Cigarette Smoking, Coffee Drinking, and Ingestion of Charcoal-Broiled Beef as Potential Modifiers of Drug Therapy and Confounders of Clinical Trials

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ABSTRACT

A pathway of research is described, leading from the finding of an inhibitory effect of 3-methylcholanthrene on the carcinogenicity of an aminoazo dye, to the induction of drug-metabolizing enzymes by 3-methylcholanthrene, benzo[a]pyrene, and other polycyclic aromatic hydrocarbons, to the demonstration of enhanced drug metabolism in cigarette smokers, coffee drinkers, and people who eat charcoal-broiled beef. The results of these studies indicate that cigarette smoking, coffee drinking, and the ingestion of charcoal-broiled beef (all resulting in exposure to polycyclic aromatic hydrocarbons) can influence the dosing regimen needed for proper drug therapy and are potential confounders of clinical trials with drugs metabolized by polycyclic aromatic hydrocarbon-inducible enzymes.

Introduction

It is well known that there are large differences in the dose-response relationship of drugs from one person to another that depend on both genetic and environmental factors that influence drug metabolism. Although genotyping patients for drug-metabolizing enzymes before drug therapy can help establish a proper initial dosing regimen for individual patients, environmental modulation of drug metabolism is also important and requires a different approach for establishing a proper dosing regimen.

Early research demonstrated that administration of the carcinogen 3-methylcholanthrene (3-MC) to rats unexpectedly inhibited the hepatocarcinogenicity of an aminoazo dye (Richardson et al., 1952). This report was followed by a research program that showed why a carcinogenic polycyclic aromatic hydrocarbon (PAH) inhibited the tumorigenicity of a carcinogenic aminoazo dye and provided a springboard that led to research showing the stimulatory effects of cigarette smoking, eating of charcoal-broiled beef, and coffee drinking on the metabolism of certain drugs. In those early studies, it was demonstrated that the administration of 3-methylcholanthrene, benzo[a]pyrene (BP), or several other PAHs induced the synthesis of hepatic enzymes that metabolized aminoazo dyes to noncarcinogenic products (Brown et al., 1954; Conney, 1954; Conney et al., 1956). That work, which was done more than 50 years ago, was the first demonstration of the induction of xenobiotic-metabolizing enzymes and led to further research demonstrating a stimulatory effect of administration of benzo[a]pyrene and other PAHs on the metabolism of several drugs and the finding of stimulatory effects of cigarette smoking, ingestion of charcoal-broiled beef, and coffee drinking on drug metabolism in animals and humans.

The stimulatory effect of intraperitoneal injections of 3-methylcholanthrene on the oxidative N-demethylation and the reductive cleavage of aminoazo dyes to noncarcinogenic metabolites by the liver is shown in Fig. 1 (Brown et al., 1954; Conney, 1954; Conney et al., 1956), and the stimulatory

ABBREVIATIONS: PAH, polycyclic aromatic hydrocarbon; BP, benzo[a]pyrene; 3-MC, 3-methylcholanthrene; 3-methyl-AB, 3-methyl-4-aminoazobenzene; FU, 5-fluorouracil.
effect of benzo[a]pyrene on its own metabolism and the hepatic metabolism of the muscle relaxant drug zoxazolamine is shown in Fig. 2 (Conney et al., 1957, 1959, 1960). A single intraperitoneal injection of benzo[a]pyrene 24 h before the administration of zoxazolamine had a dramatic stimulatory effect on the in vivo metabolism of this drug (Fig. 2), and the duration of action of zoxazolamine was decreased from 730 to only 17 min (Conney et al., 1960). Studies on the enzyme-inducing activity of a large number of PAHs indicated that many environmental PAHs with four or more rings when administered by intraperitoneal injection to rats are potent inducers of the hepatic aminoazo dye N-demethylase (Arcos et al., 1961). Modest inducing activity was also observed in mice fed 3-methylcholanthrene, benz[a]anthracene, pyrene, or phenanthrene for 1 week (Brown et al., 1954; Conney, 1954). Later studies indicated that PAHs were potent inducers of cytochromes P450 1A1 and 1A2. Because PAHs that induce certain drug-metabolizing enzymes are present in tobacco smoke and charcoal-broiled beef (Table 1) (Lijinsky and Shubik, 1964; Hoffmann et al., 1978), the effects of cigarette smoking and eating charcoal-broiled beef on the metabolism of substances that are metabolized by PAH-inducible enzymes were evaluated in humans.

Cigarette smokers had greatly elevated levels of placental benzo[a]pyrene hydroxylase, aminoazo dye N-demethylase, and zoxazolamine hydroxylase (Table 2) (Welch et al., 1968, 1969; Kapitulnik et al., 1976), which are PAH-inducible enzymes in rodents. In additional studies, cigarette smoking or eating charcoal-broiled beef lowered the plasma levels of phenacetin (a CYP1A1/2 substrate) without altering its half-life (Fig. 3) (Pantuck et al., 1972, 1974b, 1976; Conney et al., 1976). In these studies, the ratio of N-acetyl-p-aminophenol (phenacetin’s major metabolite) to phenacetin was increased by 2- to 5-fold at the different time intervals after the administration of phenacetin, and it was suggested that cigarette smoking or eating charcoal-broiled beef enhanced the intestinal metabolism of phenacetin. In accord with this concept, the administration of benzo[a]pyrene, exposure to cigarette smoke, or the feeding of charcoal-broiled beef stimulated the metabolism of phenacetin by rat intestine (Pantuck et al., 1974a,b, 1975). It is likely that eating other charcoal-broiled
TABLE 2
Effects of cigarette smoking on the metabolism of xenobiotics by human placenta

<table>
<thead>
<tr>
<th>Reaction Measured</th>
<th>Enzyme Activity in Placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsmokers</td>
</tr>
<tr>
<td>Benzo(a)pyrene hydroxylation</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>3-Methyl-4-monomethylanisobenzene</td>
<td>&lt;4</td>
</tr>
<tr>
<td>N-demethylation</td>
<td></td>
</tr>
<tr>
<td>Zoxazolamine hydroxylation</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>7-Ethoxycoumarin O-dealkylation</td>
<td>29 ± 3</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E. The data were obtained from Welch et al. (1968, 1969) and Kapitulnik et al. (1976).

foods such as charcoal-broiled chicken or fish will also stimulate the metabolism of drugs that are metabolized by PAH-inducible enzymes.

PAHs are formed during the roasting of coffee beans (Houessou et al., 2007), and the concentrations of various PAHs in several coffee brews are shown in Table 3 (Orecchiao et al., 2009). Administration of coffee to rats increased the level of hepatic CYP1A2, and this effect of coffee was partially lost in decaffeinated coffee, suggesting that both caffeine and noncaffeine components (probably PAHs) play a role for the effect of coffee administration to increase the level of hepatic CYP1A2 in rats (Turesky et al., 2003). A recent study indicated that coffee drinkers have enhanced 3-deethylation of caffeine, which occurs predominantly by CYP1A2 (Djordjevic et al., 2008). Approximately 5 to 10% of commonly prescribed drugs undergo metabolism by CYP1A2. Examples of drugs that are metabolized to a significant extent by CYP1A2 include caffeine, theophylline, phenacetin (no longer marketed), tizanidine, clozapine, fluvoxamine, olanzapine, zoxazolamine (no longer marketed), and tacrine. In summary, cigarette smoking, coffee drinking, and the ingestion of charcoal-broiled beef can stimulate drug metabolism and alter the dosing regimen needed for proper drug therapy, and these modifiers of drug metabolism are potential confounders during clinical trials with drugs that are metabolized by PAH-inducible enzymes.

Other sources of environmental exposure to PAHs are from the burning of coal, wood, and gasoline as well as from petroleum refining, so people heavily exposed to these sources of PAHs would be expected to have enhanced metabolism of drugs that are metabolized by cytochromes P450 1A1 and 1A2.

These data on common environmental factors that increase the rate of drug metabolism raise three important clinical concerns.

The first concern is related to the variability of dose response among different individuals in a population. This is especially relevant in a clinical trial. Figure 4 (Asberg, 1974) shows the range of concentrations of nortriptyline in the plasma of 248 people taking 150 mg/day of the drug. This more than 6-fold range from the lowest to the highest concentration is representative of what occurs with many drugs eliminated by metabolism. In an additional study, the plasma level of desmethylimipramine varied by 36-fold in 11 different individuals receiving the same 75-mg daily dose of the drug (Hammer et al., 1967). Although variability in the metabolism of nortriptyline and desmethylimipramine that occurs in different individuals is related primarily to genetic polymorphisms in CYP2D6, the metabolism of these compounds can also be regulated by environmental factors, and person-to-person differences in plasma levels described in Fig. 4 illustrate the variability in metabolism that can occur from both genetic and environmental factors that regulate drug metabolism. For nortriptyline, the best efficacy is when the concentration is 50 to 140 ng/ml (Asberg et al., 1971) or approximately a 3-fold range. Although the dose of 150 mg/day had most patients in this range, many patients had lower or higher levels causing less efficacy or increased risk of an adverse effect, respectively. Adjusting the dose to allow for a person’s rate of metabolism can bring the drug level into the therapeutic range with the intended intensity of effect. One study of only 10 patients receiving a dose of 8 mg of tizanidine found a mean peak concentration of 25 pg/ml with a range of 15 to 43 pg/ml, a 3-fold range (Mathias et al., 1989). Because induction speeds drug metabolism, an induced population of subjects in a trial will lack the very slow metabolizers that occur in the general population. The trial will fail to detect subjects who would have had high drug levels at the studied dose if they were not induced. The interpretation of the trial will be that the observations represent the drug levels and effects of this dose for the general population. It will fail to recognize that the fraction of the population that are uninduced slow metabolizers will have higher levels than seen in the trial and possibly overdose at the studied dose. Thus, unanticipated toxicity may occur after marketing.

A second concern relates to the size of the population needed to determine the effectiveness of an agent being studied in a clinical trial. The presence of a moderate number of individuals with induced drug-metabolizing activity may magnify variability of response in the population studied, thereby requiring more subjects to determine effectiveness of the substance being evaluated. Measurement of blood or saliva levels of drug during the course of the trial will help in interpretation of the trial.

A third concern relates to patient care. A person taking a medicine who then ingests an inducer may have an increase
in the rate of drug metabolism. The extent of the increase depends on the dose of inducer and the sensitivity of the person to it (Mathias et al., 1989). This induction will increase the rate of metabolism of the drug and decrease the drug level and efficacy and benefit from taking the medicine. If the dose is then raised to allow for the new rate of metabolism and the inducer is subsequently stopped, such as when the patient finally quits smoking or stops eating charcoal-broiled meat or drinking coffee, the induced state will subside. If the dose is then raised to allow for the new rate of metabolism and the inducer is subsequently stopped, such as when the patient finally quits smoking or stops eating charcoal-broiled meat or drinking coffee, the induced state will subside. This was reported for a patient taking clozapine and another taking olanzapine. Each stopped smoking and developed toxicity from their medicine (Brekenridge et al., 1973).

Research in patients has found that smokers have lower concentrations of many of the clinically important drugs metabolized by PAH-inducible enzymes than nonsmokers when given the same dose. This has been shown for haloperidol (Jann et al., 1986) and tacrine (Zevin and Benowitz, 1999). Other examples are: fluvoxamine levels in smokers are 1/3 that of nonsmokers (Spigset et al., 1995), clozapine levels are 1/3 lower for men, 1/5 for women compared with nonsmokers (Haring et al., 1989), and smoker olanzapine levels are 1/5 that of nonsmokers (Carrillo et al., 2003). Clearly cessation of smoking can cause “overdose” toxicity for a patient taking any of these medications and then stops smoking.

Thus, the experimental animal studies indicating an inhibitory effect of one carcinogen (a polycyclic aromatic hydrocarbon) on the action of another carcinogen (an aminoazo dye) led to additional research demonstrating polycyclic aromatic hydrocarbon-induced synthesis of drug-metabolizing enzymes that has relevance for clinical trials and the care of sick people.

**Perspectives for the Future**

Although we have focused here on early basic research that led to the discovery of the effects of cigarette smoking, coffee drinking, and charcoal-broiled beef ingestion on drug metabolism in humans, there are many factors that influence rates of drug metabolism that are important for explaining differences in drug response in different individuals receiving the same dose of drug and in the same individual on different occasions. In earlier studies, we found substantial differences in the metabolism of phenacetin in individuals leading an unrestricted lifestyle when given phenacetin on several occasions over several months (Conney et al., 1979). Factors affecting drug metabolism in humans include genetic factors (polymorphisms such as mutations, insertions, deletions, and gene duplications), epigenetic factors (gene methylations and histone acetylation), disease states (liver disease, kidney disease, and viral infections), and a variety of environmental factors (cigarette smoking, coffee drinking, alcohol consumption, drug administrations/drug interactions, herbal remedies such as St. Johns wort, exposure to dichlorodiphenyltrichloroethane (DDT) or gasoline fumes as observed in gasoline station attendants, and dietary factors such as the ratio of protein to carbohydrate, cruciferous vegetables, charcoal-broiled beef, and grapefruit juice). Although genotyping individuals for the cytochrome P450s or other drug-metabolizing enzymes has been helpful in identifying genetically determined poor or rapid metabolizers of many drugs, the prevalence of environmental factors that influence drug metabolism suggests the importance of drug-level monitoring for effective therapy.

**Measurement of Drug Levels.** Before the initiation of an extensive program for drug-level monitoring to guide the dosing regimen in individual patients, there is a need for further research to develop simple, rapid, and inexpensive blood or saliva drug-level tests that can be used and evaluated rapidly during each visit to the patient’s physician or the monitoring of clinical trials. These tests could be developed by pharmaceutical companies that develop the drugs, and this activity could develop into a new business for the pharmaceutical industry that would help achieve more effective drug therapy. To achieve rapid information on blood or saliva drug levels and an appropriate starting dosing regimen, the analysis of samples by high sensitivity and specific methodology should be done and interpreted rapidly at the point of care.

Although we are suggesting a greater emphasis on the use of blood or saliva drug-level monitoring for measuring rates of drug metabolism and effective drug use, more attention should also be given to variations in drug transporters and their importance for person-to-person differences in drug response. Some drugs interact with transporters that modify the drug’s passage into tissues and the drug’s ability to reach receptors so that measuring blood levels of these drugs may not be a good index for the amount of drug that reaches a receptor. Both genetic and environmental factors influence

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**TABLE 3**

Representative polycyclic aromatic hydrocarbons in 13 coffee brew samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration* (ng/l)</th>
<th>Enzyme-Inducing Activity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>220 (83–542)</td>
<td>–</td>
</tr>
<tr>
<td>Anthracene</td>
<td>44 (9–75)</td>
<td>–</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>73 (37–171)</td>
<td>–</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>53 (1–161)</td>
<td>+</td>
</tr>
<tr>
<td>Chrysene</td>
<td>51 (2–129)</td>
<td>+</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>12 (1–47)</td>
<td>+</td>
</tr>
<tr>
<td>Benzo(e)pyrene</td>
<td>19 (1–78)</td>
<td>+</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>4 (1–10)</td>
<td>+</td>
</tr>
<tr>
<td>Dibenzo(a,h)pyrene</td>
<td>14 (4–35)</td>
<td>+</td>
</tr>
<tr>
<td>Total from 28 PAHs</td>
<td>1000 (520–1800)</td>
<td></td>
</tr>
</tbody>
</table>

*Data were obtained from Orecchio et al. (2009).

**Induction of azo dye N-demethylase activity was measured at 24 h after a single 1.86 μmol i.p. injection (Acros et al., 1961). In another study, feeding 0.05% phenanthrene in the diet to mice for 1 week stimulated azo dye N-demethylase activity (Brown et al., 1954).

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**Fig. 4.** Plasma levels of nortriptyline in patients receiving 150 mg daily (Aasberg, 1974).
the level or function of transporters (Wang and Morris, 2007; Wesolowska, 2011; Nakanishi and Tamai, 2012; Sissung et al., 2012), so developing methods for evaluating the levels or effects of these transporters by suitable methodology in humans will be important. Can we develop imaging methods that will allow us to measure drug levels at or near receptors? Finally, it would be an important advance to develop pharmacologically active drugs that do not undergo substantial metabolism but are excreted unchanged. It is likely that these drugs would be highly water soluble and excreted by the kidney with fewer person-to-person variations in blood levels and action than drugs that undergo metabolism. Variation of elimination rates of drugs excreted unchanged by the kidney is related to renal function. This is easily estimated by present methods. Because highly water-soluble drugs are poorly absorbed, the use of lipophilic prodrugs such as esters that would be readily absorbed and rapidly metabolized by tissue esterases before excretion by the kidney is a possible approach.

Clinical Trials. Clinical trials can be made more efficient by enriching the study population in three ways: 1) selecting subjects that are likely to have the condition or event being treated or prevented, 2) selecting subjects that are likely to respond to the intervention, and 3) reducing the heterogeneity of the subject population (Temple, 2010). A way to decrease heterogeneity caused by enzyme induction and the other causes of differences in the rate of elimination of the study drug is by doing a concentration-controlled clinical trial rather than the usual dose-controlled trial. In the concentration-controlled trial, the investigators determine what concentrations of drug in plasma they want to evaluate, measure drug concentrations in the subjects early in the trial, and then adjust the dose for each subject to achieve the desired concentration (Kraičzi et al., 2003).

Medical Practice. In medical practice, the use of drug concentration measurements to adjust doses for individual patients has been in use for over 40 years since Kutt and McDowell (1968) showed the value of this in adjusting the dose of phenytoin in patients with epilepsy. Despite this lengthy experience with using drug levels to adjust dose to achieve the desired intensity of effect, many areas of medical practice remain in which this concept of adjusting the dose to correct for an individual's unaverage pharmacokinetics is underused. For example, patient-controlled analgesia is now commonly used to treat postoperative pain. The patient pushes a button on an electronic device when a supplemental dose of an analgesic is desired. The device then administers a prespecified dose of medicine, usually by injection into an ongoing intravenous infusion. This patient-controlled analgesia corrects for both pharmacokinetic variability affecting dose to drug-level relationships and the variation of drug level to intensity of effect relationships. It is a way to individualize or personalize drug therapy. It is clear that both types of variability affect the dose-response relationships. Yet despite all of this knowledge about individualizing dosing at least for the pharmacokinetic variability, it is rarely done in managing chronic or cancer pain in ambulatory practice. One reason is lack of readily available measurements of opioid analgesics. For example, a research study done with hospitalized patients receiving opioids for chronic severe pain found that 60% of patients with bone or soft tissue pain had plasma opioid concentrations lower than the lowest concentration in patients having pain relief (Reidenberg et al., 1988). There are large person-to-person differences in the metabolic inactivation of 5-fluorouracil (FU) by dihydropyrimidine dehydrogenase, and a study (Gamelin et al., 2008) using blood-level monitoring to adjust dosing regimens throughout drug therapy was shown to improve therapy. The investigators indicated that “individual FU dose adjustment based on pharmacokinetic monitoring resulted in significantly improved objective response rate, a trend to higher survival rate, and fewer grade 3 or 4 toxicities. These results support the value of pharmacokinetically guided management of FU dose in treatment of metastatic colorectal cancer patients.”

Many drugs have large interindividual differences in rates of elimination with plasma concentrations, ranging from ineffective through therapeutic to toxic at standard dose. Individualizing doses to achieve the desired or therapeutic drug concentrations will get patients at the extremes of the elimination rate distribution into the therapeutic range with better outcomes for these patients.

To put the knowledge and concepts of pharmacology explaining some reasons for individual variability of dose response into practice, better methods of measuring drug concentrations in plasma, especially at the point of care, are needed. When these measurements should be made, such as early in therapy or at steady state, depends on the specifics of the drug and disease being treated. By applying our knowledge to control for the pharmacokinetic variability from patient to patient, the response can be made more predictable. And when it is more predictable, drug therapy is both safer and more effective.

Authorship Contributions

Performed data analysis: Conney and Reidenberg.
Wrote or contributed to the writing of the manuscript: Conney and Reidenberg.

References

Conney and Reidenberg


Richardson HL, Stier AR, and Borson-Nachtnebel E (1952) Liver tumor inhibition and adrenal histologic responses in rats to which 3 ′-methyl-4-dimethylaminoazobenzene and 20-methylcholanthrene were simultaneously administered. *Cancer Res* 12:356–361.


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