocrine problems are more acute and should be used in education efforts in the anti-smoking campaign.

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