Comparative Pharmacokinetics of Butylated Hydroxyanisole and Butylated Hydroxytoluene in Rabbits

RAGAB EL-RASHIDY * **and SARFARAZ NIAZI** ˣ

Received November 19, 1979, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680.* Accepted for publication June 16, 1980. *Present address: G. D. Searle & Company, Skokie, IL 60076.

Abstract □ The widespread use of butylated hydroxyanisole (I) and butylated hydroxytoluene (II) as food antioxidants recently has been criticized by the Food and Drug Administration because of their pharmacological and toxicological effects. Interest also has arisen recently in the use of these compounds as anticancer agents. The purposes of this study were to evaluate the pharmacokinetics of I and II in rabbits and to compare their physicochemical properties with their disposition kinetics. It was found that I has a disposition half-life of ~1 hr, compared to 11 days for II. These differences are explained in terms of their lipid solubility and protein binding characteristics.

Keyphrases □ Butylated hydroxyanisole—pharmacokinetics, rabbits □ Butylated hydroxytoluene—pharmacokinetics, rabbits □ Antioxidants—butylated hydroxyanisole and butylated hydroxytoluene, pharmacokinetics, rabbits □ Pharmacokinetics—butylated hydroxyanisole and butylated hydroxytoluene, rabbits

Butylated hydroxyanisole (I) and butylated hydroxytoluene (II) are the most commonly used food antioxidants in the United States (1). Although I and II generally are regarded as safe, recent actions by the Food and Drug Administration have been directed toward restricting their use (2) because of their pharmacological and toxicological effects (3–7). Moreover, these compounds recently were reported to possess anticancer characteristics (8, 9). Although no specific biochemical pathways have been suggested for their action in inhibiting chemical carcinogenesis, these mechanisms may involve alteration of the metabolism of the chemical carcinogens (10) and blocking of hydrocarbon free radical–DNA complex formation (11).

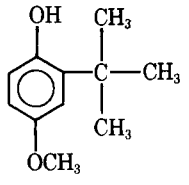
Despite widespread interest in I and II, whose structures differ only by a *tert*-butyl group, there have been no reports of their pharmacokinetic properties based on blood levels and no reports of their effectiveness and toxicity. The purposes of this study were to monitor the disposition kinetics of I and II following single intravenous dosing in rabbits and to relate their pharmacokinetic parameters to their physicochemical properties.

EXPERIMENTAL

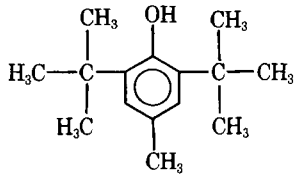
Materials and Equipment—Butylated hydroxyanisole[1] (I), butylated hydroxytoluene[1] (II), dibutylated hydroxyanisole[2], polyethylene glycol 400[3], an infusion set[4], a catheter placement unit[5] (20 gauge), sterile disposable syringes[6], an infusion pump[7], and a gas chromatograph[8] were used.

Formulations of Intravenous Dosage Form—Solutions of I and II (10 mg/ml) were prepared in polyethylene glycol 400–normal saline (1:1), which was sterilized by heating at 100° for ~1 hr over a hot plate.

[1] Nutritional Biochemical Co., Cleveland, Ohio.
[2] Universal Oil Product Co., Des Plaines, Ill.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Abbott Laboratories, North Chicago, Ill.
[5] Jelco Laboratories, Raritan, N.J.
[6] Becton, Dickinson and Co., Rutherford, N.J.
[7] Sage Instruments, Cambridge, Mass.
[8] Varian Aerograph model 1400, Varian, Park Ridge, Ill.



I



II

Animal Procedure—New Zealand White male rabbits, 2.9–3.2 kg, were used; food and water were withheld prior to and for 12 hr after drug administration. The marginal ear vein was cannulated using a 20-gauge catheter for sample collection. The I and II solutions were infused for <2 min at a constant rate of 2 ml/min to provide a total dose of 10 mg/kg. At the end of infusion, the cannula was flushed with 5 ml of heparinized sterile normal saline.

The blood samples (1.5 ml) were collected from the catheter placed in the other ear at 0, 0.085, 0.173, 0.33, 0.50, 0.75, 1, 1.5, 2.0, 3.0, 4.0, 6.0, and 12 hr and thereafter twice per day for 2 days and daily for 3 days. The zero time for sampling was assigned when the infusion was completed. A blank blood sample was obtained before the solution was infused to obtain the baseline on the gas chromatogram.

Blood Analysis—All blood samples were analyzed for their concentration of I and II using a highly sensitive and specific GLC method (12).

RESULTS AND DISCUSSION

The absorption of I and II was reported to vary significantly when given orally as a suspension in milk–olive oil and as a gelatin capsule to humans (13). Therefore, an intravenous dosage form was chosen to avoid complications due to absorption variability. The maximum allowable intake of I and II is 2 mg/kg/day, but there are indications that the per capita daily intake is >5 mg/kg (2). Thus, the dose of 10 mg/kg used in this study may represent the level of exposure for numerous people who consume many processed foods.

Pharmacokinetics of Butylated Hydroxyanisole (I)—The plasma concentrations obtained at different time intervals following a single intravenous dose (10 mg/kg) were plotted on a semilogarithmic scale, assigning time zero as the midpoint of infusion, and the data were fitted by a monoexponential model using a programmable calculator (14) (Fig. 1). This model suggests that the body behaves as a homogeneous compartment for I in terms of the equilibrium rates among various body tissues (15):

$$C_p = C_p^0 e^{-kt} \quad \text{(Eq. 1)}$$

It was reported that at low concentration (<100 μg/ml), the percent of

Table I—Pharmacokinetic Parameters of I in Rabbits after a Single Intravenous 10-mg/kg Dose

Parameter	Mean ± *SEM* (n = 4)
Body weight, kg	2.98 ± 0.20
C_p^0, mg/liter	1.298 ± 0.095
k_{el}, hr^{-1}	0.7427 ± 0.14
$t_{1/2}$, hr	1.055 ± 0.23
V_d, liters/kg	7.48 ± 0.64
TBC^a, liters/hr/kg	5.24 ± 0.50
AUC^b, (mg hr)/liter/kg	0.685 ± 0.13

[a] Total body clearance. [b] Area under the curve.

0022-3549/80/1200-1455$01.00/0

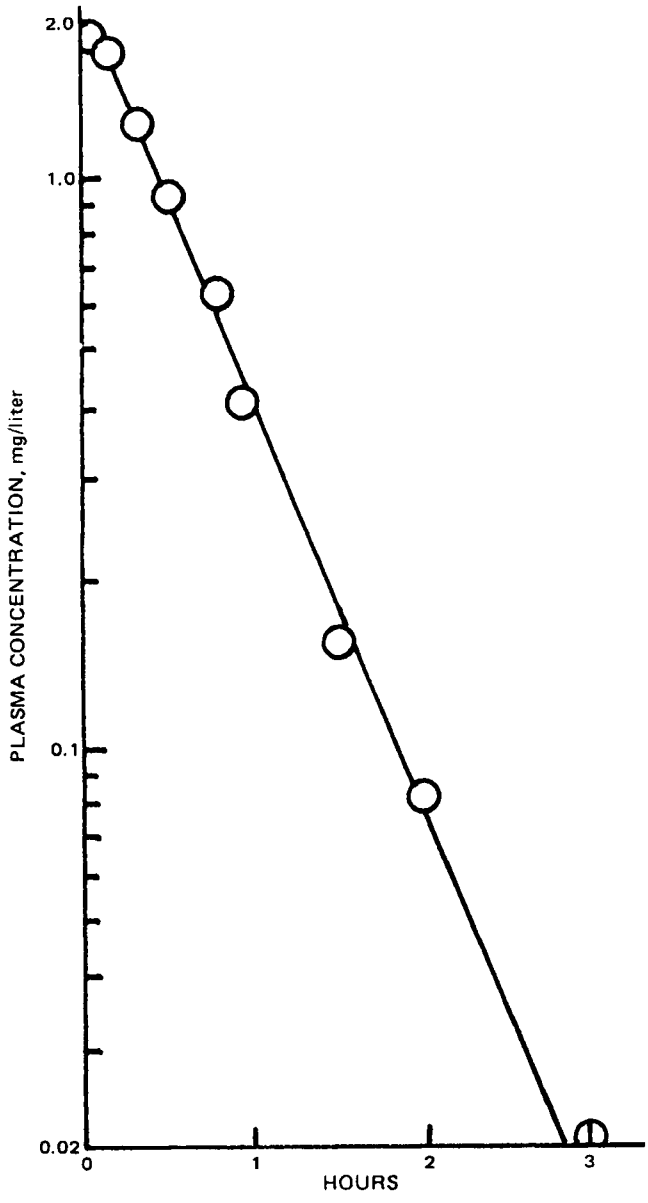


Figure 1—*Plasma concentration profile of I in a rabbit following intravenous administration of a 10-mg/kg dose.*

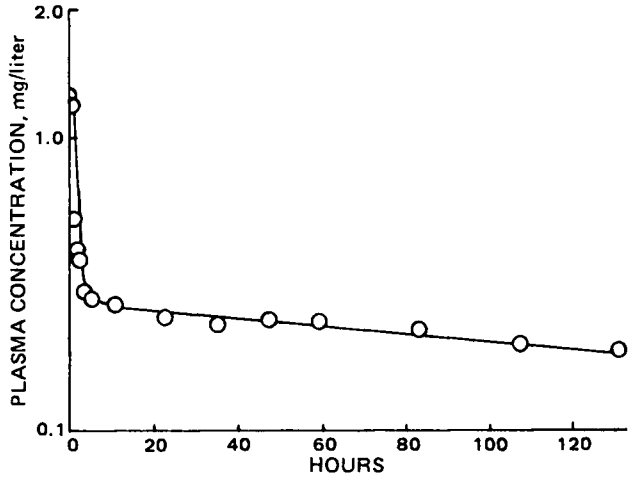


Figure 2—*Plasma concentration profile of II in a rabbit following intravenous administration of a 10-mg/kg dose.*

total I bound to human serum albumin was inversely proportional to the total concentration (16). Since >59% of the total exchangeable albumin is located in the extravascular compartments such as the skin and muscles (17), the human body can be conceived of as a large pool for I. In addition, the probable binding of I to other components of different tissues and fluids makes its distribution more extensive throughout the body, causing exposure of deep body tissues.

The estimates of C_p^0, $t_{1/2}$, V_d, and k_{el} reported in Table I were obtained from least-squares data fitting. In spite of the large volume of distribution (mean = 7.48 liters/kg), I disappears from the plasma very rapidly (mean $t_{1/2}$ = 1 hr). Thus, despite extensive distribution, fast clearance is achieved due to the highly reversible nature of its storage in the body tissues.

Pharmacokinetics of Butylated Hydroxytoluene (II) (Fig. 2)—The disposition of II in animals and in humans is complicated by the presence of two *tert*-butyl groups at *ortho*-positions of the phenolic group. More than 10 metabolites of II have been isolated (18, 19). Significant biliary excretion also has been reported in rats, rabbits, and dogs. Daniel *et al.* (5) recovered ~67% of an oral dose of [^{14}C]II in human urine samples collected over an 11-day period. They concluded that intact II and/or its metabolites first are stored in the tissues and then are slowly released after a pseudoequilibrium, which takes ~5 days, indicating extensive uptake by tissues (5).

The plasma concentration–time profiles of II were, unlike I, characterized by:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 2)}$$

where A and B are constants and α and β are complex rate constants. The estimation of A, B, α, and β was made using a programmable calculator (14). The pharmacokinetic parameters are reported in Table II. The fast disposition phase half-life of II was ~1 hr, and the slow disposition phase decayed with a half-life of >11 days. These data suggest rapid accumulation and slow clearance from the body, in agreement with earlier speculations for II (5).

A comparison of the disposition of I and II in rabbits clearly reveals large differences between their distribution and elimination characteristics. Whereas the accumulation of I does not appear to be a serious problem, II tends to be stored in body tissues upon multiple dosing, as encountered in daily life exposure to this compound. Based on the following equation, more than a 16-fold accumulation of II is possible on daily exposure whereas the accumulation is negligible for I:

$$\text{accumulation} = 1/1 - e^{-k\mathrm{T}} \qquad \text{(Eq. 3)}$$

where k is the terminal disposition rate constant and T is the frequency of exposure.

Such large differences in the *in vivo* kinetic properties of I and II occur because of the additional *tert*-butyl group in II. This structural change renders II more lipid soluble, explaining the approximately threefold increase in the final volume of distribution while the volume of the central compartment remains identical for both compounds. Since the volume of the central compartment does not change, an increase of over 100-fold in the total body clearance results from a change in the elimination rate constant from the central compartment (k_{el}).

The distribution characteristics of I and II also can be related to their plasma protein binding characteristics. The volumes of distribution often are inversely related to the extent of plasma protein binding (17), especially for compounds that have weaker association constants, such as I

Table II—Pharmacokinetic Parameters of II in Rabbits after a Single Intravenous 10-mg/kg Dose

Parameter[a]	Mean ± *SEM* (n = 4)
A, mg/liters	1.0402 ± 0.26
B, mg/liters	0.4535 ± 0.025
α, hr^{-1}	0.7841 ± 0.073
β, hr^{-1}	0.00258 ± 0.0008
k_{21}, hr^{-1}	0.2639 ± 0.06
k_{10}, hr^{-1}	0.00760 ± 0.0013
k_{12}, hr^{-1}	0.5152 ± 0.057
V_d, liters/kg	7.31 ± 1.18
TBC[b], liters/hr/kg	0.0551 ± 0.013
V_{deg}, liters/kg	21.13 ± 1.29
AUC[c], (mg hr)/liter/kg	208.9 ± 35.5

[a] $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where t is in hours and C_p is in milligrams per liter. [b] Total body clearance. [c] Area under the curve.

and II (16, 18). The binding constant for I reported from this laboratory was $2 \times 10^{-3} M^{-1}$ and that for II was $3 \times 10^{4} M^{-1}$. The number of binding sites was ~2.6 for I and 0.25 for II (20). Thus, increased lipid solubility of II results in its increased strength of interaction with albumin molecules, but the steric hindrance due to the additional *tert*-butyl group makes the extent of binding only about 10% of that observed for I. Thus, the total exchangeable albumin present in the extravascular compartments provides a large pool for possible storage of I but not for II, which probably is taken up by the body fatty tissues due to its higher lipid solubility (5).

REFERENCES

(1) WHO Technical Report Series, No. 488, 1971.

(2) *Fed. Regist.*, 42(104, May 31) (1977).

(3) P. H. Grantham, J. H. Weisburger, and E. K. Weisburger, *Food Cosmet. Toxicol.*, **11**, 209 (1973).

(4) R. Tye, J. D. Engel, and I. Rapien, *ibid.*, **3**, 547 (1965).

(5) J. W. Daniel, J. C. Gage, D. J. Jones, and M. A. Stevens, *ibid.*, **5**, 475 (1967).

(6) W. Snipes, S. Person, A. Keith, and J. Cupp, *Science*, **188**, 64 (1975).

(7) I. F. Gaunt, D. Glibert, and D. Martin, *Food Cosmet. Toxicol.*, **3**, 445 (1965).

(8) T. J. Slaga and W. J. Brecken, *Cancer Res.*, **37**, 1631 (1977).

(9) L. K. T. Lam and L. W. Wattenberg, *J. Natl. Cancer Inst.*, **58**, 413 (1977).

(10) P. A. Craven and F. R. DeRubertis, *Cancer Res.*, **37**, 4088 (1977).

(11) E. Krzywansaka and L. Pierkarski, *Neoplasma*, **24**, 4 (1977).

(12) R. El-Rashidy and S. Niazi, *J. Pharm. Sci.*, **68**, 103 (1979).

(13) B. D. Astill, J. Mills, D. W. Fassett, R. L. Roudabush, and J. Terhaar, *Ag. Food Chem.*, **10**, 315 (1962).

(14) S. Niazi, *Int. J. Biomed. Comput.*, **10**, 245 (1979).

(15) S. Niazi, "Textbook of Biopharmaceutics and Clinical Pharmacokinetics," Appleton-Century-Crofts, New York, N.Y., 1979.

(16) R. El-Rashidy and S. Niazi, *J. Pharm. Sci.*, **67**, 967 (1978).

(17) W. J. Jusko and M. Gretch, *Metab. Rev.*, **5**, 43 (1976).

(18) M. Akagi and L. Aogi, *Chem. Pharm. Bull.*, **10**, 161 (1962).

(19) *Ibid.*, **10**, 200 (1962).

(20) R. El-Rashidy, Ph.D. dissertation, University of Illinois at the Medical Center, Chicago, Ill., 1979.

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by R. El-Rashidy to the University of Illinois in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by a grant from the Graduate College, University of Illinois at the Medical Center.

Human Whole Blood and Parotid Saliva Concentrations of Oral and Intramuscular Promethazine

G. JOHN DiGREGORIO ˣ and EILEEN RUCH

Received February 13, 1980, from the *Department of Pharmacology, Hahnemann Medical College, Philadelphia, PA 19102.* Accepted for publication June 20, 1980.

Abstract □ Ten healthy male subjects received 25 mg of promethazine intramuscularly, followed within 3 weeks by oral administration of 25 mg. Whole blood and parotid saliva were collected over a 12-hr period after drug administration. Promethazine was quantitated by high-performance liquid chromatography. After intramuscular administration, the promethazine concentrations were 3.0–22.4 ng/ml in blood and 0.9–2.8 ng/ml in parotid saliva. After oral administration, the promethazine concentrations were 1.4–5.5 ng/ml in blood and 0.2–0.8 ng/ml in parotid saliva. The peak blood promethazine concentration after the intramuscular dose was four times higher than that following the oral dose, which indicates that promethazine possesses an extensive first-pass effect. The mean parotid saliva to whole blood ratio (S/B) was calculated to be 0.24 after the intramuscular route and 0.20 after the oral route over the 12-hr period. The calculated percentage of free drug in the blood was 20–24% of the whole blood concentration determined from the S/B ratio.

Keyphrases □ Promethazine—whole blood and parotid saliva concentrations following oral and intramuscular administration □ High-performance liquid chromatography—analysis, promethazine, whole blood and parotid saliva concentrations following oral and intramuscular administration □ Phenothiazines—promethazine, high-performance liquid chromatographic analysis in whole blood and parotid saliva following oral and intramuscular administration

Several investigators have reported analytical methods for the determination of phenothiazines, including colorimetric (1), spectrofluorometric (2), spectrophotometric (3), GLC (4, 5), and liquid chromatographic (6) procedures. However, little or no information is available on the quantitation of promethazine in therapeutic doses in human whole blood or parotid saliva.

This report presents a sensitive method for the detection and quantitation of promethazine in whole blood and parotid saliva after the administration of 25 mg of promethazine orally and intramuscularly in human volunteers. Parotid saliva to blood promethazine concentration ratios were calculated, and the bioavailability of promethazine was compared for the two routes of administration.

EXPERIMENTAL

Materials—Powdered promethazine[1] and promazine[1], the internal standard, were used without further purification as the analytical standards. Commercially available promethazine tablets[1] and injectable liquid[1] were obtained from a local pharmacy. All solvents and reagents were analytical reagent grade, except for methanol[2], which was high-performance liquid chromatographic (HPLC) grade. All glassware was coated with silicone[3] by the method described on the package insert.

Apparatus—The liquid chromatograph[4] was fitted with a loop injector and a fixed-wavelength UV detector (254 nm). A 1-mv recorder[5] was attached to the chromatograph. The detector was attenuated at 0.005 aufs.

Column—A prepacked, 300 × 3.9-mm i.d., μBondapak CN column[6] was run at ambient temperature.

[1] Wyeth Laboratories, Philadelphia, Pa.
[2] Fischer Chemical Co., King of Prussia, Pa.
[3] Siliclad, Clay Adams, New York, N.Y.
[4] Model 200, Waters Associates, Milford, Mass.
[5] Perkin-Elmer Corp., Norwalk, Conn.
[6] Waters Associates, Milford, Mass.

0022-3549/80/1200-1457$01.00/0