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THE EFFECT OF ACTIVATED CHARCOAL ON MOUSE SLEEP TIMES INDUCED BY INTRAVENOUSLY ADMINISTERED HYPNOTICS

LAURI J. ADLER, DONALD H. WATERS AND PETER R. GWILT*

School of Pharmacy, West Virginia University Medical Center, Morgantown, West Virginia 26506, U.S.A.

ABSTRACT

The effect of orally administered activated charcoal (AC) on the sleep times of mice following intravenous injection of various hypnotics was investigated. Preliminary studies with phenobarbital (Pb) showed that a linear relationship exists between the Pb-induced sleep time and the logarithm of the Pb dose in both control and AC treated mice. Half-lives of Pb in the two groups were estimated to be 8·1 and 0·9 h, respectively. A linear decline in Pb-induced sleep time with increasing dose of AC was observed up to a maximum effective dose of AC beyond which dose increments caused no further reduction in sleep time. A similar relationship was observed between sleep time and the concentration of sodium sulfate in which the AC was suspended.

AC treatment resulted in an 82–88 per cent reduction in sleep time induced by administration of phenobarbital, methyprylon, glutethimide, ethchlorvynol, and methaqualone. AC had no significant effect on sleep time following amobarbital or pentobarbital administration.

KEY WORDS Mouse sleep time Activated charcoal Intestinal clearance Hypnotics

INTRODUCTION

Activated charcoal (AC) has proved useful in the treatment of acute drug intoxication largely due to its effectiveness in reducing drug absorption. Recent studies have demonstrated that large, multiple, oral doses of AC increase the rate of elimination of the following drugs from the body: phenobarbital,[1] carbamazepine,[2] phenylbutazone,[2] digitoxin,[3] methotrexate,[4] theophylline,[5] nadolol,[6] proscillaridin,[7] and dapsone.[8] The clearance of certain other drugs, however, does not appear to be affected by AC administration. For example, while the half-life of phenobarbital is

*Addressee for reprints.

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dramatically reduced by AC administration, preliminary studies indicated that the effect of orally administered AC on the elimination of amobarbital, cyclobarbital or pentobarbital in intoxicated patients was not very promising.[9] At the present time the factors influencing the rate of removal of a drug from the body by AC have not been systematically studied. It is not possible therefore to predict which drugs are susceptible to AC treatment and which are not. Studies evaluating AC treatment on the disposition of individual drugs will require as many experiments as there are drugs: furthermore many subjects will be exposed to an unpleasant study protocol involving parenteral administration of drug accompanied by oral ingestion of large amounts of AC. Animal experiments may therefore prove useful in predicting which drugs are eliminated more rapidly from the body as a result of AC treatment. In addition, by monitoring the change in a well-defined and easily measured pharmacologic end-point, whole classes of drugs could be rapidly screened as to their susceptibility to AC treatment without resorting to chemical assays for individual drugs.

Using mice, we have examined the effect of orally administered AC on the sleep times induced by various intravenously administered hypnotics. In addition we have examined some of the factors that might influence the effect of AC on drug clearance.

MATERIALS AND METHODS

Female Swiss Webster mice (Hilltop Lab Animals, Inc., Scottdale, PA.), weighing 18–22 g were used. Mice had access to food and water at all times. Animals receiving AC treatment were administered 1 ml of a suspension of 25 mg AC in a 2 per cent sodium sulfate solution by oral intubation every 20 min beginning 1 h before drug administration and ending at the time of injection. This protocol was followed to ensure that AC reaches the intestines, the presumed site of interaction of AC with the drug. This was verified in initial experiments by examining intestinal contents. Once such an interaction has been established additional experiments relating the time of AC application to drug administration can be undertaken. Sodium sulfate was used because in preliminary experiments administration of AC suspended in water caused the AC dose to be deposited in the stomach. Addition of sodium sulfate facilitated passage into the intestines. Control mice received no prior treatment.

The mice received intravenous injections of the following drugs via the tail vein:

1. Phenobarbital (118 mg kg^{-1}) as the sodium salt dissolved in water with an injection volume of 10 ml kg^{-1}.
2. Methyprylon (159 mg kg^{-1}) dissolved in water with an injection volume of 10 ml kg^{-1}.

3. Glutethimide (100 mg kg^{-1}) dissolved in mixture of 40 per cent polypropylene glycol, 10 per cent ethanol, and 50 per cent water with an injection volume of 10 ml kg^{-1}. The solution was injected at a rate of 0·01 ml min^{-1}.
4. Methaqualone (60 mg kg^{-1}) dissolved in a mixture of 30 per cent polypropylene glycol, 30 per cent ethanol, and 40 per cent water with an injection volume of 2·5 ml kg^{-1} and an injection rate of 0·0075 ml min^{-1}.
5. Ethchlorvynol (125 mg kg^{-1}) mixed with 35 per cent ethanol and 60 per cent water with an injection volume of 2·5 ml kg^{-1} and an injection rate of 0·0075 ml min^{-1}. The ethchlorvynol constituted 5 per cent by volume of the total solution.
6. Pentobarbital (45 mg kg^{-1}) as the sodium salt dissolved in water with an injection volume of 10 ml kg^{-1}.
7. Amobarbital (75 mg kg^{-1}) as the sodium salt dissolved in water with an injection volume of 7·5 ml kg^{-1}.

Sleep times were measured in terms of loss and re-establishment of the righting reflex. Mice were placed in a V-shaped trough so that the action of righting was a purposeful movement. Re-establishment of the righting reflex was based on the ability of the mouse to right itself three times in 30 s.[10] The effect of the dose of phenobarbital on the sleep times of both control and AC treated mice was examined. Five mice in each group received injections of 105, 115, 118, and 130 mg kg^{-1} phenobarbital as the sodium salt. The AC treated mice received AC in the manner described above. Sleep times for both groups were measured and compared with the dose according to equation (1)[11]

$$t_d = \frac{2{\cdot}303}{K} \log X_o - \frac{2{\cdot}3030}{K} \log X_{min} \qquad (1)$$

where t_d is the duration of pharmacologic effect, in this case sleep time, K the apparent elimination rate constant, X_o the dose of phenobarbital administered, and X_{min} the minimum dose required to elicit a pharmacologic effect. Data were fitted to the equation using least-squares regression and the slopes of the lines yielded values for the half-life of phenobarbital with and without AC treatment.

The effect of varying the AC dose on sleep time was investigated. Groups of five mice received 118 mg kg^{-1} phenobarbital as the sodium salt following treatment with AC doses of 0, 1, 2, 4, and 5 g kg^{-1} administered in a 2 per cent sodium sulfate solution. Sleep times at each dose level were recorded and compared.

The effect of the sodium sulfate concentration on mouse sleep time was also studied in order to determine the concentration of sodium sulfate which would provide maximum reduction in sleep time. Groups of five mice received a 25 mg dose of AC as described above. The concentration of the

sodium sulfate was varied from 0, 1, 2, and 3 per cent. A dose of 118 mg kg^{-1} phenobarbital as the sodium salt was injected immediately following the last AC administration.

The effect of AC on mouse sleep times induced by various hypnotics was examined. Twenty mice were randomly divided into two groups for each drug studied. One group received AC treatment and the other received no prior treatment. An identical dose of hypnotic was injected into the tail vein of both treated and control mice. Sleep times for both groups were measured and compared using Student's non-paired *t*-test.

RESULTS

Sleep times observed at different doses of phenobarbital for both AC treated and control mice are shown in Figure 1. Data were plotted according to equation (1). The lines through the points represent the best fit of the data to a straight line determined by least squares regression. The slopes of the lines yielded values for the half-life of phenobarbital in control and AC treated mice of 8·1 and 0·9 h, respectively.

The effect of the dose of AC and the concentration of sodium sulfate on phenobarbital induced sleep times are shown in Figures 2 and 3, respectively.

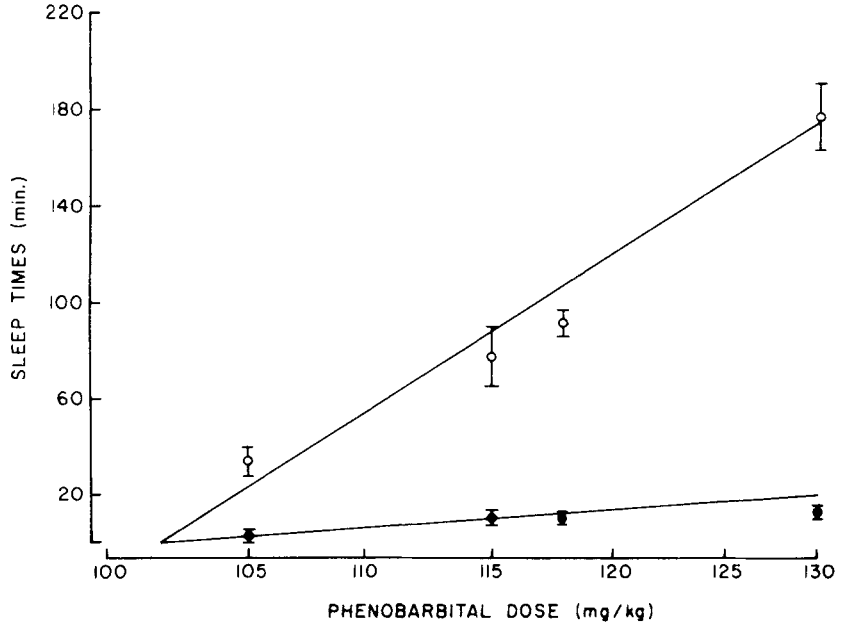

Figure 1. Mean (± standard error) sleep time as a function of phenobarbital dose. (○) control and (●) charcoal treated mice

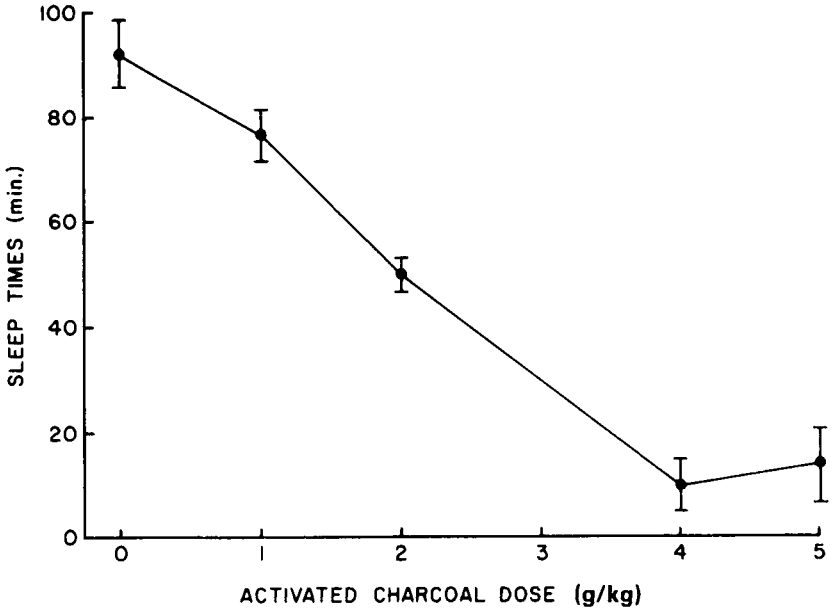

Figure 2. Mean (± standard error) phenobarbital induced sleep time as a function of charcoal dose

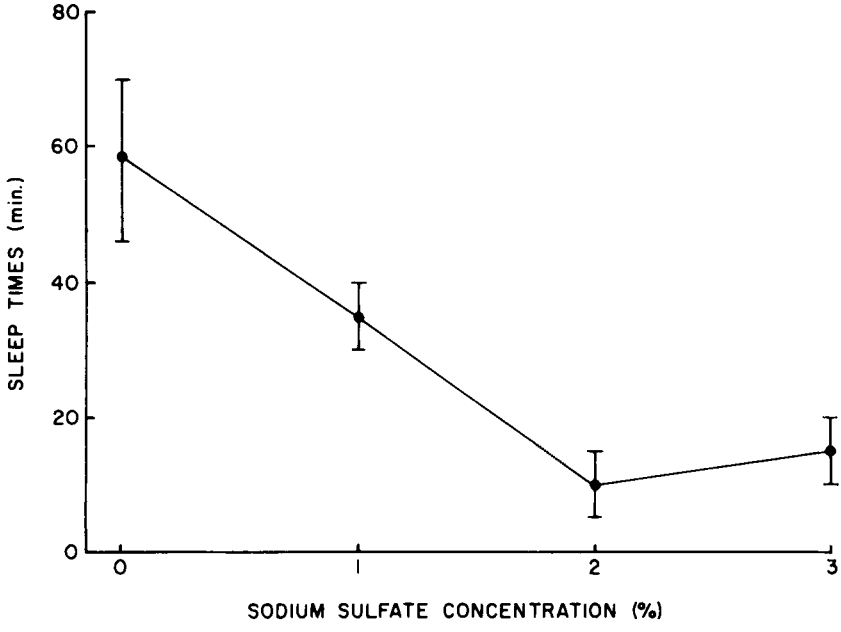

Figure 3. Mean (± standard error) phenobarbital induced sleep time as a function of sodium sulfate concentration

Table 1. The effect of orally administered activated charcoal on sleep time in mice following intravenous administration of various hypnotics

Drug	Average sleep time (h) Control	Activated charcoal
Phenobarbital	1·53* ± 0·31†	0·18* ± 0·09‡
Methyprylon	1·10 ± 0·36	0·20 ± 0·11‡
Glutethimide	1·08 ± 0·19	0·13 ± 0·11‡
Methaqualone	2·60 ± 0·55	0·33 ± 0·21‡
Ethchlorvynol	1·04 ± 0·24	0·18 ± 0·14‡
Pentobarbital	1·01 ± 0·32	0·94 ± 0·33
Amobarbital	1·23 ± 0·36	1·39 ± 0·28

*Values represent the average of 10 mice.
†Standard deviation.
‡Statistically significant difference ($p < 0·01$).

In both cases an apparently linear relationship holds between sleep time and the dose of AC or sodium sulfate until maximum value is reached. Beyond this value, increases in the amount of AC or sodium sulfate fail to produce any further reduction in sleep time.

The effect of AC on the sleep times induced by various hypnotics is reported in Table 1. AC treatment resulted in an 82–88 per cent reduction in the sleep times of all hypnotics except amobarbital and pentobarbital. The duration of sleep induced by these drugs was unaffected by oral administration of AC.

DISCUSSION

The results of this study show that orally administered AC suspended in a 2 per cent sodium sulfate solution can significantly reduce mouse sleep time induced by intravenously administered hynotics. The immediate cause of this effect is most likely an increased decline in the concentration of hypnotic in the brain associated with a similar decline in drug concentrations in the blood. This hypothesis is supported by the study examining the effects of phenobarbital dose on the sleep times of both treated and control mice (Figure 1). The data in this figure conform to equation (1) relating the duration of pharmacologic effect to the logarithm of the dose. Analysing the relationship between the duration of effect and the dose of phenobarbital by this means for both control and treated mice suggests that AC treatment affects only the elimination rate of the drug and not the relationship between the pharmacologic effect and the concentration of drug in the body. Thus the

slopes of the lines were markedly different, reflecting a tenfold decrease in the half-life of phenobarbital with AC treatment. By contrast the intercepts of both lines, as estimated by least squares regression, were identical, indicating that the minimum effective amount in the body (or minimum effective concentration in the blood) required to elicit a pharmacologic effect was the same for both control and treated mice.

This simple relationship between the duration or intensity of effect and drug concentration in the blood, however, may not hold true in the case of severe barbiturate overdose. Pond *et al.*[12] recently reported a study with 10 patients being treated for barbiturate overdose. Five of the patients received large, multiple, oral doses of AC whereas the other five patients were administered only a single AC dose. While the phenobarbital concentrations in the blood of the patients receiving multiple AC doses declined more rapidly compared to those receiving a single AC dose ($t_{1/2}$, multiple dose = 36 h; $t_{1/2}$ single dose = 93 h) the duration of respiratory depression was identical for both groups. Furthermore two similarly overdosed patients received haemoperfusion and the duration of respiratory depression was much shorter than either of the other two groups of patients. It would appear that with doses of phenobarbital much higher than those used in our study, i.e. doses that cause respiratory depression, the relationship between effect and phenobarbital concentrations in the blood is more complex.

The study relating the size of the AC dose and sleep time induced by phenobarbital indicates that there exists a maximum effective dose of AC. When amounts of AC smaller than this dose are administered, the rate of removal of drug from the body may be limited by the amount of AC available to the drug. At higher doses there is a sufficient quantity of AC to adsorb the drug and other factors such as the rate of diffusion through the gut wall or intestinal blood flow become rate limiting. Two other studies in the literature report similar findings.[13,14] Following a 60-minute 6 mg kg^{-1} aminophylline infusion, 5, 10 or 20 g AC were administered every 2 h for 6 doses. The half-life of theophylline was reduced from control values by 62, 60, and 47 per cent, respectively. While the 5 g doses of AC substantially increased the elimination rate of theophylline, only relatively small increases in theophylline clearance were seen with the additional dose increments of AC.[13] A subsequent study by the same investigators compared charcoal with a large surface area (PX-21) with the standard AC available for clinical use (Norit USP XX).[14] The area under the theophylline plasma concentration-time curve was 77·4 mg l^{-1} h^{-1} for PX-21 and 88·9 mg l^{-1} h^{-1} for an equal dose (5 g AC every 2 h for 6 doses) of Norit USP XX; again demonstrating that at low doses of AC the surface area of AC may be the limiting factor in the rate of removal of drug from the body.

A similar relationship exists between the concentration of the sodium sulfate solution used to suspend the AC and the sleep time induced by phenobarbital. Using the maximum effective dose of AC found in the

preceding experiment, the concentration of sodium sulfate was varied from 1 to 3 per cent. The maximum effective concentration was found to be 2 per cent. Concentrations of sodium sulfate greater than this produced no further reduction in sleep time.

The effect of sodium sulfate on the action of AC has also been noted by Chin *et al.* in rats.[15] AC suspended in a 6·5 per cent solution of sodium sulfate reduced concentrations of aspirin, chloroquine, and pentobarbital in the blood more than an equivalent dose of AC suspended in water. The mechanism by which sodium sulfate enhances the effect of AC on drug clearance is not certain. It is possible that osmotic retention of fluid in the gastrointestinal tract dilutes the drug in the intestinal fluids thereby decreasing the concentration gradient driving drug reabsorption. Alternatively, increased intestinal propulsion may move the AC-drug complex to more distal regions of the gut that are less permeable to phenobarbital reabsorption. Inclusion of effective concentrations of sodium sulfate could be of particular importance in the treatment of hypnotic overdose when the motility of the gut may be markedly reduced.

The administration of oral AC to mice receiving various intravenously administered hypnotics results in a notable reduction in sleep time as shown in Table 1. With the exception of amobarbital and pentobarbital, sleep times were reduced by 82–88 per cent suggesting that AC treatment may be useful in the management of acute intoxication by these compounds. Furthermore the mouse may be a useful model in predicting which drugs are significantly removed from the body by AC treatment. As mentioned before, the clearance of phenobarbital in man is greatly increased by AC whereas that of amobarbital and pentobarbital appears to be unaffected by the treatment. The reasons for the difference in the response of phenobarbital (Pb), amobarbital (Ab), and pentobarbital (Pt) to AC treatment is not clear. The plasma protein binding of the drugs in man is similar (Pb = 52 per cent,[16] Ab = 58 per cent,[17] Pt = 60 per cent[18]) and the volumes of distribution are also comparable (Pb = 49 l,[20] Ab = 64 l,[19] Pt = 63 l,[18]). The total body clearance of the drugs in the absence of AC treatment however is quite different (Pb = 0·3 $l\ h^{-1}$,[20] Ab = 2·2 $l\ h^{-1}$,[19] Pt = 2·0 $l\ h^{-1}$,[18]). Therefore if the clearance due to AC is about the same for each drug, the effect of AC on the total body clearance would be proportionately greater for phenobarbital than for either amobarbital or pentobarbital.

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