

Quantitation of Liver Function: Antipyrine Metabolism, an Update

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Introduction

For the majority of drugs, hepatic metabolism is a necessary pathway of elimination. Due to large inter- and intraindividual variations in hepatic metabolism of drugs, it is difficult to predict the actual elimination rate of a therapeutic agent. For many years it was believed that the rate of metabolism of any individual could be estimated from the elimination of a test drug; however, the failure of the antipyrine test to predict the rates of other drugs has demonstrated the inadequacy of such a simple approach. In the case of antipyrine this is probably due to its metabolism by a multiplicity of hepatic cytochrome P-450 enzymes.

Antipyrine was synthesized at the end of the last century, became one of the first important synthetic drugs, and remained popular until the 1930s. In the late 1940s Brodie (Brodie et al. 1949) Axelrod (Brodie and Axelrod 1950), and Soberman et al. (1949) performed the original studies on antipyrine metabolism in man. They determined the elimination half-life of antipyrine, showed that it is rapidly and completely absorbed following oral administration, determined its volume of distribution to be total body water, and established that it is eliminated almost exclusively by biotransformation. A large number of studies emerged concerning the influence of inheritance, and up to and during the 1970s antipyrine was extensively used in studies on the influence of inheritance, environmental factors, diseases, other drugs, etc. One of the driving forces was Elliot Vesell, who contributed many original papers and comprehensive reviews (Vesell 1979, 1982).

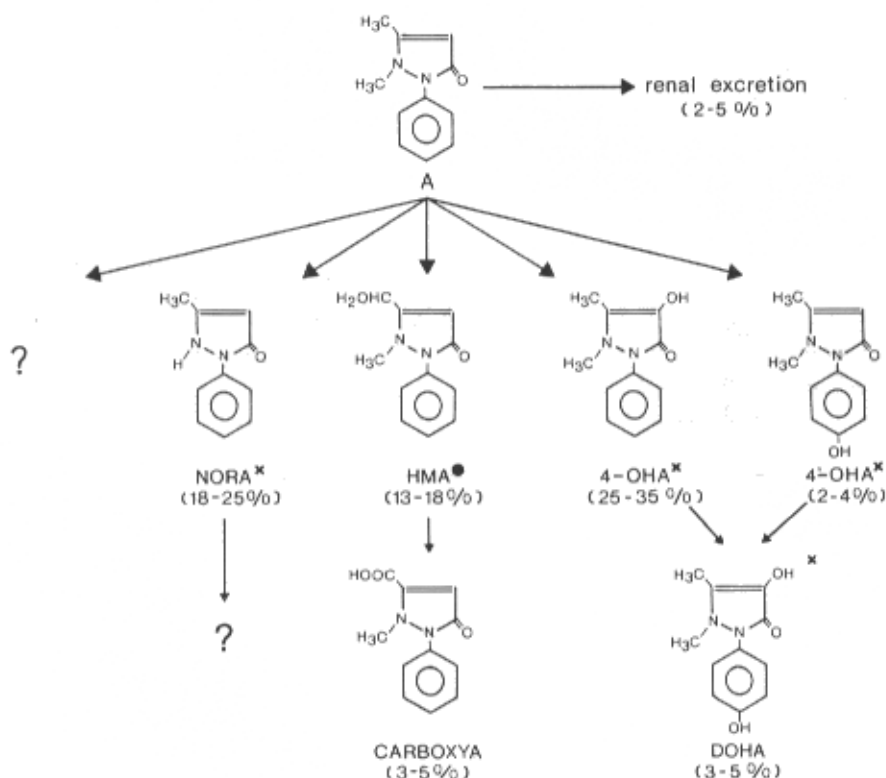
Huffmann et al. (1973) hypothesized that each of the antipyrine metabolites was formed in the liver by a single selective form of the mixed-function oxidase isozyme, cytochrome P-450. This hypothesis is now generally accepted and the underlying experiments have recently been reviewed (Danhof and Teunissen 1984).

In the last 10 years antipyrine has become more widely used and a large variety of factors influencing its elimination have emerged due to improved designs and measurements of individual metabolic pathways.

In the present review we shall give details of the present state of knowledge regarding (1) the pharmacokinetics of antipyrine, (2) determination of antipyrine using a single time-concentration point, and (3) quantitative assessment of liver function by antipyrine clearance.

Pharmacokinetics of Antipyrine

After intravenous administration, the decay of antipyrine in plasma can be described by the sum of two exponentials, suggesting two-compartment kinetics (Greisen and Andreasen 1976). The oral systemic bioavailability of antipyrine is almost complete (Andreasen and Vesell 1974; Danhof et al. 1982a). After oral administration the absorption and distribution of antipyrine occur very rapidly compared with the elimination, and the clearance determined from postabsorption/distribution data according to a one-compartment model is not different from that determined from the area under the complete plasma concentration-time curve (Greisen and Andreasen 1976; Danhof et al. 1982a). The volume of distribution equals total body water and the protein binding is negligible (Brodie and Axelrod 1950; Soberman et al. 1949). Independently of the rate of secretion and pH, saliva holds concentrations of antipyrine close to plasma and can act as a substitute in kinetic studies (Welch et al. 1975; Vesell et al. 1975). The clearance of antipyrine is independent of liver blood flow and of the dose, at least within the common range, from 250 to 1000 mg (Ohnhaus et al. 1976; Danhof and



× excreted as glucuronide or sulfate.

● partly excreted as conjugates.

Fig. 1. Fate of antipyrine in man

Breimer 1979). In the isolated perfused pig liver, saturation kinetics has been demonstrated at very high antipyrine concentrations (Andreasen et al. 1977).

Antipyrine is almost completely metabolized by hepatic cytochrome P-450 enzymes, leaving only a few percent to be excreted unchanged into urine (Brodie and Axelrod 1950). About 99% of radiolabeled doses of antipyrine has been recovered in urine (Böttcher et al. 1981; Uchino et al. 1983). The excretion into urine of unchanged antipyrine and the three major metabolites (Fig. 1) — the *N*-demethylated (NORA), the 4-hydroxylated (OHA), and the 3-methylhydroxylated (HMA) — account for 65%–70% of the dose (Danhof et al. 1979a, 1982a, c). Minor metabolites are 3-carboxy antipyrine formed from HMA, 4'-hydroxy-antipyrine, and 4,4'-dihydroxyantipyrine (DOHA), each accounting for 5% of the dose or less in man (Danhof et al. 1979a, b, 1982a, b; Inaba et al. 1981; Böttcher et al. 1981). In the rat, the excretion of DOHA accounts for about 15% of the dose administered (Danhof et al. 1979a, b; Böttcher et al. 1981). Other possible metabolic pathways include 1-phenyl-4,5-dioxypyrazoline, formed from NORA, and epoxidation may also take place (Zietz et al. 1978; Eichelbaum et al. 1981). All the 4-hydroxylated metabolites and NORA are almost completely conjugated to glucuronic acid in man, leaving a few percent to be excreted as sulphates or free metabolites (Danhof et al. 1979 a, b; Böttcher et al. 1982). In the rat, sulphate conjugates predominate (Böttcher et al. 1982). About half of the excreted amount of HMA is conjugated to glucuronic acid, whereas the other half and carboxy-antipyrine are excreted as free metabolites (Danhof et al. 1982a–c). HMA may to some extent be subject to enterohepatic recirculation (Aarbakke 1978). The excretion into urine of the three major metabolites of antipyrine has been shown to reflect their rate of formation (Boobis et al. 1981; Teunissen et al. 1985). There is increasing evidence that the formation of each of the three main antipyrine metabolites is dependent on one or more selective form(s) of cytochrome P-450. The evidence is based mainly on the differential changes in the rates of metabolite formation observed in man and rat after administration of some enzyme-inducing or -inhibiting agents (Danhof et al. 1979b, 1982b; Toverud et al. 1981; Teunissen et al. 1985). Accordingly, the saliva or plasma clearance of antipyrine reflects the aggregate activity of at least three different hepatic cytochrome P-450 drug-metabolizing isozymes. By measuring the excretion of metabolites into urine, the activity of each isozyme can be assessed.

Determination of Antipyrine Using a Single Time-Concentration Point

Since antipyrine elimination follows first-order kinetics and can be described by a one-compartment model, the minimum requirement for information to estimate antipyrine clearance (APC) is the elimination constant (k) and the volume of distribution (Vd). In order to estimate k , two time-plasma concentration points are needed. Suppose that Vd is given from body weight, height, age, and sex, and that the dose of antipyrine is D , then the theoretical plasma concentration at time $t=0$ is D/Vd and can be used as one point. If the plasma antipyrine concentration C_T is determined at a time $t=T$, then the elimination constant is given by $[\ln(D/Vd) - \ln C_T]/T$ and APC can be calculated

$$APC = Vd \cdot [\ln(D/Vd) - \ln C_T] / T$$

Intuitively, the longer the time interval T between dosing and sampling, the smaller the variation of the determination. From mathematical-statistical considerations

(Døssing et al. 1983) it can be shown that the optimal time for taking the one sample is

$$t = (1 + \sigma^2/\omega^2)/k$$

where σ^2/ω^2 is the ratio between the variation of the concentration determination and the variation of Vd . Calculated this way, the optimal time is about 24 h (Døssing et al. 1983b), which is in agreement with our empirical estimate from a study of 142 persons (Døssing et al. 1982). It should be noted that a dramatic increase in the variance of the estimate occurs when one sample is taken too early, whereas only a slight increase occurs when the sample is taken late (Døssing et al. 1983), and that bias on the Vd has a relatively small influence (Pilsgaard and Poulsen 1984).

The one-sample method can be used as a noninvasive test if saliva is collected instead of plasma, since the concentrations are almost identical (Welch et al. 1975; Vesel et al. 1975).

In our experience, patients and volunteers can perform the test at home, write the necessary information on a data sheet, and mail the saliva sample for analysis if they have performed the test once under supervision. The concentration of antipyrine in saliva is constant for 14 days at room temperature. The saliva sample is easily obtained if a piece of Para-Film is chewed; in our laboratory, conventional chewing gum gives interfering peaks when the saliva is analyzed by HPLC.

The one-sample method only gives information as to clearance. If another parameter is to be estimated, e.g., Vd , or if large changes in Vd are expected, conventional multiple sampling of plasma or saliva is preferred.

We have also determined the optimal method for multiple sampling in order to minimize the variation of the clearance determination (Døssing et al. 1983): the average sampling time should equal $1/k$. If the half-life of antipyrine is about 12 h, the corresponding optimum sampling time is about 17 h. Preferentially, the sampling times should be spaced equally around the optimum time.

Until recently, many investigators collected the samples for determination of antipyrine kinetics within 12 h after administration (Ballinger et al. 1972; Døssing and Andreassen 1981; Elin et al. 1975; Vessel and Page 1968; Uppal et al. 1980). In such cases the variation coefficient of antipyrine clearance will be at least 8%. If the half-life is 24 h and the samples are taken within 12 h the coefficient of variation will be at least 20%. If the samples are collected optimally, i.e., spaced around $1/k$, which is 17.3 h in the first example and 34.6 h in the second, then the coefficient of variation of antipyrine clearance is reduced, at best, to 2.5%.

Assuming that Vd changes very little in repeated measurements in a subject under stable environmental conditions, then one can calculate the number of samples needed to estimate antipyrine clearance by the conventional method for comparison with the one-sample method (Døssing et al. 1983). Quite surprisingly, 26 blood or saliva samples are required. One might doubt the one-sample method, but in our experience it is useful when studying adults (Døssing et al. 1983; Loft et al. 1986), children (Loft et al. 1985), and animals (Pilsgaard and Poulsen 1984; Poulsen and Pilsgaard 1985; Poulsen 1985; Hansen and Poulsen 1986a, b).

Quantitative Assessment of Liver Function by Antipyrine Clearance

Liver disease causes a sequential loss of hepatic function. It has been put forward that the "functional hepatic mass" is reduced as the disease progresses (Tygstrup and Vilstrup 1983) until a minimal residual function is reached incompatible with survival.

Eliminated almost exclusively by hepatic metabolism, antipyrine is an ideal model drug to investigate hepatic function. As intrinsic clearance is a measure of drug-metabolizing enzyme activity, this parameter is the most attractive for estimating hepatic function (Rowland 1972; Andreassen et al. 1974).

Quantitative assessment of liver function by antipyrine clearance is not of much diagnostic value, especially in the initial stage of the disease. According to the concept of functional hepatic mass, quantitative assessment can estimate (1) severity of disease, (2) prognosis, (3) risk, e.g., exposure to hepatotoxins, (4) time course, (5) need for treatment, and (6) results of therapy.

The prognostic value of antipyrine clearance is well established in the case of acute viral or toxic hepatitis (Andreassen and Ranek 1975; Ramsøe et al. 1980; Bremmelgård et al. 1983). The minimal residual function is about 10%–20% of control value, which is consistent with animal experiments, where 90% reduction in antipyrine clearance from subtotal hepatectomy is associated with mortality of about 10% (Poulsen 1985).

In patients with alcoholic liver cirrhosis, antipyrine clearance is reduced (Andreassen et al. 1974) compared with other measures of functional liver mass (Vilstrup 1980). As such, antipyrine is considered to be predictive for survival of patients with liver cirrhosis; however, completed long-term studies are still lacking, though some are in progress.

It remains to be established whether an estimate of the individual metabolic pathways in patients with liver diseases is more informative than antipyrine clearance per se.

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