In Vivo Distribution of Carbon-11 Phenytoin and its Major Metabolite, and Their Use in Scintigraphic Imaging

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Curie quantities (0.3–1.5 Ci) of $H^{11}CN$ were used in the synthesis of C-11-tagged phenytoin (C-11.DPH) and 5-(p-hydroxyphenyl)-5-phenylhydantoin (C-11.HPPH), using a modified Bücherer-Bergs reaction. The $H^{11}CN$ was produced from a mixture of 95% nitrogen and 5% hydrogen by a 45-min bombardment with 10-MeV protons at 10 μA.

Following i.v. infusions of C-11 DPH (13.7 mg/kg at a rate of 29 mg/min) into the left femoral vein of Rhesus monkeys, DPH shows persistent concentration in the brain and liver fields. Extravascular administration shows significant retention at the site of administration.

Intravenous bolus injection of [^{11}C]-HPPH into a Rhesus monkey, at a dose of 6.4 mg/kg, resulted in localization of this compound in the liver, gallbladder, urinary bladder, and intestinal fields. Loss of activity from the liver region, with appearance of this activity in the intestinal field, suggests that [^{11}C]-HPPH is secreted into the intestine via the bile.

Further investigation is needed to study the potential of [^{11}C]-DPH as a brain-scanning agent and [^{11}C]-HPPH as a possible cholescintigraphic agent.

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Phenytoin (diphenylhydantoin, DPH) has been used as an anticonvulsant in doses of 300–1000 mg daily (*1*). Recently it has been used as an antiarrhythmic in doses of 125 mg up to 1000 mg intravenously every 5 min (*2*). It has been recommended that the i.v. administration rate be no greater than 50 mg/min. Rapid administration has produced immediate toxicity, including transient agitation, nystagmus, ataxia, and cardiopulmonary depression (*3*). Harris and Kokernot (*4*) reported that a dose of 20 mg/kg of body weight proved fatal when injected quickly, but doses as large as 50 mg/kg were given repeatedly without incident when administered at slow infusion rates.

Pharmacokinetic studies in the literature (*5,6*), and at the University of Kentucky College of Pharmacy, indicate that the plasma concentration vs. time curves after i.v. administration present a series of anomalies. These consist of spiking in the distributive phase, time 0–30 min, a flat region between 2–4 hr after i.v. administration, and nonlinear elimination. A series of possibilities have been suggested to explain these phenomena. It has been shown by in vitro simulation (*7*) that rapid i.v. administration might cause precipitation of DPH in blood. Redissolution of these crystals, or a rapid local discharge of phenytoin from some tissue into the circulation, might explain the abrupt changes observed in the plasma concentration curves. The flat region and the spiking could also be explained in terms of entero-hepatic recycling. The nonlinearity observed in the terminal portion of the plasma concentration curves has been explained in terms of nonlinear elimination attributable in part to inhibition by the major metabolite of phenytoin, 5-(p-hydroxyphenyl)-5-phenylhydantoin

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(HPPH) (*8,9*). It was desirable to conduct experiments to determine the effect of HPPH on the in vivo distribution of phenytoin after i.v. administration. We thought that if one could follow tissue changes as a continuous function of time after i.v. administration of [^{11}C]-DPH and could correlate these changes with plasma levels, it would give an indication of the possible cause of these anomalies in the plasma concentration curves. This could result in a better understanding of phenytoin kinetics.

Rosenblum and Stein (*10*) demonstrated that phenytoin localizes preferentially in human primary brain tumors when compared with adjacent normal brain tissue. Thus, evaluation of DPH as a potential brainscanning agent was of interest.

MATERIALS AND METHODS

Synthesis of [^{11}C] phenytoin and its major metabolite. Several methods for the synthesis of DPH and HPPH are described in the literature (*11–13*), but they do not allow introduction of carbon-11, which decays by positron emission with a half-life of 20.4 min. Despite its short half-life, carbon-11 has been introduced into some complex compounds by chemical synthesis (*14–16*). Substantial quantities (300–1500 mCi) of H^{11}CN produced with the Sloan-Kettering cyclotron (*11*) were collected in NaOH/DMSO following a 45-min bombardment of a 95% nitrogen, 5% hydrogen mixture with a 40-μA beam of 10-MeV protons. The Na^{11}CN produced was used in the synthesis of [^{11}C]-DPH and [^{11}C]-HPPH, using a modified Bücherer-Bergs reaction. The detailed syntheses have been reported elsewhere (*18*).

[^{11}C]-DPH and [^{11}C]-HPPH solutions. Solutions of [^{11}C]-phenytoin and [^{11}C]-HPPH were prepared immediately after their synthesis. The drug or its metabolite was dissolved in an appropriate volume of 0.1 N sodium hydroxide, no greater than 4 ml. These solutions were filtered through a Millipore filter (0.22 μ) and kept in sterile vials prior to injection. Aliquots of these solutions were kept for analysis of DPH or HPPH to determine the dose administered to the animals.

Subjects, dose, and route of administration. Four mature, healthy male Rhesus monkeys were used in the study. The monkey labeled C was rested for a period of 2 wk and then used in a second experiment (referred to in the text as monkey 2C). The average body weight was 5.28 kg (range 5.04–5.52 kg). The animals were allowed free access to food and water until the time of the experiment. Their preparation included anesthesia (phencyclidine), 1.5 mg/kg, and catheterization of the right and left femoral veins, to allow administration of the radiolabeled compounds and removal of blood samples.

In order to study the in vivo distribution of phenytoin, a dose of 13.7 mg/kg of [^{11}C]-DPH was infused into the left femoral vein of monkey C, at a rate of 29 mg/min. The total radioactive dose was 9.02 mCi.

The influence of HPPH on the in vivo distribution of DPH was performed on monkey 2C, which received i.v. bolus injections of HPPH*, 27.2 mg/kg at 0, 15, and 30 min. Twenty-eight minutes after the last dose of HPPH, 13.7 mg/kg of C-11 DPH was infused into the left femoral vein at a rate of 29 mg/min. The total radioactive dose was 4.17 mCi. Monkey 2A, which was used to study the in vivo distribution of HPPH, received an i.v. bolus injection of [^{11}C]-HPPH (6.9 mCi) into the left femoral vein (a dose of 6.4 mg/kg). In all cases the cannulas at the site of administration were removed at the end of the infusions or the i.v. bolus injection.

Blood specimens and analytical methods. Three ml of blood were withdrawn into a syringe through a three-way stopcock, and transferred to a 3-ml heparinized vacutainer. Three ml of heparinzed saline solution was injected to rinse the cannula and to replace the volume of blood withdrawn. The blood was collected at appropriate intervals up to 42 hr for monkey C and 2C, and up to 4 hr for monkey 2A.

The blood was centrifuged and plasma specimens were analyzed for DPH and HPPH concentrations by the gas chromatographic method developed by MacGee (*19*). Plasma specimens from monkey 2A were assayed in a gamma counter to determine [^{11}C]-HPPH concentration. All samples were corrected for radioactive decay.

Scanning procedure. The total-organ kinetic imaging monitor (TOKIM), was employed for the dynamic tracer-distribution studies (*20*). Data were obtained as a continuous function of time and stored on computer tapes for further reference. For the first observation period (20–30 min) the field of view included the liver, lungs, heart, and head. Following this period, the field of view was varied to obtain data in different anatomic regions of the monkeys, and the site of administration. The animals were then transferred to a high-energy rectilinear scanner system (HEG) to obtain whole-body scans (*21*). The alphanumeric whole-body scan data were displayed in dot-density images, and in alphanumeric printout for quantitation of activity in various fields.

RESULTS AND DISCUSSION

Figure 1 illustrates the plasma levels of DPH obtained for monkey C and 2C during the first 2 hr after administration, while Fig. 2 shows the levels obtained from 2–42 hr. A sharp drop in plasma levels is observed during the first 15 min, indicating a very

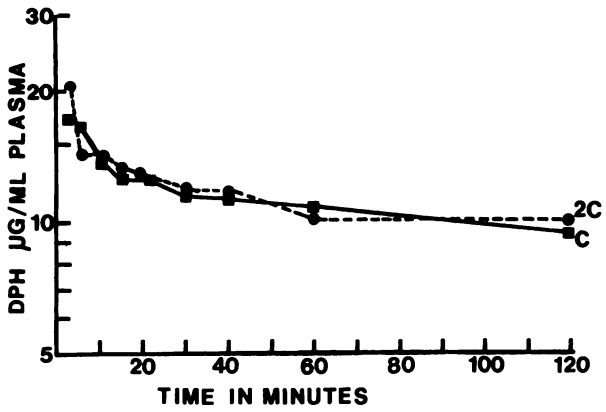

FIG. 1. Plasma levels observed in a monkey (C and 2C) during the first 2 hr after i.v. infusion of phenytoin in the presence (2C) and absence (C) of its major metabolite (HPPH).

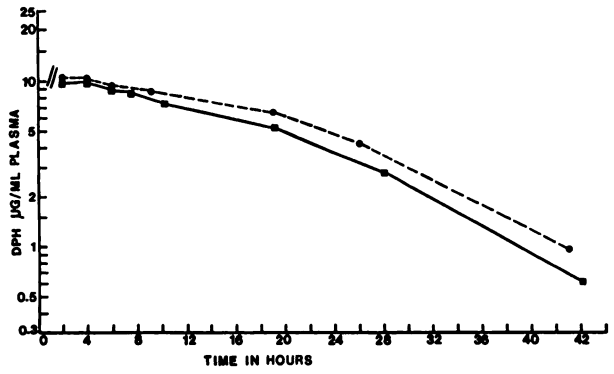

FIG. 2. Decline of plasma concentration in a monkey (C and 2C) showing that the fall-off curve is not a simple exponential process, but exhibits a flat region between 2 and 6 hr.

however, fatalities have been observed in humans (*22,23*), generally occurring through cardiopulmonary depression. Infusion rates less than 30 mg/min are recommended.

Considering the rapid uptake of drug seen in the brain, it seems likely that even slow rates of infusion should be sufficient for adequate seizure control. The slow rate of release of most of the activity initially accumulated in the heart-lung field (Fig. 3), coupled with the absence of clearly defined lung images at 19–20 min after administration (Fig. 4b), strongly suggest that blood precipitation of the drug, if it occurs, is either extremely shortlived or involves only a small fraction of the total infusion. The extended retention of DPH in this region, visible in

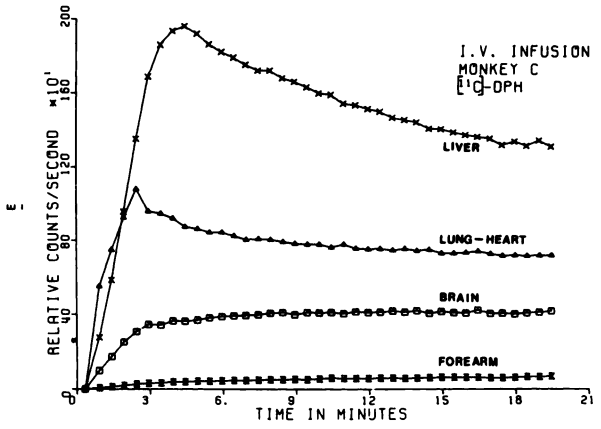

FIG. 3. Relative counts/sec in observed fields: brain, lung-heart, liver, and forearm.

fast distributive phase. The results obtained with the TOKIM in one of these studies are presented in Fig. 3, which shows the count rates summed in the indicated regions as a function of time. Note that the times to peak activity for the regions of interest differ considerably. Peak levels are reached within minutes; 2–3 min for the lung-heart field, and 4–5 min for the liver region. Plateau levels are reached at 7–8 min for the brain field, and later for the forearm region.

The fact that DPH uptake is extremely rapid in the lung-heart field may explain the cardiopulmonary depression observed after rapid intravenous infusion of this drug. It is possible that the clearance rate from the lung-heart field is slower than the infusion rate, so that fast administration would cause high initial levels of the drug in the lungs and heart, perhaps resulting in cardiopulmonary depression. In the present study, the infusion rate was 30 mg/min, and no fatalities occurred. At fast infusion rates,

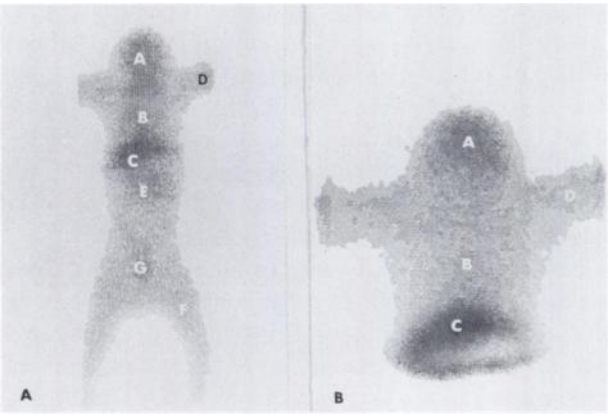

FIG. 4. (A) Whole-body scan following i.v. infusion of carbon-11 phenytoin in monkey C. Anterior ventral view, 93 min after administration. A = brain field; B = lung-heart field; C = liver field; D = forearm field; E = intestinal field; F = infusion site; and G = urinary bladder. The high-energy scanner system was used. (B) TOKIM dot display following i.v. infusion of carbon-11 phenytoin to monkey C. Anterior ventral view, head to liver, 19–20 min after administration. A = brain field; B = lung-heart field; C = liver field; and D = forearm field.

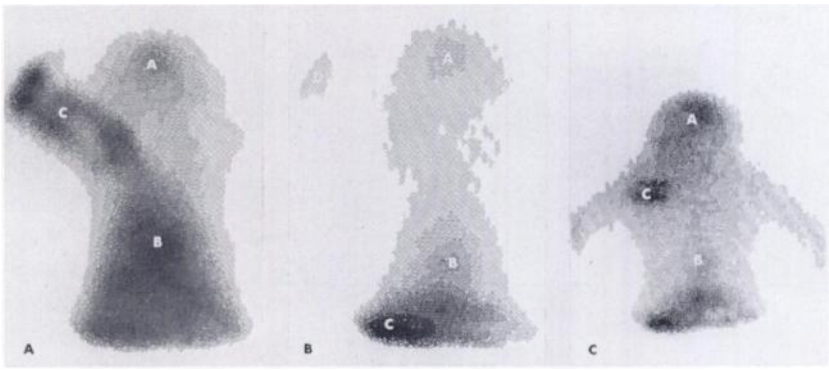

FIG. 5. (A) TOKIM dot displays obtained during rapid intravenous infusion of [^{11}C]-phenytoin to a monkey. Anterior ventral view, head to liver, 0–1 min after administration. A = brain field; B = lung-heart field; and C = forearm field. (B) As in (A) 1–2 min after administration. A = brain field; B = lung-heart field; C = liver field; and D = forearm. (C) As in (A) 20–21 min after administration. A = brain field; B = lung-heart field; and C = site of administration.

Fig. 4a, taken 93 min after administration, is likely the result of tissue binding of the drug.

In order to assess precipitation of phenytoin in the blood, an experiment was performed at high infusion rates and high doses. Following i.v. infusion of 11.9 mg/kg body weight of DPH at a rate of approximately 1,000 mg/min into the basilic vein of a monkey, it was observed that the lung-heart field is cleared very rapidly. Figure 5a, obtained 0–1 min after the start of the i.v. infusion, illustrates how DPH reaches the lung-heart field, while Fig. 5b, obtained 1–2 min postinfusion, illustrates that DPH does not remain at the site of administration. It is also qualitatively evident from these two displays that DPH is cleared very rapidly from the lung-heart field. The results of this experiment decrease, if they do not totally exclude, the possibility of DPH precipitation in blood; consequently the spiking in the distributive phase in humans cannot be attributed to DPH precipitation in blood, and its cause is at present unclear. Extravascular administration of DPH shows definite accumulation of activity at the site of administration. This is illustrated in Fig. 5c, obtained 20–21 min after administration.

The fact that the prompt liver uptake decreases with time, and the appearance of activity in the urinary bladder, indicate that the drug is being eliminated. One would then expect the levels of the lung-heart and brain fields to fall if a true equilibrium is established between all tissues and plasma. That the activity in the lung-heart field decreases very slowly, whereas that in the brain field is retained, is seen in Fig. 3. The persistence of activity in the brain region reflects a strong affinity of this compound for brain tissue, which may explain its prolonged pharmacologic action. These results correlate with Firemark's experiments (*24*), which also show persistent localization of DPH in the brain. This marked uptake in the brain field and the ability to synthesize [^{11}C]-phenytoin routinely suggest the potential diagnostic use of this compound in detection and identification of neoplastic brain lesions. Preliminary studies in humans at Sloan-Kettering Institute indicate that there is an increased uptake of [^{11}C]-DPH by brain tumors.

Figure 4a, obtained 93 min after i.v. infusion of [^{11}C]-DPH shows significant activity in the intestinal field. Reports in the literature (*25*) demonstrated that one of the possible routes of entrance of phenytoin into the intestine is through the bile. Noach reported that the maximum gastrointestinal content of DPH was considerably lower than that of HPPH, which reached more than 50% of the administered radioactivity of DPH. Furthermore, DPH and its metabolites excreted into the intestinal tract were reabsorbed almost completely since no DPH or radioactivity was found in the feces, and 95% of the administered radioactivity could be recovered in the urine (*26*). These results are consistent with the pattern of in vivo distribution of [^{11}C]-HPPH obtained in the present study in monkey 2A. Figure 6 shows the plasma levels obtained after bolus i.v. injection of [^{11}C]-HPPH. The errors associated with these determinations are $\pm 5\%$. A discontinuity in the plasma concentration-time curve is observed from 30 to 50 min after administration. This may possibly be a result of enterohepatic recycling. It is a multiphasic curve, showing distributive and eliminative phases. Correlation of this discontinuity with the kinetic tracer-distribution studies revealed a rapid loss of activity from the liver region as shown in Figures 7a, b, c, and d, obtained from 5 to 34 min after administration, and appearance of activity in the intestinal field. These results indicate that large amounts of HPPH are secreted into the intestine through the bile. It is suggested that enterohepatic recycling is responsible for the observed discontinuity in the plasma concentration curve. Figure 7d shows significant accumulation of activity in what seems to be the gallbladder, as well as in the urinary bladder. It is concluded that some of the activity present in the intestinal field of monkey C (Fig. 4a) is due to HPPH formed from phenytoin metabolism. We cannot exclude the possibility, however, that sig-

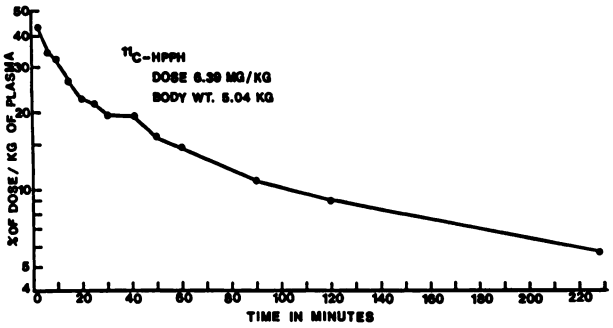

FIG. 6. Plasma levels observed in monkey 2A after bolus i.v. injection of HPPH (6.39 mg/kg) show a pause between 30 and 50 min after administration.

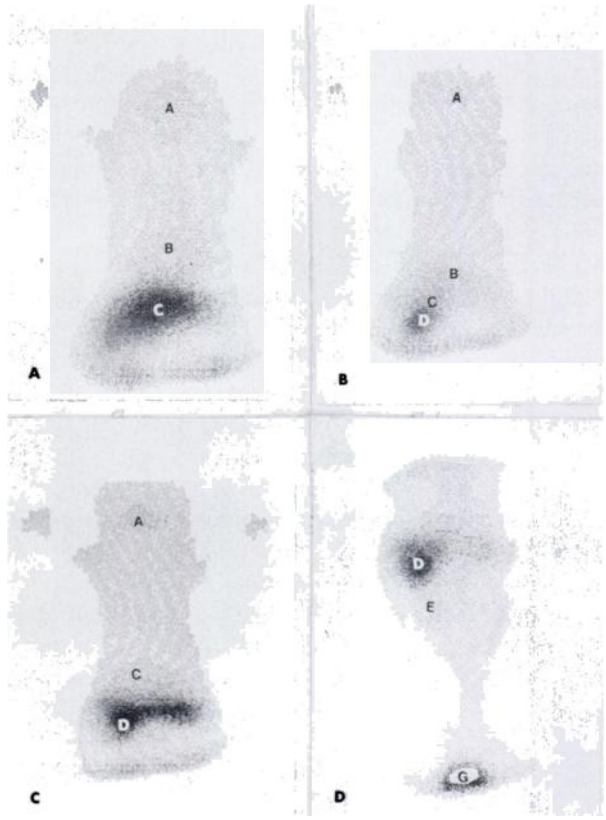

FIG. 7. (A, B, C) TOKIM dot displays following bolus i.v. injection of carbon-11 HPPH into monkey 2A. Anterior ventral view, head to liver; 5–6 min, 19–20 min, and 31 min, respectively, after administration. A = brain field; B = lung-heart field; C = liver field; D = gallbladder and possibly colon field(s). (D) Monkey 2A again, but liver to bladder, 24–29 min after administration. D = gallbladder field; E = intestinal field; and G = urinary-bladder field. White area in urinary bladder indicates high activity.

nificant amounts of phenytoin are also excreted into the intestine and then rapidly reabsorbed. This may explain the flat region seen in the plasma concentration curve of Fig. 2. This figure also shows that the decline of DPH levels is not a simple monoexponential process. This nonlinearity has previously been explained in terms of Michaelis-Menten kinetics, although the possibility of product inhibition by the major metabolite of phenytoin has been raised (*8,9*). This nonexponential decline and the flat region could also be explained, however, by a delayed reabsorption process taking place from the gut into the blood. This statement is based on the results obtained in monkey 2C when the influence of HPPH on the in-vivo distribution of DPH was studied. The results of this experiment indicate that there is a qualitative change in the distribution pattern of phenytoin in the presence of its major metabolite. The HPPH level at this time was 17 μg/ml of plasma. The plasma levels of DPH for monkey C and 2C are shown in Figs. 1 and 2. It is observed that the plasma levels of DPH are almost identical in the presence and absence of HPPH, possibly due to lack of inhibition of DPH metabolism by its major metabolite.

A closer examination of the in vivo distribution of [^{11}C]-HPPH, performed in monkey 2A, reveals that this compound is rapidly cleared from the liver and that it localizes in what seems to be the gallbladder (Figs. 7a, b, and d). These figures also show a discrete shadow suggesting the large bowel. In another experiment, a monkey received an intravenous bolus injection of [^{11}C]-HPPH and was killed at 45 min. Three or four specimens each of heart, liver, brain, colon (with contents), and pancreas were assayed for activity in a gamma counter. The results are expressed in terms of the dimensionless unit relative concentration, defined as percentage administered dose per percentage body weight, and in percentage dose per whole organ indicated by each specimen. The ranges and average relative concentrations for heart, liver, brain, pancreas, and colon and its contents are 1.3 (1.26–1.33), 1.67 (1.60–1.75), 0.47 (0.42–0.51), 0.41 (0.40–0.42), and 0.94 (0.89–1.03), respectively. Recently, Tubis et al. (*27*) suggested technetium-99m penicillamine as a cholescintigraphic agent, since the penicillamine is rapidly cleared by the liver and accumulates in the gallbladder. This behavior, as previously discussed, is analogous to that of the [^{11}C]-HPPH in vivo distribution and suggests the potential use of [^{11}C]-HPPH as a cholescintigraphic diagnostic agent.

FOOTNOTE

* Aldrich Chemical Co.

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