Age and the Pharmacokinetic–Pharmacodynamic Relationship of Phenobarbital in Rats: "Pseudo"-Longitudinal vs Cross-Sectional Study Design

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In a previous study, an apparent age-related increase in brain sensitivity to the anesthetic effect of phenobarbital was observed in BN/BiRij rats. However, since this study was conducted according to a cross-sectional design, the observed change could, in principle, also have been the result of a cohort effect. The purpose of the present investigation was to exclude the role of such a cohort effect by adopting a "pseudo"-longitudinal study design. In this design 45 animals out of one cohort were reserved, and one subgroup was investigated at five ages (7, 14, 21, 29, and 34 months). A decrease in the anesthetic threshold dose of phenobarbital was found during aging, which appeared to be due mainly to an increase in the brain sensitivity. It is concluded that the previously observed increase in brain sensitivity is indeed the result of the aging process rather than a cohort effect.

KEY WORDS: pharmacokinetics; pharmacodynamics; aging; phenobarbital; cross-sectional; "pseudo"-longitudinal.

INTRODUCTION

Increasing age appears to be associated with changes in sensitivity to a large number of drugs, which is reflected in alterations in dose requirement (1,2). In order to investigate further the mechanism of these changes in sensitivity in terms of pharmacokinetics and pharmacodynamics, we recently conducted a series of studies with drugs acting on the central nervous system in an animal model of aging. In one of these studies, an apparent increase in brain sensitivity to the anesthetic effect of phenobarbital as measured on the basis of the "loss of righting reflex technique" was observed (3). This investigation, however, was conducted according to a cross-sectional design (different groups of rats aged between 4 and 36 months), which means that the observed change could, in principle, also have been the result of a "cohort" effect. In a cross-sectional study, groups of different ages are investigated (ideally at one time point). The animals originate therefore from different cohorts. Considerable cohort differences have been observed with respect to physiology (4). Moreover, differences between the outcome of cross-sectional and that of longitudinal study designs have been reported for the body composition in young and old Sprague–Dawley rats (5).

The purpose of the present investigations was to exclude the role of such cohort effects by adopting a "pseudo"-longitudinal study design.

MATERIALS AND METHODS

Animals

Forty-five male BN/BiRij rats (TNO-IVVO, Leiden, The Netherlands), born in the 45th, 46th, or 48th week of 1988 were used. At the time of the start of the study, they were randomly assigned to five subgroups to be studied at the ages of 7, 14, 21, 28, and 34 months, respectively. A total of eight rats died before study initiation. All animals were healthy at the time they were studied. Five groups of eight 5- to 7-month-old animals served as controls. Together with every subgroup a control group was included to detect possible differences caused by the experimental conditions at the time of the year during which the experiments were performed or for potential cohort differences. The 10, 50, and 90% survival ages of the BN/BiRij rats are 38.1, 31.7, and 22.8 months, respectively.

Chemicals

Phenobarbital sodium was purchased from Brocacef (Maarssen, The Netherlands).

Animal Experiments

Pharmacokinetic Evaluation. Phenobarbital was administered as an intravenous bolus dose of 20 mg/kg via the penal vein under light halothane anesthesia. From an incision in the tail, 11 blood samples of 120 μl were taken in a period of 48 hr after administration.

Pharmacodynamic Evaluation. The evaluation was performed 2 weeks after the pharmacokinetic evaluation. One day before the experiment, the jugular vein was cannulated under halothane anesthesia. Phenobarbital was infused via the jugular vein cannula at a rate of 3 mg/min until the point of loss of righting reflex. At this point, cerebrospinal fluid, blood, and brain tissue were collected (6).

Drug Analysis

The concentrations of phenobarbital in plasma, cerebrospinal fluid, and brain tissue were measured by an HPLC method described by Danhof and Levy (6). Protein binding of phenobarbital was determined by means of ultrafiltration and the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands). The concentrations of phenobarbital in the ultrafiltrate were measured in the same way as that for the plasma samples. The HPLC system used was described before (3).

Data Analysis

Pharmacokinetics. The area under the phenobarbital concentration–time curve (AUC) was calculated using the

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linear trapezoidal rule extrapolated to infinity, on the basis of the elimination rate constant k. This elimination rate constant was determined using the slope of the terminal phase of the log concentration vs time profile. The elimination half-life was calculated as $0.693/k$. Total-body clearance was calculated as dose/AUC, and apparent volume of distribution as dose/AUC*k.

Statistics. The effect of aging on the pharmacokinetic and pharmacodynamic parameters was statistically tested by one-way analysis of variance with multiple comparisons. P values lower than 0.05 were judged to be significant.

RESULTS

Pharmacokinetic Evaluation

The values of the pharmacokinetic parameters are summarized in Table I. In the "pseudo"-longitudinal aging study, only minor variation was observed in the values of the clearance. The volume of distribution was found to be increased in the 34-month-old animals, and there was a tendency toward an increased elimination half-life.

In the control groups very similar observations were made, with no significant changes in clearance between groups and an increase in volume of distribution in the control group studied at 34 months (the volume of distribution appears to be inconsistent).

Parameters related to the distribution of phenobarbital between age groups are summarized in Table II. Generally, only minor differences in plasma protein binding and the distribution ratios between plasma, cerebrospinal fluid, and brain tissue were observed.

Pharmacodynamic Evaluation

The values of the pharmacodynamic parameters are summarized in Table III. In the pseudo-longitudinal aging study a significant decrease in the threshold dose of phenobarbital was observed, from 264 ± 3 mg/kg (mean ± SE) at the age of 7 months to 185 ± 5 mg/kg at the age of 34 months. Similar decreases in the threshold concentrations in plasma, plasma ultrafiltrate, cerebrospinal fluid, and brain were observed. The control groups showed a minor variation in the threshold dose and the threshold plasma concentration, but this variation did not reflect the decrease in these parameters in the age groups.

DISCUSSION

In the present study a "pseudo"-longitudinal study design was applied in order to verify that the age-related increase in brain sensitivity to phenobarbital, measured in a previous study with a cross-sectional study design (6), was indeed the result of the aging process, and not a cohort effect.

It is generally accepted that in pharmacological and gerontological investigations the adaption of a longitudinal study design can offer significant advantages (7). Specifically the reduction in variability and the increased power to detect statistically significant differences in longitudinal studies, in which every animal is repeatedly studied at different ages, are important considerations in this respect. It should be realized, however, that apart from certain advantages, there are also limitations associated with longitudinal studies, especially in aging research. From a practical point of view it is important that cannulas (used for infusion of drugs or sampling of blood or cerebrospinal fluid) and electrodes (necessary for pharmacodynamic investigations) rarely remain patent over the lifetime of the animal.

A more fundamental problem is that in aging studies it is impossible to exclude the role of carryover effects from one experiment to the other, since the rank order of the experiments cannot be randomized. Moreover, it has been demonstrated that, in addition to aging, the occurrence of concurrent disease may markedly influence the outcome of

Table I. The Influence of Aging on the Pharmacokinetic Parameters of Phenobarbital in Rats Following an Intravenous Bolus Dose of 20 mg/kg[a,*]

	Age (months)				
	7	14	21	28	34
Number of animals	8	8	8	8	5
Total-body clearance (ml/hr · kg)	37.1 ± 1.9	41.7 ± 1.7	44.9 ± 2.5	37.2 ± 2.9	33.9 ± 2.8
Volume of distribution (ml/kg)	497 ± 14	550 ± 37	463 ± 22	534 ± 29	645 ± 30[a,c]
Elimination half-life (hr)	9.4 ± 0.4	9.2 ± 0.5	7.2 ± 0.1[a]	10.6 ± 1.2	13.5 ± 1.1[c]
	Control group				
	c7[b]	c14[c]	c21	c28	c34
Number of animals	8	8	8	8	8
Total-body clearance (ml/hr · kg)	37.1 ± 1.9	47.1 ± 2.9	46.9 ± 3.5	47.7 ± 1.8[e]	39.8 ± 1.6
Volume of distribution (ml/kg)	497 ± 14	609 ± 32	528 ± 18	597 ± 49	633 ± 17[e,g]
Elimination half-life (hr)	9.4 ± 0.4	9.2 ± 0.7	8.0 ± 0.4	8.7 ± 0.7	11.1 ± 0.9[g]

[a] The results are presented as mea ± SE.

[b] Group c7 is the 7-month-old group in the upper part of the table.

[c] Group C14 is the 7-month-old control group evaluated together with the 14-month-old age group.

* Superscript a, significantly different from 7-month value, $P < 0.05$; c, significantly different from 21-month value, $P < 0.05$; e, significantly different from value of group c7, $P < 0.05$; g, significantly different from value of group c21, $P < 0.05$.

Table II. The Influence of Aging on the Relative Distribution of Phenobarbital at Onset of Loss of Righting Reflex[a,*]

	Age (months)				
	7	14	21	28	34
Number of animals	8	8	8	6	5
Conc. CSF/conc. plasma free[b]	0.54 ± 0.02 (n = 7)	0.58 ± 0.01 (n = 7)	0.61 ± 0.01 (n = 7)	0.56 ± 0.02 (n = 5)	0.44 ± 0.02[b,c,d]
Conc. CSF/conc. plasma total	0.44 ± 0.01 (n = 7)	0.44 ± 0.01 (n = 7)	0.49 ± 0.01 (n = 7)	0.43 ± 0.02 (n = 5)	0.36 ± 0.01[a,b,c]
Conc. CSF/conc. brain	0.63 ± 0.02 (n = 7)	0.66 ± 0.01 (n = 7)	0.74 ± 0.04 (n = 7)	0.71 ± 0.04 (n = 5)	0.62 ± 0.03
Plasma protein binding (%)	20.5 ± 1.7	24.2 ± 1.7	19.9 ± 1.0	23.2 ± 1.1	19.4 ± 2.2
Conc. brain/conc. plasma free	0.88 ± 0.03	0.89 ± 0.02	0.83 ± 0.03	0.82 ± 0.04	0.72 ± 0.03[b]
Conc. brain/conc. plasma total	0.70 ± 0.02	0.66 ± 0.01	0.74 ± 0.03	0.63 ± 0.03	0.58 ± 0.02[a]
	Control group				
	c7[c]	c14[d]	c21	c28	c34
Number of animals	8	8	8	8	8
Conc. CSF/conc. plasma free	0.54 ± 0.02 (n = 7)	0.58 ± 0.03	0.68 ± 0.04 (n = 7)	0.65 ± 0.05 (n = 7)	0.59 ± 0.02 (n = 7)
Conc. CSF/conc. plasma total	0.44 ± 0.01 (n = 7)	0.46 ± 0.02	0.51 ± 0.01 (n = 7)	0.52 ± 0.05 (n = 7)	0.49 ± 0.01 (n = 7)
Conc. CSF/conc. brain	0.63 ± 0.02 (n = 7)	0.64 ± 0.03	0.72 ± 0.03 (n = 7)	0.76 ± 0.02[e,f] (n = 7)	0.70 ± 0.03 (n = 7)
Plasma protein binding (%)	20.5 ± 1.7	20.5 ± 1.0	23.8 ± 3.1	21.2 ± 3.7	17.5 ± 1.6
Conc. brain/conc. plasma free	0.88 ± 0.03	0.91 ± 0.02	0.94 ± 0.06	0.87 ± 0.05	0.85 ± 0.03
Conc. brain/conc. plasma total	0.70 ± 0.02	0.73 ± 0.01	0.70 ± 0.01	0.68 ± 0.05	0.70 ± 0.04

[a] The results are presented as mean ± SE.
[b] Conc., concentration; CSF, cerebrospinal fluid.
[c] Group c7 is the 7-month-old group in the upper part of the table.
[d] Group c14 is the 7-month-old control group evaluated together with the 14-month-old age group.
* Superscript a, significantly different from 7-month value, $P < 0.05$; b, significantly different from 14-month value, $P < 0.05$; c, significantly different from 21-month value, $P < 0.05$; d, significantly different from 28-month value, $P < 0.05$; e, significantly different from value of group c7, $P < 0.05$; f, significantly different from value of group c14, $P < 0.05$.

pharmacological investigations (8). Therefore, a postmortem tissue examination, performed immediately after the experiment, is essential for the interpretation of the results. Obviously such an examination cannot be conducted in a longitudinal study within one animal.

These considerations led us to conduct the present study on the basis of an adapted pseudo-longitudinal design, as described before. In this design the advantage of a longitudinal study concerning the reduction of the variability is not valid. However, it should be considered that the interindividual variability in the pharmacodynamic parameter (threshold phenobarbital concentration at onset of loss of righting reflex) is small (9).

Using the pseudo-longitudinal design, a decrease in the threshold dose of phenobarbital needed for onset of loss of righting reflex was found during aging, without important changes in the pharmacokinetics. The concentration in cerebrospinal fluid at onset of loss of righting reflex decreased during aging. Together with the fact that the relative distribution of phenobarbital at onset of loss of righting reflex did not show important changes (Table II), this shows an increased brain sensitivity during aging (6), which is the main reason for the decreased dose requirement of phenobarbital during aging.

The values of the threshold dose of phenobarbital and the concentration in cerebrospinal fluid at onset of loss of righting reflex in the control animals showed some variation in time but did not decrease to the same extent as the age groups (Table III). This implied that the decrease in the threshold dose with increasing age is not caused by the different times at which the experiments for the different age groups were performed and that the animal model does not show a tendency for a higher sensitivity to phenobarbital.

Both the decrease in phenobarbital dose requirement for onset of loss of righting reflex and the increase in brain sensitivity measured in the present pseudo-longitudinal study design were comparable to that found in the cross-sectional study (6). These findings confirmed the results of the cross-sectional study, thereby excluding the interference of cohort differences.

The fact that the results of the pseudo-longitudinal study confirm those of the cross-sectional study justifies the use of a cross-sectional study design in these kind of experiments using phenobarbital as a model drug and the technique of loss of righting reflex to measure the anesthetic effect. In addition, the pseudo-longitudinal design offers a good alternative for the common longitudinal and the cross-sectional design by overcoming disadvantages of both these designs.

Table III. The Influence of Aging on Dose and Concentrations of Phenobarbital at Onset of Loss of Righting Reflex During an Intravenous Infusion at a Rate of 3 mg/min[a,*]

	Age (months)				
	7	14	21	28	34
Number of animals	8	8	8	8	5
Threshold dose (mg/kg)	264 ± 3	239 ± 5[a]	224 ± 5[a]	206 ± 9[a,b]	185 ± 5[a,b,c]
Plasma concentration (mg/L)	425 ± 9	419 ± 14	357 ± 11[a,b]	356 ± 8[a,b]	316 ± 6[a,b,d]
Plasma ultrafiltrate concentration (mg/L)	339 ± 12	313 ± 5	286 ± 8[a]	273 ± 8[a,b]	255 ± 3[a,b,c]
Cerebrospinal fluid concentration (mg/L)	187 ± 6 (n = 7)	186 ± 6 (n = 7)	177 ± 6 (n = 7)	154 ± 10 (n = 5)	113 ± 4[a,b,c,d]
Brain concentration (mg/kg)	296 ± 7	280 ± 7	237 ± 10[a,b]	222 ± 9[a,b]	184 ± 8[a,b,c]
	Control group				
	c7[b]	c14[c]	c21	c28	c34
Number of animals	8	8	8	8	8
Threshold dose (mg/kg)	264 ± 3	258 ± 7	250 ± 8[e]	273 ± 4[g]	258 ± 4
Plasma concentration (mg/L)	425 ± 9	420 ± 9	384 ± 5[e,f]	421 ± 16[g]	380 ± 9
Plasma ultrafiltrate concentration (mg/L)	339 ± 12	334 ± 7	293 ± 14	329 ± 9	313 ± 6
Cerebrospinal fluid concentration (mg/L)	187 ± 6 (n = 7)	194 ± 9	193 ± 4 (n = 7)	213 ± 10 (n = 7)	184 ± 4 (n = 7)
Brain concentration (mg/kg)	296 ± 7	305 ± 9	270 ± 5	281 ± 8	266 ± 10

[a] The results are presented as mean ± SE.

[b] Group c7 is the 7-month-old group in the upper part of the table.

[c] Group c14 is the 7-month-old control group evaluated together with the 14-month-old age group.

* Superscript a, significantly different from 7-month value, $P < 0.05$; b, significantly different from 14-month value, $P < 0.05$; c, significantly different from 21-month value, $P < 0.05$; d, significantly different from 28-month value, $P < 0.05$; e, significantly different from value of group c7, $P < 0.05$; f, significantly different from value of group c14, $P < 0.05$; g, significantly different from value of group c21, $P < 0.05$.

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