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Diurnal Exposure Profile in Rats from Dietary Administration of a Chemical (Doxazosin) with a Short Half-life: Interplay of Age and Diurnal Feeding Pattern

C. Charuel, P. Comby and A. M. Monro†
Centre de Recherche, Laboratoires Pfizer, 37400 Amboise, France

Key words: doxazosin; rats; toxicokinetics; dose-dependent; age-dependent; diurnal pattern; feeding pattern.

Doxazosin, an α-adrenergic blocking agent, has a plasma half-life in male rats of 1–2 h after i.v. administration. Plasma concentrations of doxazosin were measured in male rats receiving the drug mixed in the diet at dose levels from 5 to 40 mg kg^{-1}. Samples taken at 4-h intervals during the light (0700–1900) and dark phases revealed peak concentrations at 0400 which were only about three times higher than the trough concentrations observed ca. 12 h later. The 24-h area under the curve (AUC) values increased disproportionately with dose and with age from 2 months up to 8 months of age; thereafter they were fairly stable to 24 months of age. This age-related effect may have been due to a reduction in clearance and/or a change in the feeding pattern of the rats. Young rats consumed ca. 84% and old rats only 45% of their daily feed during the nocturnal (active) phase. Given the known diurnal rhythms in absorption, protein binding and enzyme metabolising activity, such a change in feeding pattern with age may have wider toxicokinetic implications.

INTRODUCTION

In chronic toxicology studies, chemicals are commonly administered to rodents by mixing them with the diet. Since rodents ingest most of their diet during the nocturnal phase, it would be reasonable to assume that rapidly cleared chemicals will achieve only relatively low plasma concentrations during the quiescent (light) phase. The toxicokinetic studies reported here for doxazosin (plasma half-life in rats ca. 1–2 h) show that dietary administration may result in relatively little fluctuation in plasma concentrations throughout 24 h; further more, an age-related change in the feeding pattern of rats indicates that this may be a hitherto unrecognized factor in apparent age-related kinetic changes of chemicals administered to rodents via the diet.

Doxazosin [1-(4-amino-6,7-dimethoxy-2-quinazolin-yl-4-(1,4-benzodioxan-2-ylcarbonylpiperazine] is a post-synaptic α-adrenergic blocking agent used for the treatment of hypertension.[1,2] In most patients it is effective in a once-daily oral dose of 2–4 mg, with the maximum recommended clinical dose being 16 mg (0.25 mg kg^{-1}). Doxazosin is extensively metabolized in laboratory animals and in man, with the pattern of metabolites being essentially similar in all species.[3]

In accordance with present-day requirements for the development of a chronic-use drug, doxazosin has been subjected to a battery of experimental toxicology studies in rodents and non-rodents to underwrite its safety in man. Since doxazosin has relatively low toxicity and has a plasma clearance which is much greater in rodents than in higher species,[3] the dose levels used in toxicology (in order to satisfy the criterion of demonstrating toxicity) ranged up to more than 150 times those required for an anti-hypertensive effect. The pharmacokinetics of a xenobiotic can differ significantly over such a dose range. In addition, a toxicology programme in rodents includes 'life-span' studies for the evaluation of carcinogenic potential. In such studies the test substance is administered successively to weanlings, young animals, mature adults and finally to geriatric animals. Drug kinetics are obviously liable to change during the course of such a 'life-span' toxicology study.

We report here the toxicokinetics of doxazosin in rats under the conditions of a 'life-span' toxicology study which employed dietary administration of the drug, and describe the potentially important changes in kinetics which occurred with dose, age and duration of dosing. Preliminary studies (data not shown) led to a choice of dose levels of 10, 20 and 40 mg kg^{-1} for the studies.

METHODS

Animals

Rats were a Sprague-Dawley-derived strain from Charles River (France or Italy). They were housed singly in shoe-box cages in a conventional environmentally controlled animal room. The lighting was 12/12 h, with the light phase 0700–1900 h. A standard commercial rat diet and tap water were supplied *ad libitum*.

† Author to whom correspondence should be addressed at: Pfizer Central Research, Eastern Point Road, Groton, CT06340, USA.

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Drug administration

Drug–diet mixes were prepared by mixing doxazosin mesylate with the diet, first as a concentrated premix and then by stepwise dilution in a mechanical mixer. The concentration required to give the desired dose in terms of doxazosin base kg^{-1} body weight was calculated from the average food consumption over the previous week for each dose group. Representative samples of the drug–diet mixes were analysed for drug content to ensure adequate accuracy and homogeneity of the mixes. The drug was shown to be stable for up to 7 days at ambient temperature when dispersed in the diet.

Blood samples and assays

Animals were bled from the orbital sinus under light ether anaesthesia. Plasma was prepared and stored frozen until analysed. The assay method was based on the HPLC method of Kaye *et al.*[3] Satisfactory calibration curves were obtained by spiking control plasma with doxazosin over a concentration range up to 1000 ng ml^{-1}. The lower limit of quantitation was ca. 5 ng ml^{-1}. The area under the curve (AUC) was calculated by the trapezoidal rule. Clearance was calculated from dose AUC^{-1} (assumes that the fraction of dose absorbed is constant).

Protocols

Study A was a 2-month study in which two groups of 24 male rats initially aged ca. 1 month and 7 months, respectively, were bled at 2000, 2400, 0400, 0800, 1200 and 1600 h (4 rats per dose per time point) on study days 21/22 and 58/59. Study B was a 12-month study in which male rats entered the study at 12 months of age. They were bled at age 13, 15, 18, 21 and 24 months at 2000, 2400, 0400, 0800, 1200 and 1600 h (3–4 rats per dose per time point). In both studies each rat was bled once in 24 h. At every sampling interval food hoppers were weighed for estimation of food consumption.

Statistics

AUC values within each dose group were compared by a Wilcoxon rank test.

RESULTS

In study A, the 4-h sampling schedule (Fig. 1) allowed good estimates of the AUC. While plasma concentrations of doxazosin were lower during the light phase, the trough levels were about one-third the peak levels. Thus, significant concentrations of doxazosin were present throughout the 24-h period. The AUC values (Fig. 2) were greater at 40 mg kg^{-1} than would be predicted from the values at 10 and 20 mg kg^{-1}. Furthermore, from comparison of the AUC values of rats treated for similar periods (ages 2 and 8 months and 3 and 9 months), it is apparent that the AUC at all dose levels increased with the age of rats.

In study B, the AUC values (Table 1) observed from 13 to 24 months of age were similar to those observed at 9 months of age in study A, and appeared to undergo just random fluctuations over this second year of life. During this period the AUC values were generally proportional to dose.

The food consumption measurements from studies A and B have been consolidated in Fig. 3. It is clear that there was a steady change in feeding pattern throughout the age-span studied: young rats consumed ca. 84% of their diet (and therefore of their daily dose) during the dark phase, while 2-year old rats only consumed ca. 45% of their diet (and drug dose) during the dark phase.

DISCUSSION

Although the pattern of results raises several questions specific to the disposition of doxazosin, a result of more general toxicological application also emerges. The young rats ingested the greater part of their diet by night, yet the trough plasma concentrations of doxazosin, generally observed in the afternoon, were about one-third the peak values observed at 0400. Thus, the plasma concentration of doxazosin showed relatively little fluctuation over 24 h, despite the plasma half-life in rats after i.v. administration being only 1–2 h.[3,4] Theoretically, if drug intake ceased completely at the beginning of the light phase (0700), by mid-afternoon (say six half-lives later) the concentrations should have diminished by 64-fold. While the pattern of food intake (see below) may partly explain the sustained concentrations of drug observed during the light phase, additional factors may be diurnal differences in absorption, plasma protein binding and microsomal enzyme activity, all of which can vary significantly between the active (nocturnal) and the resting (light) phases.[5,6]

Another feature of the results with potentially broader ramifications for chronic toxicology studies in rats is the gradual change observed from a predominantly nocturnal feeding pattern to one in which less than half the daily ration was ingested during the dark phase. Investigators may need to remain alert to the possibility that as rats advance into their second year of life, differences in feeding pattern taken together with diurnal variations in xenobiotic disposition may affect the overall toxicokinetics of the test substance.

The pattern of results raises several questions specific to doxazosin: why do the plasma concentrations increase disproportionately with dose and, at all dose levels, with the age of the animals? Plasma concentration (or the AUC) can be higher than predicted for one or more of three basic reasons: increased absorption, smaller volume of distribution (V_D) or reduced clearance.

Absorption seems unlikely to be an important factor. Doxazosin is generally well absorbed after oral administration to all species investigated, including young rats.[3] While it is possible that admixture with the diet could impair oral absorption, this would have to be a marked effect to allow for the increases of absorption with age and dose observed in this study.

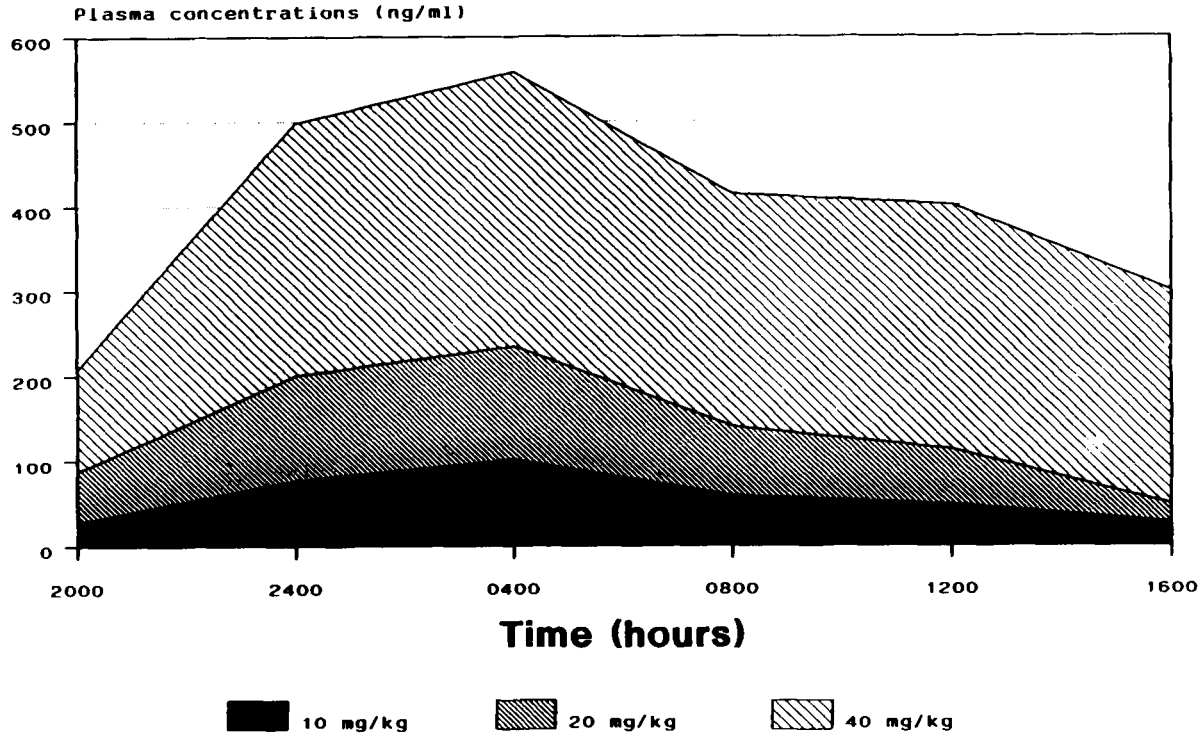

Figure 1. Plasma concentrations of doxazosin over a 24-h period in male rats receiving 10, 20 or 40 mg kg^{-1}. The rats were ca. 3 months of age and had received the drug mixed in the diet at appropriate concentrations for 2 months.

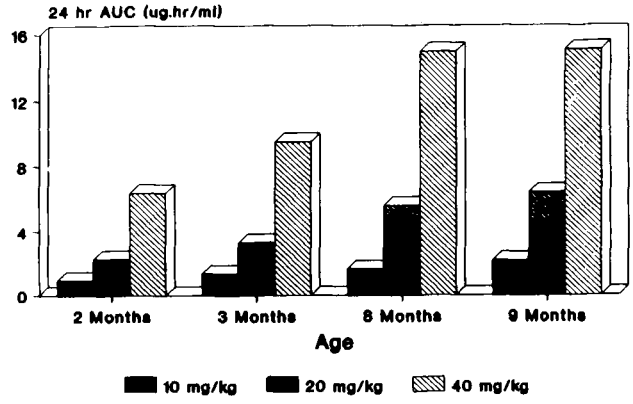

Figure 2. Plasma 24-h AUC values for doxazosin in male rats receiving doxazosin via the diet at dose levels of 10, 20 or 40 mg kg^{-1}. At the 2- and 8-month points rats had been treated for 1 month, and at the 3- and 9-month points for 2 months. Statistical analysis showed that within each dose group the AUC at 3 months was greater than at 2 months, and at 9 months greater than at 3 months; also, at 3 and 8 months the values for 40 mg kg^{-1} were more than twice those for 20 mg kg^{-1}.

Age-related changes of absorption in rats tend to occur only after 18 months of age,[7] while the changes revealed in the present study occurred mainly in the first 9 months of life.

Similarly, a decrease in V_D with increase in age does not seem likely. Kaye reported that after i.v. administration of doxazosin, old rats showed a slightly greater V_D than young rats, while plasma protein binding was unchanged.[4] Although it may not always be valid to extrapolate V_D values after i.v. dosing to the p.o. situation, it does seem unlikely that a reduction in V_D with age is an important factor in explaining

Table 1. Mean AUC (μg h ml^{-1}) of doxazosin in rats aged 13–24 months (study B)

Dose (mg kg^{-1})	Age in months[a] 9[b]	13	15	18	21	24
10	2.2	1.5	1.8	1.7	2.1	2.9
20	6.3	5.5	4.2	6.0	7.4	7.6
40	15.0	23.0	15.0	15.0	16.0[c]	15.0[c]

[a] Dosing started at month 12.
[b] Data from study A, for comparison.
[c] At several sample times only 2 or 3 rats were available.

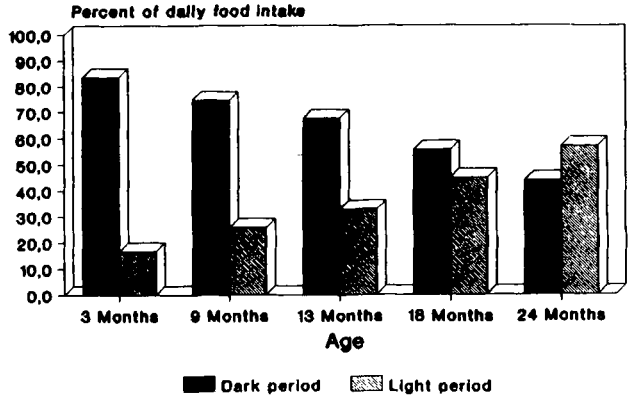

Figure 3. The distribution of food consumption of male rats during the light and dark periods over an age range of 3–24 months.

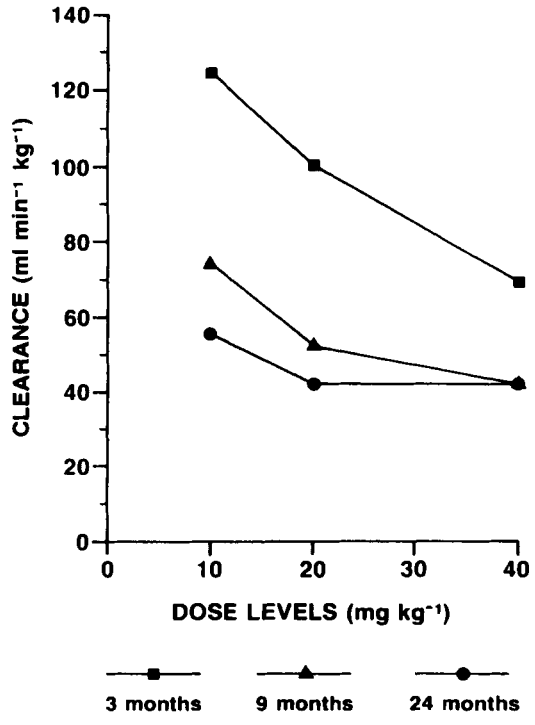

Figure 4. Plasma clearance of doxazosin (calculated from the AUC values) in male rats receiving doxazosin via the diet at 10, 20 or 40 mg kg^{-1}. The rats at 3, 9 and 24 months of age had received the drug for 2, 2 and 12 months, respectively.

our present results after dietary administration of doxazosin.

The clearance was calculated at 10, 20 and 40 mg kg^{-1} for animals aged 3, 9 and 24 months (Fig. 4). This reveals clear dose- and age-related trends. In 3- and 9-month-old animals the clearance declines as the dose and age increase. In 24-month-old animals, however, the clearance seems to have reached a trough level of ca. 45 ml min^{-1} kg^{-1}, irrespective of dose. This is reflected in the approximately dose-proportional values for the AUC (Table 1) found at 24 months, in contrast to the lack of proportionality in the first 12 months of life (Fig. 2).

Thus, dose- and age-related changes in the clearance of doxazosin remain as the most plausible explanation for the results. Since doxazosin is eliminated mainly by metabolism,[3] clearance could be reduced by a decrease in hepatic blood flow or in intrinsic activity of the metabolizing enzymes. The former would be a predictable consequence of the principal pharmacodynamic property of doxazosin—the lowering of systemic blood pressure. Added to this is the possibility of an age-dependent factor, since Yates and Hiley[8] report that the hepatic blood flow in rats aged 12 months is ca. 30% less than that of rats aged 4 months.

A reduction in the rate of biotransformation with increase in dose would not be unexpected, as such phenomena are well-recognized.[9–11] However, an age-related change in metabolizing activity, while well known in general terms,[7] may not be pertinent in the present case: most of our changes occurred between 2 and 12 months of age, while senescent changes in metabolism in rats tend to occur after 12 months of age.[12]

If changes in clearance lie behind the dose- and age-related trends, one must ask what repercussions this might have on the interpretation of the toxicology of doxazosin. For example, does a change in the drug/metabolite ratio have any importance? The main feature of the chronic toxicity studies in rats was an exacerbation in the top-dose male group of the myocardial fibrosis which occurs spontaneously in aged rats of this strain. This phenomenon is explicable in terms of an ischaemia resulting from prolonged hypotension produced by dose levels of doxazosin more than 100 times those required for a therapeutic anti-hypertensive effect. Two metabolites were found to have hypotensive properties in rats.[13] One, UK-58, 993, was equipotent with doxazosin but was present in the plasma in only ca. 1/50 the concentration of doxazosin; the other, UK-28,805, had only ca. 1/10 the potency of doxazosin and was present in the plasma at ca. 1/5 the concentration of doxazosin. It is concluded, therefore, that the rate or extent of formation of metabolites of doxazosin has negligible impact on the interpretation of the toxicology of doxazosin in rats at these dose levels. Instead, the toxicity observed reflects variations in the concentration of doxazosin itself, rather than of its metabolites. Looked at in these terms, one can conclude that not only does the nominal increase in dose levels underestimate the increase in pharmacological insult, but also that in the second year of the study the rats were exposed to considerably greater concentrations of pharmacologically active parent drug than would have been predicted from data acquired in the first few months of the study.

Finally, it should be noted that the kinetics of doxazosin are unaffected by dose in mice or dogs,[14] and unaffected by dose (over the whole therapeutic range of 2–16 mg) or by age in man.[15] The results in rats, however, illustrate the potential importance of pursuing a systematic approach to toxicokinetic studies, paying attention to variables which include, *inter alia*, dose, route of administration, sex, age and diurnal patterns of feeding.

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REFERENCES

1. V. A. Alabaster and M. J. Davey, The α-adrenoceptor antagonist profile of doxazosin: preclinical pharmacology. *Br. J. Clin. Pharmacol.* **21**, 9S–17S (1986).
2. D. A. Cox, J. P. Leader, J. A. Milson and W. Singleton, The anti-hypertensive effects of doxazosin: a clinical overview. *Br. J. Clin. Pharmacol.* **21**, 83S–90S (1986).
3. B. Kaye, N. J. Cussans, J. K. Faulkner, D. A. Stopher and J. L. Reid, The metabolism and kinetics of doxazosin in man, mouse, rat and dog. *Br. J. Clin. Pharmacol.* **21**, 19S–25S (1986).
4. B. Kaye (personal communication) reports that an i.v. dose of 5 mg kg^{-1} of doxazosin to male rats aged 9 months gave a V_D of 5.4 l kg^{-1}, and a plasma half-life of 2.1 h; plasma protein binding was similar in rats aged 2 and 12 months and did not vary with drug concentration.
5. A. Reinberg, Clinical chronopharmacology. In *Biological Rhythms and Medicine. Cellular, Metabolic, Physiopathologic and Pharmacologic Aspects*, ed. by A. Reinberg and M. H. Smolensky, pp. 211–263. Springer-Verlag, New York (1983).
6. P. M. Bélanger, M. Lalande, A. Labrecque and F. M. Dore, Diurnal variations in the transferases and hydrolases involved in glucuronide and sulfate conjugation of rat liver. *Drug. Metab. Dispos.* **13**, 386–389 (1985), and references cited therein.
7. D. L. Schmucker, Aging and drug metabolism: an update. *Pharmacol. Rev.* **37**, 133–148 (1985).
8. M. S. Yates and C. R. Hiley, The effect of age on cardiac output and its distribution in the rat. *Experientia* **35**, 78–79 (1979).
9. P. J. Gehring, P. G. Watanabe and G. E. Blau, Pharmacokinetic studies in evaluation of the toxicological and environmental hazard of chemicals. In *New Concepts in Safety Evaluation*, Vol. 1, Part 1, ed. by M. A. Mehlman, R. E. Shapiro and H. Blumenthal, pp. 195–270. Hemisphere, Washington, DC (1976).
10. F. K. M. Dietz, W. T. Stott and J. C. Ramsay, Non-linear pharmacokinetics and their impact on toxicology: illustrated with dioxane. *Drug. Metab. Rev.* **13**, 963–981 (1982).
11. B. Clark and D. A. Smith, Pharmacokinetics and toxicity testing. *CRC Crit. Rev. Toxicol.* **12**, 343–385 (1984).
12. S. Fujita, H. Kitagawa, M. Chiba, J. Suzuki, M. Ohta and K. Kitani, Age and sex associated differences in the relative abundance of multiple species of cytochrome P-450 in rat liver microsomes. *Biochem. Pharmacol.* **34**, 1861–1864 (1985).
13. V. Alabaster, personal communication.
14. C. Charuel, unpublished observations.
15. H. L. Elliott, P. A. Meredith and J. L. Reid, Pharmacokinetic overview of doxazosin. *Am. J. Cardiol.* **59**, 78G–81G (1987).