exposure of humans to enzyme inducers such as polychlorinated biphenyls may lower the oral bioavailability of facile liver-metabolized drugs and require dosage adjustments.

The apparent agreement of the bioavailability estimation from intravenous or oral data with that determined experimentally from *AUC* values tends to support the validity of the clearance model in assessing systemic availability due to the first-pass effect for drugs exclusively metabolized by the liver. Estimation of the liver blood flow for the control and polychlorinated biphenyl-treated animals showed excellent agreement with literature values. This finding suggests that the blood flow rate on a liver weight basis does not change with induction but that the total hepatic blood flow may be affected by enzyme inducers.

REFERENCES

(1) J. Marniemi, M. Nokkala, H. Vainio, and K. J. W. Hartiala, *Chem. Biol. Interact.*, **18**, 247 (1974).

(2) J. V. Bruckner, K. L. Khanna, and H. H. Cornish, *Toxicol. Appl. Pharmacol.*, **28**, 189 (1974).

(3) K. Akiyama, G. Ohi, K. Fujitani, H. Yagyu, M. Ogino, and T. Kawana, *Bull. Environ. Contam. Toxicol.*, **14**, 588 (1975).

(4) J. V. Bruckner, K. L. Khanna, and H. H. Cornish, *Toxicol. Appl. Pharmacol.*, **24**, 434 (1973).

(5) R. W. Riseborough, P. Riche, D. B. Peakall, S. B. Herman, and M. N. Kirven, *Nature (London)*, **220**, 1098 (1968).

(6) A. P. Alvares and A. Kappas, *Clin. Pharmacol. Ther.*, **22**, 809 (1977).

(7) J. G. Babish and G. S. Stoewsand, *J. Toxicol. Environ. Health*, **3**, 673 (1977).

(8) J. A. Goldstein, P. Hickman, V. W. Burse, and H. Bergman, *Toxicol. Appl. Pharmacol.*, **32**, 461 (1975).

(9) G. J. Stone, D. J. Ecobichon, and O. Hutzinger, *ibid.*, **28**, 66 (1974).

(10) L. S. Birnbaum and M. B. Baird, *Exp. Gerontol.*, **13**, 469 (1978).

(11) J. R. Allen and L. J. Abrahamson, *Arch. Environ. Contam. Toxicol.*, **8**, 191 (1979).

(12) B. B. Brodie, J. J. Burns, L. C. Mard, and P. A. Lief, *J. Pharmacol. Exp. Ther.*, **109**, 26 (1953).

(13) A. H. Conney and J. J. Burns, *Science*, **178**, 576 (1972).

(14) F. W. Ossenberg, M. Peignoux, D. Bourdiau, and J. P. Benhamou, *J. Pharmacol. Exp. Ther.*, **194**, 111 (1975).

(15) V. J. Stella and C. K. Chu, *J. Pharm. Sci.*, **69**, 1274 (1980).

(16) C. K. Chu, V. J. Stella, J. V. Bruckner, and W. D. Jiang, *ibid.*, **66**, 238 (1977).

(17) M. Virolainen, *Transplantation*, **5**, 1530 (1967).

(18) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, p. 293.

(19) D. Perrier and M. Gibaldi, *J. Pharmacol. Exp. Ther.*, **191**, 17 (1974).

(20) D. M. Kornhauser, A. J. J. Wood, R. E. Vestal, G. R. Wilkinson, R. A. Branch, and D. G. Shand, *Clin. Pharmacol. Ther.*, **23**, 165 (1978).

(21) M. Gibaldi, R. N. Boyes, and S. Feldman, *J. Pharm. Sci.*, **60**, 1338 (1971).

(22) W. A. Colburn and M. Gibaldi, *Drug. Metab. Disp.*, **6**, 193 (1978).

(23) M. Rowland, *J. Pharm. Sci.*, **61**, 70 (1972).

(24) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).

(25) B. J. Winer, "Statistical Principles in Experimental Design," McGraw-Hill, New York, N.Y., 1962, pp. 89–91.

(26) A. Hume, M. Bush, J. Renick, and B. H. Douglas, *Arch. Int. Pharmacodyn. Ther.*, **171**, 122 (1968).

(27) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.*, **15**, 1883 (1967).

(28) *Ibid.*, **15**, 1705 (1967).

(29) K. S. Pang and J. R. Gillette, *Drug Metab. Disp.*, **6**, 567 (1978).

(30) J. Grayson and D. Mendel, "Physiology of the Sphlanchnic Circulation," Williams & Wilkins, Baltimore, Md., 1965, p. 74.

(31) R. Schulte-Hermann, *CRC Crit. Rev. Toxicol.*, **3**, 97 (1974).

ACKNOWLEDGMENTS

Supported by National Institutes of Health Grant GM 22357.

The authors thank Dr. J. V. Bruckner for the Aroclor 1254 and Dr. G. Schlager, Dr. Y. Yamaoka, and Dr. K. Yamaoka for help with the statistics.

Effects of Antibiotics on Platelet Functions in Human Plasma *In Vitro* and Dog Plasma *In Vivo*

M. I. GENUA *, J. GIRALDEZ *, E. ROCHA *x, and A. MONGE ‡

Received November 20, 1979, from the **Clínica Universitaria and the* ‡*Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain.* Accepted for publication May 1, 1980.

Abstract □ The effects of 31 antibiotics on platelet aggregation in human plasma in the presence of adenosine 5′-diphosphate were studied. The marked activity of tetracycline hydrochloride led to a study of its effects on various platelet functions *in vivo* in dogs.

Keyphrases □ Antibiotics—effects on platelet functions □ Platelet aggregation—effect of antibiotics

Many compounds, including some antibiotics (1–6), interfere with platelet activity under certain conditions. The present investigation was concerned with whether antibiotics in current clinical use affect platelet aggregation, either *in vitro* in human plasma or *in vivo* in dog plasma. When such an effect was found, the mechanism was studied; the investigation was broadened to include other platelet functions such as platelet adhesiveness, platelet factor 3 liberation, platelet mobility in an electric field, and the aggregation of platelets filtered in Sepharose gel.

EXPERIMENTAL

The *in vitro* experiments were carried out with plasma from apparently healthy subjects. The aggregation results correspond to the mean values of at least three trials.

The *in vivo* experiments were carried out on five dogs with similar physical characteristics. The results correspond to the mean values of three trials.

Plasma Samples—Human plasma samples were obtained by forearm venipuncture. The blood was collected in silanized tubes containing 3.8% sodium citrate (1:9) to obtain both platelet-rich plasma and platelet-poor plasma for the aggregation studies.

Dog plasma samples were obtained in a similar manner from mongrel dogs weighing 16–20 kg.

Platelet-rich plasma was obtained by sedimentation or centrifugation of samples at 800 rpm for 10 min for human plasma and for 8 min for dog

0022-3549/80/1100-1282$01.00/0

Table I—Percent Inhibition of Human Platelet Aggregation Induced by Adenosine 5′-Diphosphate *In Vitro*[a]

Compound	*a*, mg/ml	*A*	*b*, mg/ml	*B*
Penicillin G sodium	12	70	0.12	10
Penicillin G potassium	8	95	0.08	10
Penicillin G benzathine	30	100	0.3	10
Cloxacillin sodium	14.28	80	0.14	0
Ampicillin sodium	25	100	0.25	0
Metampicillin sodium	16.6	100	0.16	0
Carbenicillin disodium	28.5	90	0.28	0
Cephalothin sodium	25	100	0.25	0
Cephaloridine	33.3	70	0.33	25
Cefazolin	25	85	0.25	10
Cephapirin sodium	10	75	0.1	10
Cephacetrile	40	90	0.4	0
Cephradine	50	100	0.5	10
Cefamandole	10	100	0.1	0
Cefoxitin	100	95	1	15
Streptomycin sulfate	25	55	0.25	0
Kanamycin sulfate	25	100	0.25	0
Gentamicin sulfate	4	100	0.04	0
Amikacin sulfate	25	100	0.25	10
Dibekacin sulfate	1.25	100	0.01	0
Colistin sulfate	1.13	0	0.01	0
Lincomycin hydrocloride	30	100	0.3	15
Rifamycin sodium	7.57	100	0.07	20
Spectinomycin	62.5	100	0.62	0
Tetracycline hydrochloride	12.5	100	0.12	30
Oxytetracycline	5	100	0.05	10
Erythromycin	5	100	0.05	0
Chloramphenicol	10	100	0.1	0
Fosfomycin	25	100	0.25	0
Vancomycin	5	100	0.05	10
Amphotericin B	0.5	100	0.005	20

[a] The *a* value is 10% of the concentration of the drug in its dosage form, *A* is the percent inhibition of adenosine 5′-diphosphate-induced platelet aggregation by the antibiotic at concentration *a*, *b* is the maximum concentration that the drug reaches in human plasma through the administration of a therapeutic dose, and *B* is the percent inhibition of adenosine 5′-diphosphate-induced platelet aggregation by the antibiotic at concentration *b*.

plasma, with platelet-poor plasma added to give a concentration of 250,000 platelets/mm^3.

Platelet-poor plasma was obtained by centrifugation of human and dog samples at 4000 rpm for 30 min.

Antibiotics—The 31 antibiotics used were penicillin G sodium, penicillin G potassium, penicillin G benzathine, cloxacillin sodium, ampicillin sodium, metampicillin sodium, carbenicillin disodium, cephalothin sodium, cephaloridine, cefazolin, cephapirin sodium, cephacetrile, cephradine, cefamandole, cefoxitin, streptomycin sulfate, kanamycin sulfate, gentamicin sulfate, amikacin sulfate, dibekacin sulfate, colistin sulfate, lincomycin hydrochloride, rifamycin sodium, spectinomycin, tetracycline hydrochloride, oxytetracycline, erythromycin, chloramphenicol, fosfomycin, vancomycin, and amphotericin B[1].

Aggregating Agents—Adenosine 5′-diphosphate was used at a concentration of 2×10^{-5} *M* in pH 7.4 phosphate buffer for the *in vitro* tests and at 10^{-4} *M* in pH 7.4 phosphate buffer for the *in vivo* tests. Epinephrine at a concentration of 10^{-4} *M* in phosphate buffer was used. An initial collagen solution was prepared by the addition of 5 ml of 0.1 *N* acetic acid (pH 3.5) to 10 mg of collagen, followed by dilution with pH 7.4 phosphate buffer to give a final concentration of 0.2 mg of collagen/ml. The solution then was incubated at 37° for at least 30 min until opalescence was observed.

Platelet Aggregation—Platelet aggregation was measured by the absorbance method of Born and Cross (7) using a platelet aggregation meter[2,3].

In the *in vitro* tests[2], 0.1 ml of the test sample was added to a polyethylene cell containing 0.8 ml of platelet-rich plasma. The cell was incubated for 90 sec at 37°. At this point, 0.1 ml of a solution of adenosine 5′-diphosphate, epinephrine, or collagen in buffer was added.

[1] The trade names are Unicilina, Cidan-cilina, Bencetacil Simple, Orbenin, Cilleral, Actuapen, Pyopen, Keflin, Kefloridine, Cefamecin, Brisfirina, Celospor, Velocef, Mandokef, Mefoxitin, Neodual Estrepto, Kanahidro, Genticina, Biclin, Klobamicina, Colimicina, Lincocin, Rifocina, Kempi, Tetralen, Terramicina, Pantomicina, Chloromycetin, Fosfocina, Diatracin, and Fungizona, respectively.

[2] Evans EE1 169.

[3] Dual-channel Aggro-Meter.

Table II—Percent Inhibition of Human Platelet Aggregation Induced by Adenosine 5′-Diphosphate, Epinephrine, and Collagen *In Vitro*

Antibiotic	Adenosine 5′-Diphosphate	Epinephrine	Collagen
Cephaloridine	25	0	15
Rifamycin sulfate	20	5	15
Tetracycline hydrochloride	30	10	55
Amphotericin B	20	0	30

In the *in vivo* tests[3], 0.05 ml of the aggregating agent was added to 0.4 ml of platelet-rich dog plasma treated with tetracycline, using a procedure similar to that used for the *in vitro* tests.

Platelet Factor 3—The liberation of platelet factor 3 was studied by the Hardisty–Hutton method (8).

Platelet Electrophoretic Mobility—Alteration in the charge of the platelet cell membrane was studied by the determination of platelet mobility in an electric field using a cytopherometer[4]. Platelet-rich dog plasma (0.5 ml) was diluted with 9.5 ml of isotonic saline. The time required for platelets to travel a specified distance at the current strength used was determined.

Platelet Adhesiveness—Platelet adhesiveness was determined by the method of Salzman (9).

Platelet Filtration—Silanized K 15/30 columns, 30 cm × 1.5 cm i.d.[5], were used. Nylon filters with 40-μm diameter pores were placed in the bottom of the columns. The 2B Sepharose gel[5] was dissolved 1:4 in normal saline, kept at 4° for 24 hr, and cleared of bubbles before use.

The columns were filled with the gel to a height of ~16 cm. Then 6 ml of normal saline was added to cover the gel until the columns were used. From 4 to 6 ml of platelet-rich plasma was used for each filtration, which was carried out at a rate of 6–9 drops/min. The eluate was collected in 2-ml fractions in polyethylene tubes in ethylenediaminetetraacetic acid (0.077 *M*) and subsequently was centrifuged for 20 min at 3000 rpm.

The sedimented platelets were diluted with phosphate buffer to a final concentration of 400,000 platelets/mm^3. The aggregation of normal filtered platelets in plasma from dogs treated with tetracycline and of platelets from untreated dogs was studied, and the results were compared.

Statistical Analysis—The statistical analysis was performed using a *t* test.

RESULTS AND DISCUSSION

Most of the antibiotics studied inhibited the aggregation of platelets induced by 2×10^{-5} *M* adenosine 5′-diphosphate (Table I) at 10% of the concentration of the dosage form. At the maximum drug concentration in human plasma after the administration of a therapeutic dose, four drugs (cephaloridine, rifamycin sodium, tetracycline hydrochloride, and amphotericin B) inhibited adenosine 5′-diphosphate-induced aggregation by more than 20% and only one compound (tetracycline hydrochloride) clearly maintained its inhibitory effect *in vitro* in the presence of epinephrine (10%) and collagen (55%) (Table II).

The study of the various aggregating agents in dog blood led to the

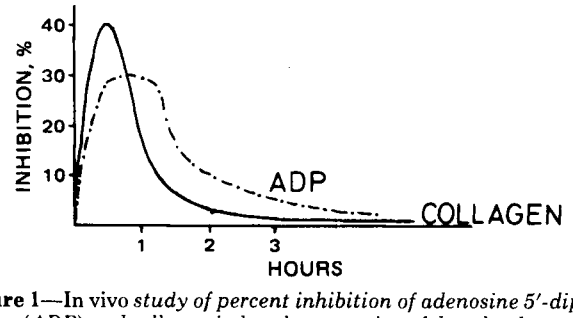

Figure 1—In vivo *study of percent inhibition of adenosine 5′-diphosphate- (ADP) and collagen-induced aggregation of dog platelets treated with tetracycline hydrochloride according to the time elapsed since its administration.*

[4] Zeiss 477500.

[5] Pharmacia, Uppsala, Sweden.

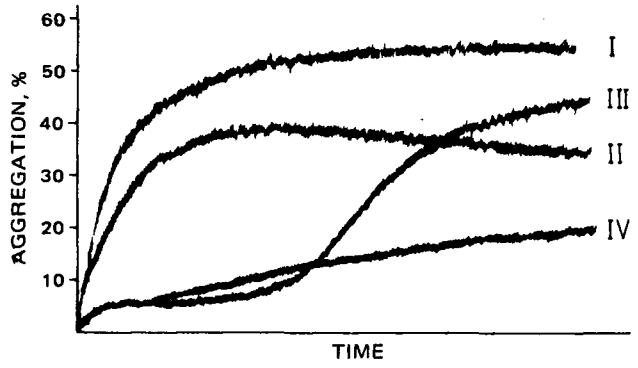

Figure 2—*Aggregation induced by adenosine 5′-diphosphate in filtered dog platelets (I) and in filtered dog platelets previously treated with tetracycline hydrochloride (II) and aggregation induced by collagen in filtered dog platelets (III) and in filtered dog platelets previously treated with tetracycline hydrochloride (IV).*

conclusion that solutions of 10^{-4} *M* adenosine 5′-diphosphate gave identical results to solutions of 2×10^{-5} *M* adenosine 5′-diphosphate in human plasma. The 0.2-mg/ml collagen solutions used in human plasma gave similar results in dog plasma, although the latency period for aggregation with platelet-rich dog plasma was approximately twice that obtained with platelet-rich human plasma. No significant aggregation in platelet-rich dog plasma was obtained with epinephrine, so this aggregating agent was not used for *in vivo* tests in dogs.

Platelet aggregation *in vivo* was studied at 0, 30, 60, and 90 min and 2, 24, 48, and 72 hr after intramuscular administration of tetracycline hydrochloride. As shown in Fig. 1, tetracycline hydrochloride inhibited 40% of collagen-induced platelet aggregation in blood extracted 30 min after the intramuscular administration of 5 mg of antibiotic/kg and 30% of adenosine 5′-diphosphate-induced platelet aggregation in blood extracted 30 and 45 min after a similar dose of the antibiotic. Platelet factor 3 values in dog plasma were similar to those found in human plasma. In dogs treated with tetracycline hydrochloride, factor 3 release was decreased significantly ($p < 0.0001$), with a mean value of 32.5 sec, compared to liberation in untreated dogs, where the mean value was 26 sec.

Electrophoretic mobility was identical for platelets from treated and untreated dogs, indicating unaltered platelet membrane potentials. The results were $x/N = 12.65 \pm 0.05$ and $x_1/N = 13.15 \pm 0.05$, where x is the time required for dog platelets to travel 64 μm (32 μm to the left and 32 μm to the right by varying the polarity), x_1 is the time required for tetracycline-treated dog platelets to travel the same distance, and N is the number of determinations (in this case, 20).

To determine whether the alteration in platelet aggregation produced by tetracycline was due to the platelets themselves or to the surrounding plasma, the platelets were filtered. The activity of normal filtered platelets with tetracycline-treated dog plasma and of tetracycline-treated platelets with untreated dog plasma in the presence of adenosine 5′-diphosphate and collagen was studied; the aggregation of untreated filtered platelets was greater than that of tetracycline-treated platelets (30% inhibition in the presence of adenosine 5′-diphosphate and 85% in the presence of collagen) (Fig. 2).

In conclusion, high concentrations of antibiotics usually completely inhibit the *in vitro* aggregation of platelets induced by adenosine 5′-diphosphate. At concentrations equivalent to plasma levels after administration of a therapeutic dose, only cephaloridine, rifamycin sodium, tetracycline hydrochloride, and amphotericin B partially inhibit adenosine 5′-diphosphate-induced aggregation and only tetracycline hydrochloride maintains this activity in the presence of other aggregating agents. In addition, tetracycline hydrochloride exerts an inhibitory effect *in vivo* on platelet aggregation in dog blood, affecting the platelets themselves and not the surrounding plasma, as shown by the results obtained with platelets filtered in Sepharose columns. It appears to alter the liberation of platelet factor 3.

REFERENCES

(1) J. P. Cazenave, M. A. Guccione, M. A. Packham, and J. F. Mustard, *Br. J. Haematol.*, **35**, 135 (1977).

(2) K. Andrassy, E. Ritz, B. Hasper, M. Scherz, E. Walter, H. Storch, and W. Vomel, *Lancet*, **1**, 1039 (1976).

(3) L. Houbouyan, J. F. Stolz, and A. Goguel, *Acta Haematol.*, **53**, 300 (1975).

(4) J. P. Cazenave, M. A. Packham, M. A. Guccione, and J. F. Mustard, *Proc. Soc. Exp. Biol. Med.*, **142**, 159 (1973).

(5) J. F. Mustard and M. A. Packham, *Chem. Pharmacol.*, **22**, 3151 (1973).

(6) H. Johnson and P. M. Niklassom, *Thromb. Res.*, **11**, 237 (1977).

(7) G. V. R. Born and M. J. Cross, *J. Physiol.*, **168**, 178 (1963).

(8) R. M. Hardisty and R. A. Hutton, *Br. J. Haematol.*, **11**, 258 (1965).

(9) E. W. Salzman, *J. Lab. Clin. Med.*, **62**, 724 (1963).

ACKNOWLEDGMENTS

Supported by a grant from the Comisión Asesora de Investigación Cientifica y Técnica, Spain.

Binding of Toxic Metabolites of Isoniazid by Aconiazide

HANS R. HELD * and SILVIO LANDI *‡x

Received October 17, 1979, from **Connaught Laboratories Limited, Willowdale, Ontario, Canada*, and the ‡*Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.* Accepted for publication May 12, 1980.

Abstract □ Isoniazid, the hydrazide of isonicotinic acid, is widely used in the treatment and prophylaxis of tuberculosis. The toxicity and carcinogenicity of isoniazid have been attributed to the action of its metabolites, hydrazine and acetylhydrazine. Aconiazide, the isonicotinylhydrazone of 2-formylphenoxyacetic acid, has been used in the treatment and prophylaxis of tuberculosis. Aconiazide is hydrolyzed in the body to isoniazid and 2-formylphenoxyacetic acid. 2-Formylphenoxyacetic acid has been shown to bind hydrazine and acetylhydrazine. This binding could explain the lower toxicity of aconiazide and also could provide a reason for postulating its lack of carcinogenicity.

Keyphrases □ Isoniazid—binding of toxic metabolites by aconiazide □ Aconiazide—binding of toxic metabolites of isoniazid, potential as antitubercular agent □ Antitubercular agents, potential—aconiazide, binding of toxic metabolites of isoniazid

Isoniazid (isonicotinic acid hydrazide) was introduced in 1952 as a drug for the treatment of tuberculosis and it soon became recognized as the most effective drug for the treatment and prevention of tuberculosis. It later became apparent that prolonged treatment with isoniazid may affect the peripheral and central nervous systems, and

0022-3549/80/1100-1284$01.00/0