Stability of Aspirin in Different Media

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Received December 3, 1981, from the *Department of Pharmacodynamics, College of Pharmacy, University of Illinois Health Sciences Center, Chicago, Ill 60612.* Accepted for publication May 12, 1982.

Abstract □ Aspirin rapidly hydrolyzes in various aqueous, organic, and biological media. The purpose of this investigation was to study the decomposition of aspirin in the media that comes in contact with it during analysis in biological fluids for pharmacokinetic studies. These media included water, water–polyethylene glycol 400, water–methanol–acetic acid, phosphate buffer, freshly drawn blood and plasma from control rats and rats deprived of water for 36 hr, and blood precipitated with acetonitrile. Studies were also conducted to determine the decomposition as a function of temperature and pH. Of the various solvent systems studied, aspirin was found most stable in water–polyethylene glycol (4:1, v/v), which provides an excellent medium for preparation of intravenous dosage forms. Phosphate buffer showed significant catalysis of aspirin hydrolysis. A more than fivefold increase in the hydrolysis of aspirin was noted when the temperature was raised to 37° from 22.5°. The hydrolysis of aspirin in rat blood was 13 times faster than that in plasma, with an average half-life in blood of ~13 min. This creates significant problems in aspirin disposition kinetic studies. Mixing the blood sample immediately after collection with twice the volume of acetonitrile and then centrifuging gives a plasma–acetonitrile mixture in which no lysis of blood cells is observed.

Keyphrases □ Aspirin—stability in media, human blood and plasma, hydrolysis kinetics □ Kinetics—aspirin hydrolysis in media, human blood and plasma, stability □ Stability—of aspirin in media, human blood and plasma, hydrolysis kinetics

The spontaneous hydrolysis of aspirin in suspensions can be stabilized by the addition of polyethylene glycol 6000, povidone, or sorbitol (1). The former two additives form viscous insoluble masses; sorbitol is therefore the only potential stabilizer of aspirin in suspensions. The hydrolysis rate constant of aspirin decreases in the presence of surfactants due to the migration of aspirin molecules into the micelles where they are unavailable for hydrolysis (2, 3), but the undissociated molecules of aspirin are often less stable in the micellar phase than in the aqueous bulk phase (4). The stability of aspirin in polyethylene glycols, both substituted and unsubstituted, has been studied in great detail (5–8). Aspirin is more stable in unsubstituted polyethylene glycols, where the hydrolysis occurs through transesterification which is retarded when the substituted polyethylene glycols are used.

Aspirin is also rapidly hydrolyzed by a group of enzymes broadly termed as aryl esterases. These enzymes are widely distributed in blood, plasma, liver, kidney, and GI tissues (9) and are present in lesser amounts in the other tissues (10). All organs of the human fetus exhibit aspirin-hydrolyzing esterase activity, with the activity increasing during intrauterine development (11).

Sex differences have been observed in the aspirin esterase activity in human serum: activity is more prolonged in males than in females (9,12). However, a sex difference has not been observed in the esterase activity of serum albumin (12). The aryl esterase activity is significantly reduced in chronic hepatitis, liver cirrhosis, and nephrosis (12). The differences between choline, pseudocholine, and the so-called aspirin esterases in serum have been reviewed by Morgan and Truitt (10). A recent finding shows that pseudocholine esterases also participate in the hydrolysis of aspirin in serum (12).

The *in vitro* hydrolysis of aspirin in dilute blood and serum shows significant inter- and intraspecies variability, probably due to differences in the aryl esterase activity (10). At 25° the half-life of aspirin in serum diluted 30 times is 8.1, 12.6, 13.1, 14.7, and 23.3 hr in the rat, rabbit, cat, human, and dog, respectively. Thus, the hydrolysis rate of aspirin in human serum is comparable with that in the rabbit and the cat, while rats and dogs are at the extreme ends of the range. In human blood diluted 30 times, the half-life is calculated to be ~10 hr at 25°.

Significant variations have been observed in the hydrolysis rate of aspirin in serum of different human individuals (10). In human blood or plasma, diluted by 10% with aspirin in isotonic saline, the hydrolysis of aspirin follows apparent first-order kinetics at body temperature with half-lives of 32 and 66 min in blood and plasma, respectively (13).

Compared with aspirin, the enzymatic hydrolysis of salsalate, a dimer of salicylic acid, is much slower in both plasma and blood (14). For example, the half-life of aspirin in 95% whole blood (95 ml of blood plus 5 ml of ethanol containing 10 mg of aspirin) is 50 min, which compares well with the previously reported value (13). But the half-life of salsalate hydrolysis under similar conditions is 854 min, 17 times longer than the half-life of aspirin.

The routine analysis of aspirin in biological fluids involves contact with blood, acetonitrile-precipitated blood, plasma, and other solvent systems such as water–methanol–glacial acetic acid mixtures for high-performance liquid chromatographic (HPLC) analysis, water–polyethylene glycol 400 mixtures, phosphate buffer, *etc.* Because of the instability of aspirin in solution, both biological or nonbiological, it was decided to investigate the rate of hydrolysis of aspirin in these media to estimate the loss of aspirin due to the time lag between blood sampling and analysis. This information would help devise proper storage conditions for blood and plasma samples, minimizing the loss of aspirin during sample preparation. The determination of the rate constants for the hydrolysis of aspirin in blood (both *in vivo* and *in vitro*), plasma, and precipitated plasma would also permit the estimation of the relative contribution of the liver (and other aspirin-hydrolyzing tissues), blood cells, and plasma to the overall disposition of aspirin in the body.

EXPERIMENTAL

Analytical Method—One-hundred microliters of the reaction mixture was diluted with 200 μl of acetonitrile[1], mixed thoroughly and centrifuged[2] for 5 min at 15,000 rpm. Twenty microliters of the supernatant

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Eppendorf Microcentrifuge Model 5412, Brinkmann Instruments, Westbury, N.Y.

0022-3549/ 83/ 0900-1024$01.00/ 0
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Table I—Half-Lives for the Hydrolysis of Aspirin in Different Solvent Systems

Solvent System	Temperature	N^a	Half-life $\pm$ SEM^b, hr	k_{obs} $\pm$ SEM^b, hr^{-1}
Distilled water, unbuffered	22.5°	4	153.30 ± 3.70	$4.53 \times 10^{-3} \pm 1.12 \times 10^{-4}$
Water–propylene glycol 400 (4:1), unbuffered	22.5°	4	359.80 ± 7.80	$1.92 \times 10^{-3} \pm 4.15 \times 10^{-5}$
Mobile phase (water–methanol–acetic acid; 64:35:1, v/v/v)	22.5°	4	178.50 ± 4.70	$3.89 \times 10^{-3} \pm 9.98 \times 10^{-5}$
Phosphate buffer, 0.1 *M*, pH 7.0	22.5°	4	75.30 ± 3.00	$9.25 \times 10^{-3} \pm 7.29 \times 10^{-4}$
Phosphate buffer, 0.1 *M*, pH 7.4	22.5°	4	82.40 ± 1.70	$8.43 \times 10^{-3} \pm 3.49 \times 10^{-4}$
Phosphate buffer, 0.1 *M*, pH 7.4	37.0°	4	15.40 ± 0.40	$4.50 \times 10^{-2} \pm 2.36 \times 10^{-3}$
Acetonitrile-precipitated blood	22.5°	6	105.70 ± 2.70	$6.58 \times 10^{-3} \pm 1.69 \times 10^{-4}$
Plasma from control rats[c]	22.5°	6	7.60 ± 0.50	$9.39 \times 10^{-2} \pm 7.01 \times 10^{-3}$
Plasma from control rats[c]	37.0°	6	2.80 ± 0.20	$2.55 \times 10^{-1} \pm 1.94 \times 10^{-2}$
Plasma from water-deprived rats[d]	37.0°	6	2.50 ± 0.10	$2.83 \times 10^{-1} \pm 1.08 \times 10^{-2}$
Blood from control rats[c]	22.5°	6	0.46 ± 0.04	$2.57 \times 10^{-2} \pm 1.91 \times 10^{-3}$
Blood from control rats[c]	37.0°	6	0.21 ± 0.01	$6.59 \times 10^{-2} \pm 5.94 \times 10^{-3}$
Blood from water-deprived rats[d]	37.0°	6	0.18 ± 0.02	$5.59 \times 10^{-2} \pm 3.52 \times 10^{-3}$

[a] Number of experiments. [b] Standard error of the mean. [c] Food and water *ad libitum*. [d] Deprived of water for 36 hr; food available *ad libitum*.

was analyzed by HPLC as described in the previous paper (15). The mobile phase was an isocratic mixture of water–methanol[1]–glacial acetic acid[3] (64:35:1, v/v/v), and the flow rate was 2 ml/min on a reverse-phase column[4]. The separated components were detected at 238 nm. The concentrations of aspirin and salicylic acid were calculated from the peak heights using appropriate standard curves.

Hydrolysis of Aspirin in Different Solvents—The solvent systems used to study the rate of hydrolysis of aspirin were: (*a*) water at 22.5°, (*b*) water–polyethylene glycol 400[5] (4:1, v/v) at 22.5°, (*c*) water–methanol–glacial acetic acid (64:35:1, v/v/v) at 22.5°, (*d*) phosphate buffer (0.1 *M*, pH 7.0) at 22.5°, and (*e*) phosphate buffer (0.1 *M*, pH 7.4) at 22.5 and 37°. The aspirin stock solution was prepared by dissolving 1 g of aspirin in 25 ml of polyethylene glycol 400, then diluting with sterile normal saline to 100 ml. Forty microliters of the aspirin stock solution was diluted with 1960 μl of the solvent system to make the final reaction mixture of volume 2 ml. Therefore, the concentration of aspirin was 10 mg/ml in the stock solution and 200 μg/ml in the reaction mixture. Samples were collected at appropriate intervals for up to 100 hr and analyzed by HPLC.

Hydrolysis of Aspirin in Plasma, Blood, and Precipitated Blood—Approximately 6 ml of blood was collected from each rat. Two milliliters of this sample was used for the study of aspirin hydrolysis in blood; from the remainder, ~2 ml of plasma was obtained to study the hydrolysis of aspirin in plasma. The following experimental media were used to study aspirin hydrolysis:

1. Fresh plasma from control rats (food and water *ad libitum*) at 22.5°.
2. Fresh plasma from control rats (food and water *ad libitum*) at 37°.
3. Fresh plasma from rats deprived of water for 36 hr (food *ad libitum*) at 37°.
4. Blood precipitated with acetonitrile at 22.5°. Whole blood was precipitated with two volumes of acetonitrile, mixed gently, centrifuged (5 min at 15,000×*g*), and the supernatant was used to prepare the reaction mixture.
5. Blood from control rats (food and water *ad libitum*) at 22.5 and 37°.
6. Blood from rats deprived of water for 36 hr (food *ad libitum*) at 37°.

The reaction mixture for all of the above kinetic determinations was 5 μl of heparin (10,000 U/ml), 40 μl of aspirin stock solution (10 mg/ml), and 1955 μl of plasma (or blood). Therefore, the final concentrations of heparin and aspirin in the reaction mixture was 25 U/ml and 200 μg/ml, respectively. The samples were collected at appropriate intervals, precipitated with two volumes of acetonitrile, and the supernatant was analyzed by HPLC (15).

[3] BASF, Wyandotte, Mich.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] C_{18} μBondapak Column, 30 cm long, 10 μm silica, Waters Associates, Milford, Mass.

RESULTS AND DISCUSSION

Hydrolysis of Aspirin in Different Solvent Systems—The rate of hydrolysis of aspirin was followed by direct measurement of the amount of intact aspirin remaining as a function of time. The hydrolysis of aspirin was found to follow apparent first-order kinetics in all solvent systems studied. A minimum of four and maximum of six replicate kinetic runs were conducted for each of the solvent systems used. The concentration–time data were fitted to the linear regression equation for pseudo-first-order kinetics.

Table I lists the observed rate constant (k_{obs}) and the half-lives of aspirin in different solvent systems at the specified temperature. Aspirin was found to be most stable in a water–polyethylene glycol 400 (4:1, v/v) mixture ($t_{1/2}$ = 360 ± 8 hr), followed by the mobile phase ($t_{1/2}$ = 179 ± 5 hr), distilled water ($t_{1/2}$ = 153 ± 4 hr), phosphate buffer at pH 7.4 ($t_{1/2}$ = 82 ± 2 hr), and phosphate buffer at pH 7.0 ($t_{1/2}$ = 75 ± 3 hr) at 22.5°. At 37.0°, the rate constant of aspirin hydrolysis in 0.1 *M* phosphate buffer (pH 7.4) was found to be more than five times the rate constant for the same buffer at 22.5°.

Although aspirin has been reported to be more stable in moisture-free polyethylene glycol (5), its stability data in a water–polyethylene glycol combination are not available in literature. In polyethylene glycol, aspirin is hydrolyzed by a process of transesterification with an activation energy of 20.1 Kcal/mole (5), resulting in much higher stability compared with water alone. Therefore, the water–polyethylene glycol 400 (4:1, v/v) solvent system is superior to water as a vehicle for aspirin in terms of both solubility and stability.

Since aspirin is highly unstable in phosphate buffer, any sample containing aspirin which is extracted with phosphate buffer should be analyzed as quickly as possible. The relatively higher stability of aspirin ($t_{1/2}$ = 179 ± 5 hr) in the mobile phase [an isocratic mixture of water, methanol, and glacial acetic acid (64:35:1, v/v/v)] compared with its stability in water ($t_{1/2}$ = 153 ± 4 hr) at room temperature ensures that there will be no significant on-the-column hydrolysis of aspirin during its analysis by the described HPLC method (15).

Hydrolysis of Aspirin in Plasma and Blood—The hydrolysis of aspirin in plasma and blood is mostly an enzymatic process caused by aryl esterases in plasma and blood cells. Although the enzymes retain their activity for at least 1 week when stored at 5° (15), only freshly drawn blood and plasma were used to conduct the hydrolysis studies of aspirin. The hydrolysis was also performed at two different temperatures, 22.5° and 37.0°, using plasma and blood from water-deprived rats (deprived

of water for 36 hr, but having access to food *ad libitum*). Since sex differences in the rate of hydrolysis of aspirin in blood have been reported (9, 12), only male rats weighing between 300 and 450 g were used in this study.

The hydrolysis of aspirin in blood and plasma follows apparent first-order kinetics. The rate constants (k_{obs}) and the half-lives are listed in Table I. The hydrolysis of aspirin in blood from control rats is more than 13 times faster than in plasma, both at 22.5° and 37.0°. For example, at 37.0°, the half-life of aspirin in blood is 0.21 hr (13 min) compared with 2.8 hr (168 min) in plasma. The corresponding values at 22.5° for blood and plasma are 28 and 456 min, respectively.

The activity of various enzymes such as acid phosphatase, β-glucosidase, β-glucuronidase, β-galactosidase, and *N*-arylamidase has been reported to increase in water-deprived rats (16). The hydrolysis rates of aspirin in both blood and plasma of water-deprived rats, however, were found to be about equal to that in the blood and plasma from control rats.

As expected, temperature had considerable effect on the rate of enzymatic hydrolysis of aspirin in blood and plasma. The half-lives at 22.5° in the blood and plasma of control rats were 0.46 and 7.6 hr, respectively, compared with 0.21 and 2.8 hr at 37.0°.

The rapid hydrolysis of aspirin in blood and plasma might create considerable problems in the handling and storage of samples for analysis. When the blood sample containing aspirin is drawn from the rat, it has a temperature of ~37°. At that temperature, the half-life of aspirin in blood is only 13 min (Table I). Unless frozen immediately, the aspirin in the sample will hydrolyze rapidly as the sample gradually cools to room temperature (~22.5°), where the half-life of aspirin is still merely 28 min. If blood at room temperature is centrifuged and the plasma separated, the half-life of aspirin in plasma at room temperature is 7.6 hr. Therefore, to avoid significant hydrolysis of aspirin in plasma samples, the enzymatic process should be stopped at the moment the blood is collected in the syringe. If the blood is collected in a syringe and transferred to a centrifuge tube to obtain plasma, considerable loss of aspirin is inevitable due to the time lag between blood collection and the end of centrifugation. This difficulty can be overcome by collecting the blood samples in a syringe containing twice the volume of acetonitrile. Acetonitrile instantly deactivates the aspirin-hydrolyzing enzymes, coagulates the blood, and precipitates the serum proteins, but does not cause hemolysis of red blood cells so that a colorless, clear solution is obtained from blood on centrifugation. Stability studies have shown that aspirin hydrolysis in blood–acetonitrile (1:2, v/v) supernatant follows pseudo-first-order kinetics with a half-life of 106 hr at room temperature (Table I), as compared with a half-life of 75 hr for aspirin in phosphate buffer, pH 7.0.

Pharmacokinetic studies of aspirin in blood or plasma have long had problems due to (*a*) rapid loss of aspirin in blood and plasma, (*b*) lack of an analytical procedure which directly measures the aspirin in blood or plasma samples, and (*c*) fast disposition of aspirin in the body. These problems have been obviated by instantly deactivating the aspirin-hydrolyzing enzymes by adding acetonitrile, developing a rapid and sensitive HPLC method for direct quantitation of aspirin in blood and plasma, and cannulating the rat permanently so that blood samples can be collected as frequently as ≤1 min.

The *in vitro* hydrolysis rate of aspirin in whole blood or serum of rats has been reported previously by Morgan and Truitt (10) using 30-fold dilute rat serum. The initial concentration of aspirin used in their study was 433 μg/ml, a toxic level. The hydrolysis rate was followed by measuring salicylic acid (not aspirin) spectrophotometrically at 300 nm. The half-life of aspirin was found to be 8.12 hr in 30-fold dilute rat serum at 25°. This value is much higher than the half-life of 168 min found in the present study in plasma, showing that the rate constant for the enzymatic hydrolysis of aspirin cannot be extrapolated to whole blood sérum, or plasma from dilute-sample studies. Morgan and Truitt (10) failed to recognize that at high serum dilution and higher aspirin concentrations, the hydrolysis may follow a different order of reaction.

Harris and Riegelman (13) have reported half-lives of aspirin in human plasma and blood to be 32 and 66 min, respectively, corresponding to 13 and 168 min in rats in our study. The concentration of aspirin was ~20 times smaller than our study, and therefore no direct comparisons can be made. But if the dilution effect is extrapolated, it is observed that aspirin is hydrolyzed by rat blood faster than human blood, whereas the opposite finding is made when plasma is used. Thus, rat blood is not a good substitute for human blood in hydrolysis studies.

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