The high temperatures used in the GLC method may lead to thermal degradation of cholesterol, especially since the 3β-hydroxyl group is not derivatized. However, no evidence for thermal degradation was observed in this study. A saturable adsorptive phenomenon for cholesterol in the column was not observed. Others reported this to be a problem (2, 6, 9). If loss on the column occurs in the method reported here, it does not manifest itself in poor accuracy or precision (Fig. 2 and Table I).

A technician may, starting with whole blood, obtain the results for total cholesterol on at least 40 samples in a working day. This assumes that manual techniques are utilized and only one column of a gas chromatograph is available. One column in these laboratories has been satisfactorily utilized for more than 1 year under the conditions specified.

REFERENCES

(1) D. B. Tonks, *Clin. Biochem.*, **1**, 12(1967).

(2) J. P. Blomhoff, *Clin. Chim. Acta*, **43**, 257(1973).

(3) D. B. McDougal, Jr., and H. S. Farmer, *J. Lab. Clin. Med.*, **50**, 485(1957).

(4) R. W. Albers and O. H. Lowry, *Anal. Chem.*, **27**, 1829(1955).

(5) F. Tishler and J. N. Bathish, *J. Pharm. Sci.*, **54**, 1786(1965).

(6) L. P. Cawley, B. O. Musser, S. Campbell, and W. Faucette, *Amer. J. Clin. Pathol.*, **39**, 450(1963).

(7) Von H.-C. Curtius and W. Bürgi, *Z. Klin. Chem.*, **4**, 38(1966).

(8) B. A. Vela and H. F. Acevedo, *Steroids*, **14**, 499(1969).

(9) J. L. Driscoll, D. Aubuchon, M. Descoteaux, and H. F. Martin, *Anal. Chem.*, **43**, 1196(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 9, 1973, from the *Sections of Clinical Pharmacology and Pharmacokinetics, Schools of Pharmacy and Medicine, University of Southern California, Los Angeles, CA 90033*

Accepted for publication January 18, 1974.

Supported by U.S. Public Health Service Grant HE-13620.

[x] To whom inquiries should be directed.

Correlation of Plasma Ibuprofen Levels with Biological Activity

DAVID G. KAISER [x] and E. MYLES GLENN

Abstract □ Investigations with ibuprofen [(±)-*p*-isobutylhydratropic acid], a well-tolerated, orally active, anti-inflammatory drug, were undertaken to: *(a)* determine the relationship among plasma drug concentrations, administered dose, and anti-inflammatory activity in developing and established polyarthritis in rats; and *(b)* compare the plasma drug disappearance half-lives in normal and polyarthritic rats. The results indicated that plasma drug concentrations in normal and polyarthritic rats were dose related. The logarithm of biological activity expressed as [% IPA/(100 − % IPA)], where % IPA is the mean percent inhibition of developing or established polyarthritis as measured by plasma inflammation units, was related to the logarithms of: *(a)* administered dose (mg kg^{-1}), *(b)* plasma drug concentrations (μg ml^{-1}) at 2 hr postadministration, and *(c)* average plasma drug concentrations (μg ml^{-1}) in a dosage interval at the equilibrium state. Half-lives for elimination of ibuprofen from the plasma of normal rats (after single-dose oral drug administration) and polyarthritic rats (after 29 doses) were essentially identical.

Keyphrases □ Ibuprofen—plasma levels correlated with biological activity □ Biological activity—correlated with ibuprofen plasma levels □ Anti-inflammatory agents—ibuprofen plasma levels correlated with biological activity

Ibuprofen[1] [(±)-*p*-isobutylhydratropic acid] (I) is a well-tolerated, orally active, anti-inflammatory agent utilized for the treatment of rheumatoid arthritis (1–3). The pharmacology, toxicology, and aspects of the absorption, distribution, and metabolism of I were reported previously (4–6). Recently, a sensitive and specific GLC procedure was described (7) for the determination of I in plasma. The present studies were conducted to: *(a)* determine the relationships among plasma I concentrations, orally administered dose, and anti-inflammatory activity in developing and established polyarthritis in rats; and *(b)* compare the elimination half-lives of I after single-dose administration to normal rats and after multiple-dose administration to rats with established polyarthritis.

EXPERIMENTAL

Rats with Developing Polyarthritis—Sixty Badger male rats (~235 g) were made polyarthritic by the intradermal injection into the tail of 0.5 mg of dead *Mycobacterium butyricum*[2] in 0.1 ml of mineral oil on Day 0. On Day 1, the animals were divided into six groups of 10 each. The groups received 0, 4.2, 9.0, 17.2, 33.6, or 67.2 mg I/kg, respectively, as a 1-ml aqueous suspension orally, bid for 14 days. On Day 15 all animals were weighed and scored. Five rats from each group received an additional (29th) oral dose of I, 2 hr prior to sacrifice. All animals were exsanguinated, and plasma specimens were obtained for the measurement of plasma inflammation units. Plasma aliquots from the animals receiving the 29th dose were stored at −18° until analyzed for I.

Rats with Established Polyarthritis—A series (180) of Badger male rats (~230 g) was made polyarthritic as described. On Day 14, the animals were visually scored, weighed, and divided into four groups of 45 each. Beginning on Day 15, the groups received 0, 16.4, 34.6, or 72.4 mg I/kg, respectively, as a 1-ml aqueous suspension orally, bid for 14 days. After 28 doses (Day 28), animals were visually scored and weighed. Beginning with the 29th dose (Day 29), two to five rats per group were sacrificed and exsanguinated at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hr. Plasma specimens were

[1] Motrin, The Upjohn Co.; Brufen, The Boots Co. Ltd.

[2] Difco.

Table I—Plasma Inflammation Units and Plasma I Concentrations in Rats with Developing Adjuvant-Induced Polyarthritis 2 hr after the Last Dose of a Multiple-Dose Regimen

Dose of I, Orally, bid, mg kg^{-1}	Plasma Inflammation Units[a]	Percent IPA[b]	Plasma I Concentrations[c], μg ml^{-1}
0	60.0 ± 4.6	0	0
4.2	46.0 ± 3.0	23	3.50 ± 0.27
9.0	46.0 ± 2.8	23	9.81 ± 0.95
17.2	32.0 ± 3.7	47	17.02 ± 3.11
33.6	27.0 ± 2.4	55	29.36 ± 5.61
67.2	21.0 ± 4.0	65	50.27 ± 10.56

[a] Mean ± *SEM* for 10 rats per group. [b] Percent inhibition of polyarthritis. [c] Mean ± *SEM* for five rats per group.

obtained for the measurement of plasma inflammation units and the remaining portions were stored at −18°.

Normal Rats—Forty-five Carworth male rats (~255 g), housed in screen-bottomed holding cages, were fasted for 16 hr prior to oral administration of 19.6 mg I/kg as a 1-ml aqueous suspension. Animals were fasted an additional 4 hr, after which food was returned *ad libitum*. Groups of five rats each were sacrificed and exsanguinated at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hr. Plasma specimens were collected and stored at −18°.

Determination of Plasma Inflammation Units—The procedure for the determination of plasma inflammation units in the adjuvant-induced polyarthritic rat described previously (8) was employed.

Determination of Plasma I Concentrations—The GLC procedure for the determination of I in plasma reported previously (7) was used.

RESULTS AND DISCUSSION

Observed plasma inflammation units and plasma I concentrations 2 hr after the last (29th) dose in rats with developing polyarthritis are summarized in Table I. Table II shows observed plasma inflammation units and plasma I concentrations over the 0–24-hr interval after the last (29th) dose in rats with established polyarthritis, as well as plasma I concentrations after single-dose administration in normal rats.

Figure 1 indicates that both the 2-hr plasma I concentrations and the mean plasma I concentrations over the 0–12-hr dosage interval were linearly related to dose. Similar observations were reported previously for the anti-inflammatory agents indoxole (9) and 4,5-bis(*p*-methoxyphenyl)-2-phenylpyrrole-3-acetonitrile (10) in polyarthritic rats. In the present study, plasma I concentrations in developing and established polyarthritic rats after multiple-dose administration were not different from those in normal rats after a single dose (Fig. 1), indicating no effect of the polyarthritis or of the chronic dosage regimen on the pharmacokinetics of I *(vide infra)*.

Earlier studies with indoxole (9) and 4,5-bis(*p*-methoxyphenyl)-2-phenylpyrrole-3-acetonitrile (10) showed that inhibition of polyarthritis in chronically treated rats was linearly related to log dose and log mean circulating drug levels over the dosage interval. Similar treatment of the data for I (Tables I and II), covering a wide range of doses and plasma I concentrations, gave sigmoid curves. Wagner (11) suggested linearization of such data by the use of Eq. 1:

$$\log\left[\frac{\%\ \text{IPA}}{100 - \%\ \text{IPA}}\right] = s \log A + \log Q \qquad \text{(Eq. 1)}$$

where % IPA is percent inhibition of polyarthritis, A is dose or circulating drug concentration, and Q is [% IPA/(100 − % IPA)] at unit value of A. Linear least-squares regression analysis of the data from Tables I and II according to Eq. 1 is shown in Fig. 2. Separate analyses of the data from rats with developing and with established polyarthritis gave slopes and intercepts not significantly different, permitting the data to be pooled. From Fig. 2, it may be calculated that 50% inhibition of polyarthritis was produced by an oral dose of 28.3 mg kg^{-1} bid, by a 2-hr plasma I concentration at equilibrium of 21.7 μg ml^{-1}, and by a mean plasma I concentration over the dosage interval of 6.95 μg ml^{-1}. Of considerable interest is the fact that I was equipotent when given during the development of polyarthritis or following establishment of the polyarthritis.

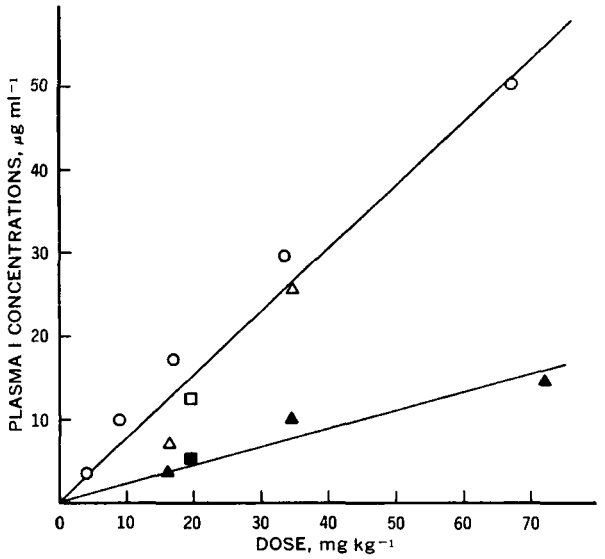

Figure 1—*Linear least-squares regressions of 2-hr plasma I concentrations (open symbols) and mean plasma I concentrations, 0–12 hr (closed symbols),* versus *dose. Key: ○, rats with developing polyarthritis; △,▲, rats with established polyarthritis; and □, ■, normal rats.*

Recent publications (12–16) have shown that marked reductions in liver microsomal drug-metabolizing enzyme systems occur during the development of adjuvant-induced polyarthritic lesions in rats. Quevauviller *et al.* (12) observed a prolongation of sleeping times after the administration of phenobarbital, pentobarbital, and thiopental. Morton and Chatfield (13) found that liver microsomal *N*-demethylase and $NADPH_2$-oxidase activities as well as P-450 levels were greatly reduced. The maximum tolerated oral dose of phenobarbital was lower than in normal rats. In addition, in rats with established polyarthritis, decreased amounts of urinary β-glucuronide and sulfate conjugates, and increased amounts of unconjugated drug were observed following

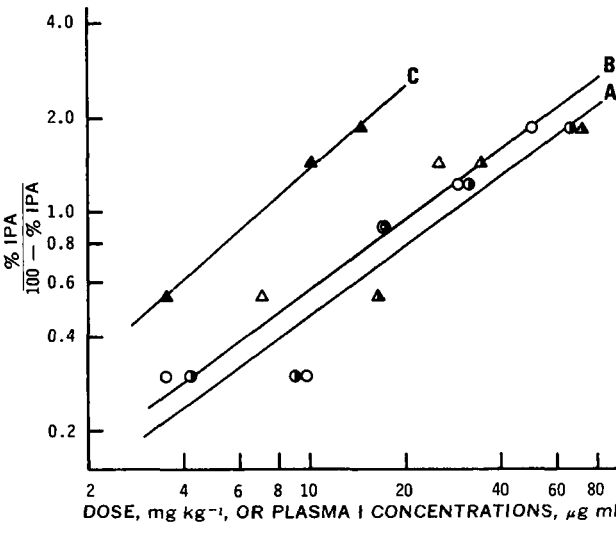

Figure 2—*Linear least-squares regressions of log [% IPA/(100 − % IPA)]* versus *log dose (mg kg^{-1}, hatched symbols, A), log 2-hr plasma I concentrations (μg ml^{-1}, open symbols, B), and log mean plasma I concentrations, 0–12 hr (μg ml^{-1}, closed symbols, C) after multiple-dose administration in rats with developing polyarthritis (circles) and with established polyarthritis (triangles).*

Table II—Plasma Inflammation Units and Plasma I Concentrations in Rats with Established Adjuvant-Induced Polyarthritis after the Last Dose of a Multiple-Dose Regimen and in Normal Rats after a Single Dose

	Polyarthritic Rats[a]			Normal Rats
Dose, mg kg^{-1}	16.4	34.6	72.4	19.6
Plasma Inflammation Units[b]	32.1 ± 3.4	20.1 ± 1.5	17.4 ± 2.4[c]	—
Percent IPA[d]	35	59	65	—
Hours	Plasma I Concentrations, μg ml^{-1}[e,f]			
0	0	0	1.88	0
1	14.99 ± 2.68	63.62 ± 16.50	49.20	30.88 ± 1.97
2	7.00 ± 1.07	25.51 ± 4.11	82.20	12.46 ± 1.94
3	5.19 ± 0.38	15.49 ± 4.06	15.08	5.96 ± 0.43
4	4.62 ± 1.20	5.48 ± 1.26	5.52	6.59 ± 1.55
6	2.03 ± 0.37	2.25 ± 0.60	6.24	1.35 ± 0.27
8	1.41 ± 0.79	1.15 ± 0.54	2.06	0.67 ± 0.17
12	0	0	0	0
24	0	0	0	0
$\int_0^{12} C\,dt$, μg ml^{-1} hr[g]	42.40	120.8	174.4	63.90
$\bar{C}$, μg ml^{-1}[h]	3.53	10.07	14.53	5.33
$T_{1/2}$, hr[i]	2.47	1.40	2.09	1.41

[a] I administered orally, bid for total of 29 doses. [b] Mean ± *SEM* for 45 rats per group except as noted. [c] Mean ± *SEM* for 17 surviving rats. At high dosage level (72.4 mg kg^{-1}), 28 of 45 animals died before Day 29. [d] Percent inhibition of polyarthritis. [e] Mean ± *SEM* for five rats per sampling except at highest dose where values are mean for two rats at each time. [f] Values <0.5 μg ml^{-1} are reported as 0. [g] Integrated area under the plasma I concentration curve over the 0–12-hr dosage interval calculated by the trapezoidal rule. [h] Mean plasma I concentration over the 0–12-hr interval. [i] Half-life for elimination of I from terminal portion (3–8 hr) of plasma concentration curves.

oral administration of acetaminophen (paracetamol). Zak *et al.* (14) observed a greatly prolonged hexobarbital sleeping time and reduced liver microsomal benzpyrene hydroxylase activity. These effects were reversed by treatment with the anti-inflammatory drugs, phenylbutazone, indomethacin, and flufenamic acid, which also improved the arthritic conditions of the animals. Treatment with phenobarbital reversed the enzymatic effects but did not affect the arthritis. Beck and Whitehouse (16) reported depressed demethylation of *N,N*-dimethylaniline or aminopyrine as well as impaired metabolism of cyclophosphamide.

To determine the possible effects of polyarthritis as well as multiple-dose administration of I on the rate of metabolism of I, drug elimination half-lives were determined from the data in Table II by linear least-squares regression analysis of the terminal (3–8-hr) portions of the log plasma I concentration *versus* time curves. Half-lives for elimination of I in normal rats and in polyarthritic rats after 29 doses were essentially identical. It may be concluded that if metabolism of I was inhibited by the adjuvant-induced arthritis, the inhibition was reversed by the anti-inflammatory effects of the drug. Multiple-dose administration of I did not appear to induce its own metabolism.

REFERENCES

(1) C. D. Brooks, F. R. Schmid, J. Biundo, S. Blau, R. Gonzalez-Alcover, J. D. C. Gowans, E. Hurd, R. E. H. Partridge, and E. L. Tarpley, *Rheumatol. Phys. Med. Suppl.*, **1970**, 48.

(2) E. F. Davies and G. S. Avery, *Drugs*, **2**, 416(1971).

(3) C. D. Brooks, C. A. Schlagel, N. C. Sekhar, and J. T. Sobota, *Curr. Ther. Res.*, **15**, 180(1973).

(4) S. S. Adams, K. F. McCullough, and J. S. Nicholson, *Arch. Int. Pharmacodyn. Ther.*, **178**, 115(1969).

(5) S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel, and R. F. N. Mills, *Toxicol. Appl. Pharmacol.*, **15**, 310(1969).

(6) S. S. Adams, R. G. Bough, E. E. Cliffe, W. Dickinson, B. Lessel, K. F. McCullough, R. F. N. Mills, J. S. Nicholson, and G. A. H. Williams, *Rheumatol. Phys. Med. Suppl.*, **1970**, 9.

(7) D. G. Kaiser and G. J. VanGiessen, *J. Pharm. Sci.*, **63**, 219(1974).

(8) E. M. Glenn and W. M. Kooyers, *Life Sci.*, **5**, 619(1966).

(9) D. G. Kaiser, E. M. Glenn, R. H. Johnson, and R. L. Johnston, *J. Pharmacol. Exp. Ther.*, **155**, 174(1967).

(10) D. G. Kaiser and E. M. Glenn, *J. Pharm. Sci.*, **61**, 1908(1972).

(11) J. G. Wagner, *J. Theoret. Biol.*, **20**, 173(1968).

(12) A. Quevauviller, M. A. Chalcat, H. Brouilhet, and F. Delbarre, *C. R. Soc. Biol. (Paris)*, **162**, 618(1968).

(13) D. M. Morton and D. H. Chatfield, *Biochem. Pharmacol.*, **19**, 473(1970).

(14) S. B. Zak, F. Honc, and G. Lukas, *Abstr. 5th Int. Congr. Pharmacol.*, **1972**, 259.

(15) M. W. Whitehouse and F. J. Beck, *Drug Metab. Disp.*, **1**, 251(1973).

(16) F. J. Beck and M. W. Whitehouse, *Biochem. Pharmacol.*, **22**, 2453(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 16, 1973, from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication January 17, 1974.

The authors thank B. J. Bowman, T. C. Koslowske, W. F. Liggett, S. C. Lyster, and N. A. Rohloff for excellent technical assistance and A. A. Forist for stimulating discussions.

[x] To whom inquiries should be directed.