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A Physiologically-Based Pharmacokinetic (PBPK) Model for Alcohol Facilitates Rapid BrAC Clamping

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Alcohol clamping is a technique that maintains a constant breath alcohol concentration (BrAC) for prolonged intervals, thereby reducing experimental variance in the time course of organ exposure to alcohol, when compared with oral alcohol administration paradigms. The technique employs an intravenous (iv) infusion of an ethanol solution at a rate that is intermittently adjusted based on real-time BrAC measurements. In earlier studies, when the clamped state was induced with an oral ethanol loading dose, the vagaries of gastric emptying and absorption were associated with a 45 min delay (RST: reliable start time) before collection of dependent measurements could be planned with confidence. The objective of the present study was to develop an induction method that provides an earlier RST, and to compare the performance of the two methods. The "quick-clamping" method replaced the oral loading dose with a preprogrammed infusion rate profile. A three-compartment physiologically-based pharmacokinetic (PBPK) model for ethanol was constructed, then tailored to each subject using individualized estimates of model parameters. The model was used to compute the infusion-rate profile that would produce the desired time course of BrAC when infused in the corresponding subject. The two clamping methods were compared in a two-session crossover study in 20 healthy young subjects (10 males, 10 females). Compared with the oral/iv method, quick clamping produced a comparable precision in the control of BrACs during the clamped interval, and provided a much earlier RST (mean ± SE for quick-clamp: 17 ± 4 min; for oral/iv clamp: 45 ± 7 min). The quick-clamping method enables, for the first time, the examination of the early-phase neuroadaptive responses to alcohol in human subjects.

Key Words: Alcohol, Breath Alcohol Clamping, Physiologically-Based Pharmacokinetic Models, PBPK Model, Intravenous Infusion.

SEVERAL FACTORS THAT influence the pharmacokinetics (PK) of alcohol are under the experimenter's complete control; notably, the dose, route, rate, and duration of alcohol administration. Other factors, such as the subject's age, body weight, body composition, genetics, gender, disease-status, and food intake are less controllable (Dubowski, 1985; Sedman et al., 1976; Marshall et al., 1983; Fraser et al., 1995; Li et al., 1998). Thus, the literature on the PK of alcohol emphasizes the large inter- and intra-individual variability in the time course of breath and blood alcohol concentrations after oral alcohol administration (Holford, 1987; Pikaar et al., 1988; Friel et al., 1995). Combining a loading dose with subsequent intravenous (iv) infusion of any drug in order to reduce such variability is a time-honored method in the pharmacokinetic literature. The availability of instruments that provide real-time measurements of the breath alcohol concentration (BrAC) represented an opportunity for refining the method. The refinement came after applying feedback control theory to the task of achieving and maintaining a predetermined target BrAC for prolonged intervals. The method was dubbed BrAC clamping: following an oral loading dose, the rate of an intravenous infusion of a 6% v/v ethanol solution is adjusted manually every 5–10 min, based on results of serial BrAC measurements. An algorithm that reduces the difference between the next BrAC and the target concentration yields a steady-state BrAC even while the blood alcohol concentration (BAC) continues to change as a result of ongoing absorption, distribution, and elimination of alcohol (O'Connor et al., 1998). Hereafter, this method is referred to as oral/iv clamping.

Oral/iv clamping was developed with a specific application in mind. The phenomenon of decreased effect with prolonged exposure to a drug is called tolerance. Acute tolerance occurs when the adaptation develops within the time course of a single exposure. In humans, the large inter- and intra-individual variability in alcohol PK adds experimental variance to the evaluation of acute tolerance to alcohol (Martin and Moss, 1993). The variability is attrib-

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utable, in part, to the unpredictability of the ethanol concentrations achieved and to the uncertainty in the timing of assessment of dependent measures. BrAC clamping substantially reduces such experimental variance and also minimizes excursions from the target concentration during the interval between postalcohol measurements of function (O'Connor et al., 1998). The long clamped interval provides ample time for the repeated collection of multiple dependent measures. Because BrAC reflects arterial blood alcohol concentration (Jones et al., 1997) and the brain is a high-flow, low-volume organ, equilibration of brain alcohol levels and BrAC requires just a few minutes. Thus, any method of BrAC clamping may be particularly useful in research on the brain's acute functional adaptation to alcohol.

Nonetheless, the oral/iv BrAC clamp required improvement in one important aspect. The "reliable start time," RST, was defined as the shortest delay, after the start of alcohol administration, after which the BrAC *reliably* could be forecast to remain within ± 5 mg% of the target. RST coincides with the earliest time that collection of a battery of dependent measures of brain function can be planned so that the timing is the same for every subject. In earlier studies using the oral/iv clamp, RST was 42 min for a target BrAC of 50 mg% (O'Connor et al., 1998). We hypothesized that a shorter RST would increase the sensitivity of the clamping paradigm to the brain's functional adaptation to alcohol. Thus, the objective of this study was to develop and document a "quick-clamping" method that would reduce RST to less than 20 min. A target BrAC of 60 mg% was chosen for the work.

Uncertainties associated with gastric emptying and absorption kinetics of alcohol were the obvious cause of the long RST using the oral/iv BrAC clamp. The basic approach to quick-clamping was to replace the oral loading dose with an aggressive intravenous infusion rate profile that produces a steep, linear ascension to the target, and a constant BrAC thereafter.

METHODS

The study was conducted in two parts: a series of 10 experiments to develop the procedures for quick-clamping, and a 40-session comparison of the quick-clamp with the original oral/iv clamp in 20 subjects.

Part I: Development of the Quick-Clamp

The time course of quick-clamping is divided into three chronological phases. The *loading* phase begins with the onset of alcohol administration and ends with the first achievement of the target BrAC. During the *distribution* phase, BrAC is held constant while the venous blood alcohol concentration (BAC) catches up. Venous BAC is closer to the concentration of alcohol in the total body water (TBW), and alcohol distribution into the TBW requires a much longer interval than simply loading the arteries which dominates the loading phase. The RST usually occurs 5–7 min after the beginning of the distribution phase. The *steady-state* phase begins when BAC and BrAC are both steady, and are equal, and can be maintained with a constant infusion rate.

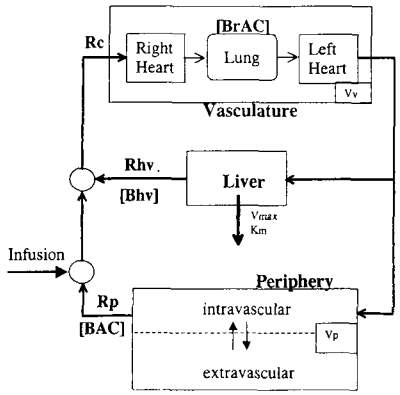

Fig. 1. The PBPK model architecture used in this study. Rc: cardiac flow rate; Rhv: hepatic flow rate; Rp: flow rate through periphery; BrAC, breath alcohol concentration; BAC, blood alcohol concentration; Bhv, hepatic alcohol concentration; Vp, volume of the periphery compartment; Vmax, maximal elimination rate; Km, Michaelis-Menten constant.

The first four developmental experiments provided data for the development of the basic scheme used to control BrAC during the rapid loading and distribution phases. Another six experiments were used to refine the loading and distribution phase procedures so that they could be performed without interfering with the scientific goals. The key to success was construction of a physiologically-based pharmacokinetic (PBPK) model for the distribution and elimination of ethanol.

The PBPK model. The PBPK model used for this study was developed using the Simulink toolbox from the Matlab® (Math Works Inc., Natick, MA) suite of mathematical script languages. Constructed as an analog computer program, lines and device symbols were connected in an intuitive way to construct the compartments out of physiologically meaningful pieces, to connect the compartments into an overall model, and to examine the time course of variables of interest. Units of the model were chosen as milligrams, minutes, and deciliters.

A three-compartment model of alcohol mass flow rate (amfr) was employed, comprising the vasculature, the liver, and the peripheral body water. The result (Fig. 1) is a simplification of a more sophisticated PBPK model developed by Rheingold et al. (1981). At the input and output of each compartment of the present model, amfr is the product of the local blood flow rate and the local ethanol concentration. The vasculature was modeled as a first-order differential equation that achieves an output amfr [(cardiac flow rate)*(BrAC)] by dynamic mixing of amfrs summed from three sources. The first source is the hepatic vein that carries a relatively low concentration of alcohol because it drains the principal site of alcohol elimination (i.e., the liver). The second source is the peripheral veins that drain the total body water compartment at a relatively high blood flow rate at a concentration equal to BAC. The third source is the infusion pump which has a relatively low rate determined by the experimenter, but carries a high ethanol concentration of 4800 mg%. The cardiac flow rate (**Rc**) and vascular volume (**Vv**) determined the time constant of the first-order differential equation, and were estimated for each subject.

The input to the liver compartment was modeled as the fraction of cardiac blood flow supplying the liver (set at 0.25), multiplied by the arterial amfr. The output of the liver compartment was the input mass flow minus the alcohol elimination rate (AER) determined by Michaelis-Menten kinetics. The hepatic Km was assumed to be small (set at 10 mg%), and the whole-body **Vmax** was attributed to the liver and estimated for each subject.

The input amfr to the peripheral body water compartment was modeled as the product of BrAC and the fraction (0.75) of Rc that did not go to the liver. The output of the peripheral compartment was the input amfr minus the net trans-vascular mass flow. The net flow was the difference between the amfr leaving the capillary bed to the extra-vascular part of TBW and the amfr entering the capillary bed from those tissues. The former was driven by the difference between BrAC and the concentration

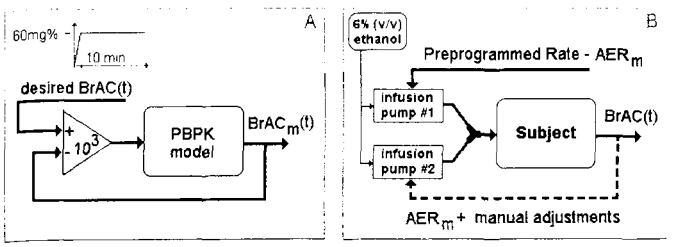

Fig. 2. A: The method for calculating the preprogrammed infusion rate profile to be used in the actual experiment forces $BrAC_m(t)$, the output of the PBPK model, to follow a desired time course by placing a large gain on any error and using the result as the infusion rate input to the model. **B**: In the actual experiment, pump #1 infuses the preprogrammed rate calculated in 2A. The rate of pump #2 is manually adjusted every 5–10 min based on feedback measurements of the subject's actual BrAC. The a priori estimate of the subject's AER used in the PBPK model is subtracted from one pump and added to the other for practicality.

of ethanol in the peripheral water space, and the latter was driven by the difference between the peripheral alcohol concentration and BAC. Each transfer pathway functioned only when the difference in concentrations driving it was greater than zero. Each transfer was modeled as a first order differential equation with a distinct partition coefficient, but with a common volume of peripheral distribution (**Vp**) that was estimated for each subject.

Four parameters of the PBPK model could be changed to characterize the kinetics of individual subjects. The Rc (dL/min) and Vv (dL) were calculated as fractions of body weight (0.8 dL/min/Kg and 0.22 dL/Kg, respectively). The volume of distribution of the peripheral compartment, Vp (dL), was estimated as TBW (dL) minus Vv, where TBW was calculated according the subject's height, weight, age, and gender (Watson, 1989). The maximum alcohol elimination rate, Vmax (mg/min), was estimated (albeit somewhat inexactly) by a combination of the subject's body weight, gender, and recent drinking history. Thus, all four model's parameters were estimated from easily assessed morphometric measures.

Computation of individual infusion profiles for quick-clamping. The PBPK model was used to precalculate the infusion rate profile necessary for each subject to achieve the desired BrAC-time profile. The calculation was performed by computing the instantaneous error, between a desired time course of BrAC(t) and the individual's modeled course, $BrAC_m(t)$, then multiplying that error by 1000 and using the result as the model's input (model infusion pump rate: see Fig. 2A). When the loop was closed, the very high gain on the instantaneous error forced that error to very small values at every point in time. Thus, the $BrAC_m(t)$ followed the desired time course, and the infusion rate profile that resulted from this forced feedback solution was recorded. Then that profile was used in the actual experiment on the corresponding subject.

Different individuals required different infusion profiles to achieve the same goal, but all the profiles had similar characteristics. The profile starts with a substantial infusion rate which then increases during the brief loading phase, ending in the range of 1400–2400 ml/hr of 6% v/v ethanol. The distribution phase begins as the BrAC reaches the target concentration, and is marked by a steep reduction in infusion rate at that moment. The reduction is followed by an exponential taper as alcohol continues to distribute into the TBW while the BrAC is held constant. In the steady-state phase, the rate of alcohol in equals the rate of alcohol out. Therefore, the asymptote of the tapering infusion rate, when multiplied by the concentration of alcohol in the infusate, is a direct measure of the subject's overall AER. Because the procedure was designed to be employed without a priori knowledge of a subject's AER, the need for feedback adjustments to the planned infusion rate profile during the distribution phase was anticipated.

For the quick-clamp, two infusion pumps were employed, with their outputs Y-connected into a single infusion line carrying the sum of the infusion pump rates (Figure 2B). In principle, the first pump was used to infuse the precalculated profile, whereas the second was used to make small manual adjustments based on real-time BrAC measurements. In practice, the estimate of the subject's AER that was used in the model was subtracted from the computed profile and used as the technician's initial pump rate. Thus, one infusion rate tapers to zero and requires no feedback modulation, while the small, intermittent adjustments to the technician's pump rate start at the best estimate of the subject's AER and achieve a precise measure of it over time. The method for calculating the adjustments was published previously (O'Connor et al., 1998). Because the actual AER was often different from the estimate, the rearrangement also compensated for inaccurate estimates of the AER in either direction. During the 10-min loading phase, the 30-sec delay associated with obtaining each BrAC sample left insufficient time for effective adjustment of the technician's pump rate, and only the preprogrammed rate profile pertained. Fortunately, inaccuracies in estimating the actual AER had little influence during the loading phase of the quick-clamp operation. Another modification to the routine published for the original oral/iv clamp was the need to preheat the infusate to near body temperature in the quick-clamp. Because fairly high infusion rates were administered in the first 15 min of the quick-clamp, some subjects perceived minor venous discomfort if the infusate was at room temperature.

For success, the sudden change in the infusion pump rate that marked the switch from loading to distribution phases must be accomplished at the precise moment BrAC reaches the target concentration. In order to predict that moment accurately, BrAC measurements must be obtained every 2 min on the ascending limb, and for a few samples thereafter. Our experience indicates the Alco- Sensor IV meter (Intoximeters Inc., St. Louis, MO) needs about 8 min to recover from a BrAC reading of ~60 mg% in order to provide an accurate estimate of the next sample. Thus, a substantial cost to the achievement of a rapid clamping capability is the need for five BrAC meters (the meters cost $700 each).

Part II: Comparison of Quick-Clamp to Oral/IV Clamp

The quick-clamp was compared with the oral/iv method with respect to RST and other indices of clamping performance in 20 subjects. Each subject underwent two clamping sessions with a target BrAC of 60 mg%, one using quick-clamping techniques, and the other using the oral/iv clamping method. The order in which the clamping sessions were conducted was alternated between subjects. Different technicians performed the two methods on any one subject, without knowledge of the AER obtained in any preceding experiment on that subject.

In every session, a 50-min data-collection block of several dependent measures of brain function was obtained three times. Collection assured that clamping procedures were subordinated to the preliminary assessment of the presumption that faster start times yielded greater sensitivity to acute adaptation of brain function to alcohol. By choosing the sample to represent different family histories of alcoholism, testing of other interesting postulates was piloted. Results pertaining to the latter two purposes will be presented elsewhere.

Subjects. The study was conducted in young, healthy, paid volunteers. Ten male and 10 female subjects were recruited by local advertisement and were considered for inclusion if they were low to moderate social drinkers ranging in age from 21 to 39 (median 24) years. Subjects were in good health, and had either no first-degree relatives who ever met DSM IIIR criteria for alcohol dependence, or else had two or more such biological relatives excluding the subject's mother. Subjects with a clinically significant history of renal, hepatic, cardiovascular, pulmonary, or gastrointestinal disease were excluded, as were subjects with a DSM-III-R Axis 1 illness, including substance abuse, history of seizure or loss of consciousness, history of mental illness requiring hospitalization, and current illness requiring psychoactive medication. Subjects provided informed consent for the protocol approved by the Institutional Review Board.

Experimental procedures. Subjects were admitted to the General Clinical Research Center at Indiana University Hospital at 7:00 AM, having been instructed to abstain from alcohol for at least 36 hr and from food for at least 12 hr. A negative urine beta-hCG test for pregnancy was obtained

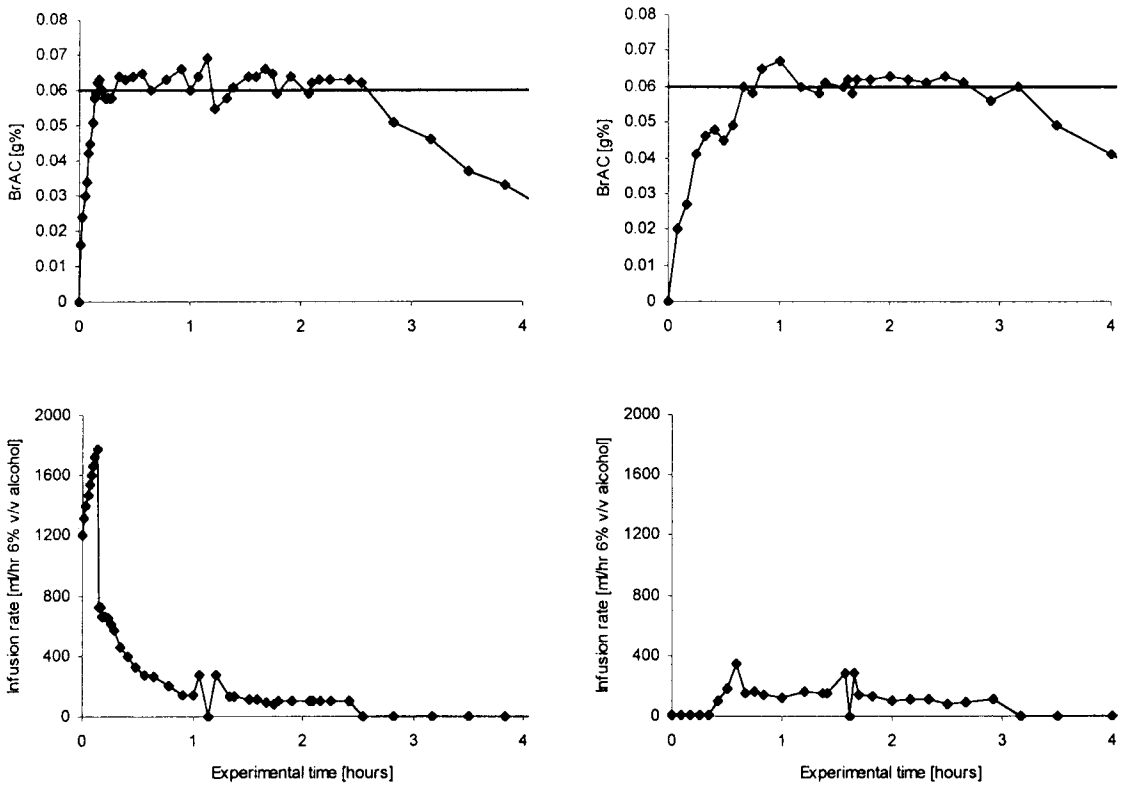

Fig. 3. Upper: BrAC vs. time profiles for the two clamping sessions in a 22 year old, 63 kg, 156 cm female. **Lower**: infusion rate vs. time profiles administered to the subject to produce the BrAC-time profiles. **Left**: quick-clamping method; **Right**: oral/iv method. The effects of accommodating the subject's need to urinate are apparent at 1:00 in the quick-clamp and at 1:45 in the oral/iv clamp, respectively.

from female subjects before the start of each session. At 7:30 AM, subjects ate a 350 calorie breakfast that consisted of eggs, toast, jelly, and juice. An indwelling catheter was inserted into a vein in the antecubital fossa of each arm: one for infusion and the other for blood sampling. Between 8:30 and 10:00 AM, preparation for, and baseline testing of, dependent measures were performed.

BrAC clamping. The oral/iv clamping method has been described in detail elsewhere (O'Connor et al., 1998). For the quick-clamping method, there was no oral loading dose. The infusion of ethanol (6% v/v in Ringer's Lactate) was administered using the procedures described in the preceding section. In both methods, RST was declared when at least three consecutive BrAC measurements were within ± 5 mg% of the target *and* the technician expressed confidence that control over the BrAC had been achieved. RST occurred 5–7 min after the BrAC reached the target, and marked the beginning of the first postethanol data-collection block of dependent measures. Clamping continued until the completion of the second postethanol data-collection block, 140 min later. During data-collection blocks, sampling of BrAC for clamping purposes was permitted only between tasks.

Data analysis. For both clamping methods, the following measures of clamping performance were calculated:

1.) RST: the time at which the BrAC was declared clamped, as described above. The measurement is made in experimental time: elapsed time after the start of alcohol administration.
2.) Mean BrAC and variation (SD) of BrAC readings (mg%) obtained during the 140 min long clamped interval (typically around 20 readings).
3.) Target Error was calculated as the difference (mg%) between the target BrAC and the mean BrAC achieved during the clamping interval.
4.) Mismatch was calculated as the average absolute difference (mg%) between the mean BrACs obtained during the two postethanol data-collection blocks.

The goal for BrAC variation, target error, and mismatch during both clamps was zero.

RESULTS

All subjects completed both clamping sessions of the study without complaint. In fact, they seemed to enjoy the

Table 1. Comparison of Clamping Performance Measures for Oral/iv vs. Quick-Clamping Methods in 20 Subjects; Target BrAC = 60mg%. The Last Column Presents the Results of Paired-t Tests of the Difference in Performance Measures for the Two Methods. A One-Tailed Criterion Was Used for RST; Two-Tailed Criteria Otherwise.

Clamping Performance Measures	Oral/iv Clamp	Quick Clamp	Significance Testing, df = 19
Mean (SD) Clamp Start Time, RST, [minutes]	45 (7)	17 (4)	$t = 8.33$; $p < 10^{-8}$
Mean (SD) BrAC during Clamp [mg%]	60.2 (1.6)	59.4 (1.3)	$t = 2.13$; $p < 0.05$
Mean (SD) BrAC Variation during clamp [mg%]	2.8 (0.8)	2.9 (0.5)	$t = 0.71$; $p > 0.4$
Median (Range) Target Error [mg%]	0.9 (0.1–3.4)	1.2 (0.1–2.8)	$t = 0.47$; $p > 0.6$
Median (Range) Mismatch [mg%]	3.6 (0.6–7.1)	1.6 (0.2–7.7)	$t = 1.46$; $p > 0.1$

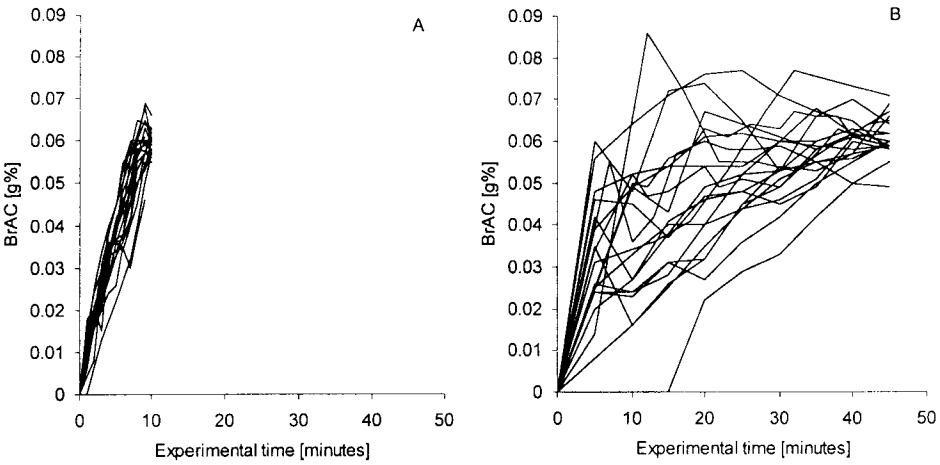

Fig. 4. A: BrAC vs. time profiles for samples taken during the ascending phase of the quick-clamp for all 20 subjects in the study. **B**: BrAC vs. time profiles for samples taken during the ascending phase of the oral/iv clamp for the same subjects.

effects of the fairly high infusion rates during the first 10 min of the quick-clamping session.

Both clamping methods were able to achieve and maintain the target BrAC for the duration of the experiment (Fig. 3). Table 1 reports clamping performance measures for both methods: the mean BrAC during the clamped interval differed by 0.8 mg%; a negligible, but statistically significant, amount (t = 2.13; p = 0.046). The primary difference was in RST; the mean (± SD) was 17 (± 4) min for the quick clamp and 45 (± 7) min for the oral/iv method. A one-tailed paired t test of the difference between mean RST yielded t = -8.33, df = 19; $p < 10^{-8}$, indicating that the quick clamping technique is able to produce significantly earlier RST compared with the oral/iv method. No other performance measure showed a significant difference between methods.

In addition to achieving earlier RST, the quick-clamping method achieved a linear ascending limb during the loading phase of the clamp. This new ability to control the shape of the ascending limb is illustrated in Fig. 4A, and is in sharp contrast to the substantial inter-individual variability in the shape of the ascending limb after an oral dose of ethanol in the same subjects, Fig. 4B. For each subject, the oral loading dose was calculated to yield a peak BrAC of 50 mg%, on the basis of the individual's TBW.

DISCUSSION

The results of this study indicated that BrAC clamping with a reliable start time less than 20 min was possible, through the application of a PBPK model of ethanol distribution and elimination. Other performance measures for "quick-clamping" are comparable to those for the previously established oral/iv clamping method (O'Connor et al., 1998). The new procedures did not interfere with the repeated collection of dependent measures of the brain's initial response and acute adaptation to alcohol (results to be published elsewhere).

In developing the PBPK model, we followed the principle that any model should describe the relevant physiology and include all essential compartments while avoiding unnecessary detail. Our model consists of only three compartments. Two of them are essential because they account for the distribution dynamics of a small, water soluble molecule (ethanol) in the intravascular and extravascular water spaces. A third compartment was required to model alcohol elimination. We labeled this compartment the liver because, in humans, it accounts for over 90% of alcohol elimination with well-known Michaelis-Menten kinetics. Researchers interested in the physiology of extra-hepatic routes of elimination (e.g., renal and transdermal excretion, pulmonary expiration, gastric or muscle metabolism, etc.) would find it necessary to add one or more compartments to the PBPK model and to measure the time course of the relevant output concentration to validate the extension. Researchers interested in modeling the intricacies of different hepatic metabolc pathways would require a more complex compartmental model compared with the one chosen in this study.

In the oral/iv clamping method, the technician treated the alcohol being absorbed from the gut as the loading mechanism, and made intermittent, small adjustments of an additional input (the infusion pump rate) to achieve and maintain the target BrAC. In the quick-clamp method, the precalculated infusion rate profile replaced the oral dose as the loading mechanism. Tailoring the PBPK model parameters to a subject's individual physiology provided an ability to understand and anticipate each subject's PK response. Application yielded a linear ascension of BrAC from 0 mg% to the target of 60 mg% in 10 min followed by steady BrACs within a ± 5 mg% window of the target for up to 3 hr. During the distribution phase of the clamp, relatively small and intermittent feedback adjustments to the infusion rates proved sufficient to compensate for any inaccuracies in the model architecture and estimation of the individual's model parameters. Thus, the PBPK model demonstrated face validity by successful application. Further validation of the PBPK model would require simultaneous measurement of breath and blood alcohol levels, and success in controlling the time course of BrAC in more complex paradigms.

Traditionally, PBPK models are developed to describe quantitatively the physiological disposition of a drug in the body (Pastino et al., 1996; Leung, 1991). The PBPK model used in this project typifies the genre by employing a series of mass balance equations to predict the concentration of the drug in various physiological compartments over time for a given input regimen (D'Souza and Boxenbaum, 1988). PBPK models can be scaled between species and across drugs, and predict the effect of physiological perturbations on drug disposition (Pastino et al., 1996, 1997; Suzuki et al., 1995; Hoang, 1995). In this study, an additional application of PBPK modeling was invented: forcing the model to follow the desired time course of BrAC resulted in computation of the infusion rate profile required for the actual task. This device can achieve BrAC versus time profiles, other than the one used for BrAC clamping (Fig. 3), that may prove useful in future research. For example, a subject's brain could be exposed to a steady concentration for 1 hr at each of four different levels in a single session. In a similar fashion, the AER could be calculated as a function of BAC in a single session. Alternatively, an individual's BrAC response to an oral dose could be recorded, then precisely replicated in the same or other subjects. A variety of shapes of the ascending limb of the BrAC-time curve could be prescribed in order to examine the brain's sensitivity to the rate of change of alcohol concentration. Other possibilities include physiologic scaling of the model parameters in order to apply the quick-clamp method to animal studies of the response to alcohol.

Compared with the method that begins with an oral loading dose, the quick-clamp method yields a shorter loading phase and an earlier RST. Nonetheless, oral/iv clamping retains some methodological advantages. Oral loading is naturalistic, and may be important in studies that investigate the expectancy or gustatory effects of the oral consumption of alcohol. Because the alcohol concentration in the oral dose is much higher than the concentration of the infusate (25% vs. 6%), there is less water-loading in the oral/iv compared with the iv-only method; about 220 ml less for the example shown in Fig. 3. One result is that the subject's need to urinate occurs later with oral/iv clamping. Establishing a steady state, during which the alcohol elimination rate and alcohol infusion rate are equal, currently requires the same amount of time with both methods. The oral/iv clamp has been used to study the effect of gender, lean body mass, and liver size on AER (Kwo et al., 1998), and ongoing studies are examining other factors such as ethnicity, genotype, and food intake.

The combination of PBPK modeling and BrAC clamping seems to be new to the alcohol research literature. An attempt to use BrAC clamping to study the acute adaptation of brain function to alcohol was reported by Lehtinen et al. (1981), and the clamped steady-state provided measurements of the AER for studies of alcohol metabolism performed later (Mascord et al., 1988, 1991). All three used iv infusion as a loading mechanism, but none employed any modeling. Up to 90 min were required to establish the clamp. Two other studies have used compartmental PK models for alcohol to calculate infusion rate profiles designed to achieve a target BrAC in 1 or 2 hr and then maintain the BrAC constant for several more hours (Hartmann et al., 1988; Ramchandani and Venitz, 1996). However, both studies required data from a separate infusion session in order to estimate the PK parameters that were used to calculate the infusion profiles for each subject. Neither study employed the power of making feedback adjustments to the precalculated infusion rate in order to compensate for modeling inaccuracies. The blending of PBPK modeling and BrAC clamping achieves an accurate clamp, with a start time less than 20 min, the first time the subject is tested. Because each testing session requires several hours, the improvement represents a nontrivial accomplishment.

The use of Matlab and Simulink was not necessary, but was more than sufficient for the development and application of the PBPK model. The ability to appreciate the impact of changes in model architecture was particularly useful during the developmental phase of this study. For example, one key to implementing the quick-clamp was the division of the infusion into two parallel pathways as shown in Fig. 2B. Recognition of the solution to an otherwise serious human factor problem was facilitated by the graphical nature of Simulink. Another example is the time saved in training technicians in clamping techniques. By simulating a manually controlled infusion pump input to the model, and then running the simulation at 10 times real time, the technician could practice on a variety of "subjects" in a single day. Once Matlab and Simulink are installed, our PBPK model can be run on virtually any modern computing platform and operating system.†

In summary, the rapid clamping method allows reliable and early establishment of the BrAC clamp. The PBPK model for ethanol allows the precalculation of individual infusion-rate profiles to achieve precise control of the breath alcohol concentration, and therefore the brain's exposure to alcohol. BrAC clamping represents a refinement of traditional methods for administering ethanol in alcohol challenge studies and provides a useful platform to study both the determinants and consequences of the pharmacological properties of alcohol.

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†Simulink scripts for our PBPK model, and for the method of computing the required infusion profiles, and that simulate on-line BrAC clamping, are available to interested investigators by email or floppy disc, gratis, provided the source is cited in consequent publications.

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