Biochemical Pharmacology, Vol. 34, No. 3, pp. 461–462, 1985.
Printed in Great Britain.

0006–2952/85 $3.00 + 0.00

THE USE OF SATURATION DOSES IN THE $^{14}CO_2$ BREATH TEST ALLOWS MEASUREMENT OF THE MIXED FUNCTION OXIDASE CAPACITY IN VIVO

C. Steffen, Jutta Friebertshäuser and K. J. Netter
Department of Pharmacology, University of Marburg, Lahnberge, D-355 Marburg/Lahn, F. R. G.

The exhalation of $^{14}CO_2$ from labeled aminopyrine has been shown to offer a valid alternative to plasma clearance measurements (1). The parameters of evaluation (discussed in ref. 2) such as elimination half life (3) or maximal exhalation rate (1) reflect the effects of liver damage or induction with phenobarbital. In an attempt to find a substrate with high affinity to cytochrome P-448 (induced by 3-methylcholanthrene, 3-MC), we evaluated methacetin as a potential model drug.

Material and Methods

(Methyl-^{14}C)-methacetin was synthetized according to (4). Methacetin was dissolved in 50 % PEG 1000 and injected intraperitoneally to male Wistar rats in a volume of 4 ml/kg. CO_2 was absorbed as described in (5).

Results and Discussion

Using a dose of 20 µmol/kg methacetin, we measured an $^{14}CO_2$-exhalation half life (EHL) of 27.6 ± 4.4 min (mean ± SD, n = 19) that was not changed by induction with either phenobarbital (PB) or 3-MC, although the serum half life (16.8 ± 3.8 min in control rats, n = 19) was decreased considerably by 30 and 50 % in animals pretreated with PB (1g/l in drinking water for 3 d) or 3-MC (30 mg/kg i.p.), respectively. As CO_2 is liberated after a series of oxidations involving formaldehyde and formate, we measured the EHL of these metabolites and found that the EHL of formate is the same as that of methacetin. This explains why changes in the metabolic rate of methacetin are reflected by the serum half life, but not by the breath test. The EHL or exhalation rate constant is therefore no meaningful parameter when the serum half life of the parent compound is shorter than the exhalation half life of formate.

However, unambiguous results can be obtained by the use of saturation doses (fig. 1) that lead to a constant $^{14}CO_2$ exhalation rate which can be converted into µmol drug-derived CO_2 exhaled · kg^{-1} · min^{-1} instead of the usual way of giving fractions of the administered dose per time unit. Induction by PB as well as by 3-MC is followed by an increase of the amount of $^{14}CO_2$ exhaled by the rat that is more pronounced than the changes of the serum half life of the drug. Performing similar saturation experiments with formate and formaldehyde, corresponding values can be given that indicate the total metabolic capacity for different substances and pretreatments (table 1). It is evident that the organism's capacity to metabolize formate is twenty times higher than the demethylation capacity.

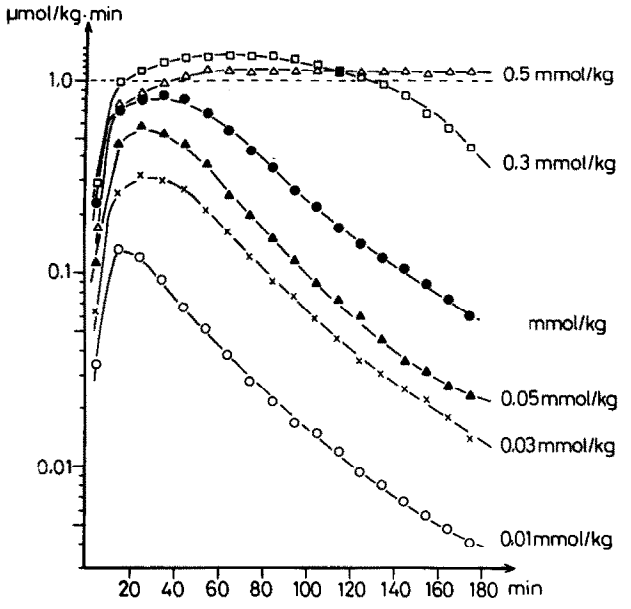

Fig. 1: $^{14}CO_2$ exhalation rate after different doses of (methyl-^{14}C)methacetin

Increase of the administered dose of (methyl-^{14}C)methacetin does not change the exhalation half life but is followed by an increased peak exhalation rate. Saturation values are attained when the dose is above 0.1 µmol/kg. Values shown are means of two animals per dose.

The acute toxicity of formaldehyde prevented the use of saturation doses, and acute toxic effects were observed when the dose of methacetin exceeded 2 mmol/kg.

Table 1. Maximal exhalation rates and saturation doses in the rat

Substrate	pretreatment	Max. exhal. rate ($\mu mol \cdot kg \cdot min^{-1}$)	Appr. satur. dose (i.p.)
Methacetin	control	1.65 ± 0.18 (n=12)	0.1 mmol/kg
	PB	3.80 ± 0.55 (n= 6)	0.5 mmol/kg
	3-MC	4.76 ± 0.81 (n= 6)	0.5 mmol/kg
Formate	control	35	< 10 mmol/kg
Formaldehyde	control	5*	> 2 mmol/kg

* Saturation could not be attained as higher doses were not tolerated

This method can be used with other drugs with short serum half lifes, provided the use of high doses is not excluded by pharmacodynamic or toxic effects.

Acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft

References:

1. B. H. Lauterburg and J. Bircher
J. Pharmacol. exper. Ther. 196, 501 (1976)
2. D. A. Henry, G. Sharpe, S. Chaplain, S. Cartwright, G. Kitchingman, G.D. Bell and M. J. S. Langman. Br. J. clin. Pharmacol. 8, 539 (1979)
3. P.V. Desmond, R. A. Branch, J. Calder and St. Schenker, Proc. Soc. exper. Biol. Med. 164, 173 (1980)
4. C. F. H. Allen and J. W. Gates, in: Organic Syntheses, Coll. Vol. 3, E. C. Horning (Edit.) J. Wiley, New York, 1955, p. 140
5. W. A. Daniel, J. Friebertshäuser, C. Steffen
Naunyn-Schmiedeberg's Arch. Pharmacol. in print.