Constant Blood Withdrawal Method for Area under Plasma Concentration–Time Curve

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Abstract □ The area under the plasma concentration–time curve (*AUC*) was measured after an intravenous injection of sodium salicylate and after intragastric administration of aspirin, using either an intermittent sampling and the trapezoid rule (*AUC*–trapezoid) or the recently introduced constant blood withdrawal method (*AUC*–integrated concentration). Six dogs were used in each study. The *AUC*–integrated concentration was significantly higher than the *AUC*–trapezoid. The difference between the two methods was more significant after the intravenous infusion than after the intragastric administration. The higher *AUC*–integrated concentration was attributed to the inclusion of the initial plasma levels, which is an inherent advantage of the constant blood withdrawal method.

Keyphrases □ Plasma concentration–time curves—*AUC* determined by constant blood withdrawal and intermittent sampling methods compared, sodium salicylate and aspirin in dogs □ *AUC*—determined by constant blood withdrawal and intermittent sampling methods compared, sodium salicylate and aspirin in dogs □ Pharmacokinetics—*AUC* determined by constant blood withdrawal and intermittent sampling methods compared, sodium salicylate and aspirin in dogs

Since the pharmacological effect of drugs can be related to their concentration in the plasma and to the duration of their presence in the plasma pool, the area under the plasma concentration peak has become an important parameter in the evaluation and quantitation of the bioavailability of various drugs (1–5). Most presently used methods for calculating the area under the plasma concentration–time curve (*AUC*) are based on a multiple sampling protocol. Each plasma sample is assayed separately. The plasma concentration–time curve is then constructed either by connecting the points with straight lines (the trapezoid method) or by fitting the function to the points and then calculating the integral of the curve.

Previously (6, 7), the constant blood withdrawal method for calculation of the *AUC* in pharmacokinetic studies was described. In this method, integration is achieved by the constant blood withdrawal apparatus. This method does not require prior knowledge of the shape of the concentration–time curve and greatly reduces the number of assays (1–5). This investigation compared the multiple sampling method with the constant blood withdrawal method.

EXPERIMENTAL

Determination of *AUC* after Intravenous Administration of Sodium Salicylate—Six adult female Beagle dogs were fasted for 24 hr prior to each experiment. Dogs 1–3 each received 800 mg of sodium salicylate in a sterile solution as a single intravenous pulse. Dogs 4–6 were injected with 400 mg of sodium salicylate. The catheter was left in place and flushed by a slow drip of saline. Constant withdrawal of blood from a vein in another limb was initiated just prior to the pulse injection, using a nonthrombogenic, presterilized, disposable set with a withdrawal pump as previously described (6). The withdrawal rate was set at 5 ml/hr and was continued for 4 hr.

In addition to the constant withdrawal, 5-ml blood samples were withdrawn from another vein every 2 min for the first 10 min and subsequently at 20, 40, 60, 80, 120, 140, 160, 180, 200, 22.0, and 240 min. The plasma salicylic acid level was measured by a previously described method (8). The salicylic acid concentration in the constant withdrawal pool was the integrated concentration (IC-4 hr). The *AUC* or $\int_0^4 c\,dt$ was then calculated as follows:

$$\int_0^4 c\,dt = \text{IC-4 hr} \times 4 \qquad \text{(Eq. 1)}$$

The *AUC* was also calculated from the intermittent samples by the trapezoid rule.

Table I—Intravenous Administration of Sodium Salicylate

Dog	*AUC*–Trapezoid	*AUC*–Integrated Concentration
1	627	745
2	615	700
3	610	688
Mean ± 1 *SD*	617 ± 8.7	711 ± 30.0
	$p < 0.01$	
4	376	400
5	374	410
6	373	384
Mean ± 1 *SD*	374 ± 1.5	398 ± 13.1
	$p < 0.05$	

Determination of *AUC* after Ingestion of Aspirin—Six dogs were used. A nonthrombogenic intravenous catheter was introduced as previously described. A lubricated gavage tube was inserted through the mouth of each dog into the stomach. Four 324-mg aspirin tablets were crushed, suspended in 80 ml of water, and administered through the gavage tube. The nonthrombogenic catheter was connected to the constant withdrawal pump, which was set to withdraw at a rate of 5 ml/hr. The blood was collected over 4 hr, spun, and separated. The salicylic acid concentration in the sample constituted the integrated concentration for the collection period (IC- 4 hr). The *AUC* was calculated as previously described.

Concurrently with the continuous withdrawal, blood samples were withdrawn every 15 min from another limb. The plasma salicylic acid concentration of these samples also was determined.

RESULTS AND DISCUSSION

The *AUC* of salicylic acid was determined in each dog by the trapezoid rule (*AUC*–trapezoid) and then by the constant blood withdrawal method (*AUC*–integrated concentration) (Table I). The *AUC* by the nonthrombogenic constant blood withdrawal method (*AUC*–integrated concentration) was significantly higher than the *AUC*–trapezoid. It was concluded that the initial plasma salicylic acid levels contributed significantly more to the *AUC* than was assumed by the trapezoid rule.

This observation is in agreement with previous conclusions (7) drawn from measurement of the sulfamethizole clearance rate using the constant infusion, the intermittent sampling, and the constant withdrawal methods. The constant blood withdrawal method yielded a clearance of sulfamethizole that was in good agreement with the clearance obtained by the constant infusion method, thereby confirming its accuracy.

Therefore, the constant blood withdrawal method yields results that are inherently more accurate than the intermittent sampling method. Even determination of the plasma level at 2-min intervals during the initial period could not fully compensate for the inaccuracy of the multiple intermittent sampling method. An additional advantage of the constant blood withdrawal method is the saving in the multiple determinations of the plasma drug concentrations. Instead of the 17 separate assays used in the intermittent method, only one determination of the plasma drug concentration was required by the constant withdrawal method.

Since the difference between the intermittent sampling method and

Table II—Intragastric Administration of 1296 mg of Aspirin

Dog	AUC after 4 hr, hr × mg/liter: AUC–Trapezoid Intermittent Sampling Method	AUC–Integrated Concentration Constant Withdrawal Method
1	555	568
2	492	492
3	491	590
4	425	513
5	717	856
6	522	590
Mean ± 1 *SD*	554 ± 100	601 ± 131
	$p < 0.05$	

the constant blood withdrawal method is mainly due to the peak plasma level observed during the initial period, the difference may become negligible when the plasma concentration–time curve does not contain a peak at the initial period. Table II contains the results of a comparison between the *AUC*–trapezoid determined by the intermittent sampling method and the *AUC*–integrated concentration determined by the nonthrombogenic constant blood withdrawal method after intragastric administration of aspirin. As expected, the difference was not as great as the difference observed in the previous experiment, but it was still significant ($p < 0.05$, paired t test). The plasma concentration–time curve of salicylic acid after intragastric administration of aspirin did not contain the sharp early peak seen after intravenous administration. Despite the gradual increase in the plasma salicylic acid level, the *AUC* was not adequately described by the multiple sampling method.

When the shape of the plasma concentration–time curve is of interest, the collection of blood for the constant blood withdrawal method can be interrupted at predetermined intervals. Therefore, a series of integrated concentrations can be obtained by determining the plasma concentration during each constant blood withdrawal interval. The use of an interruption method increases the number of determinations of the plasma drug concentration, thus reducing one advantage of uninterrupted continuous withdrawal. The advantages of this approach were discussed previously (9).

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Constituents of *Cannabis sativa* L. XIII: Stability of Dosage Form Prepared by Impregnating Synthetic (−)-Δ^9-*trans*-Tetrahydrocannabinol on Placebo *Cannabis* Plant Material

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Abstract □ Synthetic (−)-Δ^9-*trans*-tetrahydrocannabinol impregnated on placebo *Cannabis* decomposed only 6.3% after being stored for 1 year at −18°. Storage at 5° and room temperature under various conditions led to severe decomposition. The amount of cannabinol observed when (−)-Δ^9-*trans*-tetrahydrocannabinol decomposed indicates that cannabinol is not the only decomposition product.

Keyphrases □ *Cannabis sativa* L.—stability of dosage form prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material □ Stability—dosage form prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material □ (−)-Δ^9-Tetrahydrocannabinol—impregnated on placebo *Cannabis* plant material, stability of dosage form □ Dosage forms—prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material, stability

The potency of *Cannabis* preparations varies significantly according to cannabinoid ratios (1). Recently, to overcome these variations, researchers have used placebo *Cannabis* plant material impregnated with synthetic (−)-Δ^9-*trans*-tetrahydrocannabinol (I). However, this procedure also has disadvantages: (*a*) several months are required for the simplest preclinical or clinical study, (*b*) storage conditions in laboratories are not standardized, (*c*) several solvent systems may be used in the impregnation procedure, and (*d*) synthetic I and I in natural *Cannabis* preparations decompose at different rates under different storage conditions (2, 3).

In view of the disadvantages and other considerations[1], this paper reports findings on the stability of a dosage form prepared by impregnating synthetic I on placebo *Cannabis* plant material.

[1] The authors are under contract from the National Institute on Drug Abuse to provide various *Cannabis* preparation to researchers.