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# THE EFFECT OF ENZYME INDUCTION ON DIAZEPAM METABOLISM IN MAN

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**1** The elimination and metabolism of diazepam in man was investigated following the induction of the liver microsomal enzyme system by antipyrine.

**2** Seven healthy volunteers were given 1200 mg antipyrine as an inducing agent for a period of 14 days. Before and after the induction period the elimination of diazepam and desmethyldiazepam was measured in the plasma by gaschromatography. As parameters of liver microsomal enzyme activity, antipyrine elimination and $\gamma$-glutamyl-transpeptidase in the plasma, D-glucaric acid and 6-$\beta$-hydroxycortisol urinary excretion were measured on both occasions.

**3** Following the induction period most parameters of microsomal enzyme activity measured were significantly changed indicating an increase of the microsomal enzyme system. The elimination of diazepam was significantly altered having a half-life of 37 h before and 18 h afterwards combined with a significant increase in total body clearance after the induction period, although the volume of distribution remained unaltered. The formation of the main metabolite N-desmethyldiazepam was not changed, but its elimination was increased having a half-life of 139 or 58 h respectively.

**4** The elimination of unchanged diazepam and desmethyldiazepam is significantly increased by the induction of the liver microsomal enzyme system using antipyrine as an inducing agent in healthy volunteers, which might be important under certain clinical conditions.

## Introduction

Diazepam is widely used in the treatment of anxiety and tension, as an anticonvulsant and muscle relaxant. It is completely absorbed after oral administration (Kaplan, Jack & Weinfeld, 1973) and completely metabolized by demethylation and hydroxylation and after conjugation with glucuronic acid excreted in the urine. Accordingly, changes in drug metabolism, because of liver disease or following the induction of the microsomal enzyme system, can alter the elimination of diazepam. In experiments in animals it was found that diazepam given in daily doses of 400 mg/kg bodyweight to rats stimulated hepatic microsomal metabolism as evidenced by acceleration of both *p*-hydroxylation of aniline and hydroxylation of benzene (Jablonska, Knobloch, Majka & Wisniewska-Knypl, 1975). In addition, rats, mice and guinea pigs treated with phenobarbitone showed an increase in diazepam metabolism affecting the different metabolites known in the degradation of diazepam elimination (Marucci, Fanelli, Mussini & Garattini, 1970). In man diazepam was found to induce its own metabolism in a group of psychiatric patients on long term diazepam therapy (Sellman, Kanto, Raijolo & Pekkarinen, 1975). However, the effect on diazepam metabolism of other drugs like antipyrine and phenobarbitone known to be enzyme inducers in man has not been investigated. Therefore, in the present study the elimination and metabolic degradation of diazepam in man was investigated following the administration of antipyrine, a known inducing agent.

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## Methods

### 1. *Volunteers*

Seven healthy volunteers, five male and two female, ranging in age from 22 to 24 years were investigated in this study. All volunteers were informed about the aims of the study and gave their written consent. The health of the volunteers was checked by a clinical and laboratory investigation in which the following measurements were performed: haemoglobin, haematocrit, white cell and platelet count, sodium, potassium, protein, glutamic oxalacetic trans-

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aminase, glutamic pyruvate transaminase and alkaline phosphatase. In addition, a chemical urinanalysis and a sediment examination was performed. These laboratory examinations were repeated after the induction study.

2. *Evaluation of endoplasmic reticulum enzyme function*

As *in vivo* parameters of liver microsomal enzyme activity the following measurements were performed.

a. *Measurement of antipyrine elimination.* After an overnight fast antipyrine 1200 mg was given orally. One hour later the volunteers were allowed to have breakfast. Blood for the estimation of antipyrine concentration according to the method of Brodie, Axelrod, Soberman & Levy (1949) was taken at the following time intervals: 0, 3, 6, 9, 12 and 24 hours. From these plasma concentrations, the overall elimination rate constant was calculated by means of a linear regression using the method of the least squares of errors. From the overall elimination rate constant, the antipyrine half-life was calculated according to the following equation

$$T_{\frac{1}{2}} = \frac{\ln 2}{K_e} \text{ (h)} \quad (1)$$

an approximate value of the apparent volume of distribution, $V_d$, was calculated from the dose antipyrine given, D and the initial plasma concentration at zero time, $c_0$:

$$V_d = \frac{D}{c_0} \text{ (l)} \quad (2)$$

From the apparent volume of distribution, $V_d$, and the overall elimination rate constant, $K_e$, the total body clearance, $Cl_A$ for antipyrine was calculated according to the following equation:

$$Cl_A = K_e \cdot V_d \text{ (ml/min)} \quad (3)$$

b. *Measurement of γ-glutamyl-transpeptidase.* The γ-glutamyl-transpeptidase in the plasma was measured by the method of Szasz (1969). The values were expressed in $\mu$mol min$^{-1}$ l$^{-1}$ and the reference values in males are 6–28 and in females 4–18 units/l at 25°C.

c. *Estimation of the 24 h* D*-glucaric acid excretion.* For the estimation of the 24 h D-glucaric acid excretion two 24 h urine collections were performed at the time when antipyrine elimination was measured and the mean of these two measurements was taken. The determination of D-glucaric acid in urine was carried out as described by Marsh (1963) but using appropriate blanks and highly purified rat liver β-glucuronidase (600,000 Fishman units/g) in the place of the originally described rat liver homogenate.

d. *Measurement of 6-β-hydroxycortisol and 17-hydroxycorticosteroids.* For the measurement of 6-β-hydroxycortisol and the 17-hydroxycorticosteroids the same two 24 h urine collections were used as for the estimation of D-glucaric acid excretion. A ratio of the daily 6-β-hydroxycortisol and 17—hydroxycorticosteroid excretion was calculated, which represents the percentage proportion 6-β-hydroxycortisol of the total amount of 17-hydroxycorticosteroids and reduces daily variations in the urinary 6-β-hydroxycortisol excretion (Ohnhaus & Park, 1979).

*Determination of 17-hydroxycorticosteroids.* 17-Hydroxycorticosteroids (17,21-dihydroxy-20-ketosteroids, Porter–Silber chromogens) were determined by the method of Silber & Porter (1954) with some modifications. An aliquot (4 ml) of urine was diluted to 10 ml with 2.0 M sodium acetate-acetic acid buffer pH 5.0 and incubated with β-glucuronidase (2000 Fishman units/ml of urine) for 5 h at 37°C. The incubation mixture was vortexed with chloroform (50 ml) for 1 min, centrifuged and the aqueous phase discarded. The organic phase was then vortexed with 0.1 M sodium hydroxide (2 ml) for 1 min, centrifuged and the aqueous phase was again discarded. The chloroform solution was separated into two 20 ml aliquots. To one aliquot was added Porter–Silber reagent (1 ml), and to the second aliquot was added 1 ml 11.2 M sulphuric acid-ethanol (2 : 1 v/v). Each aliquot was then vortexed for 1 minute. After removal of the organic phase the remaining aqueous phase was incubated at 60°C for 30 min. Optical density readings were determined for 370, 410 and 450 nm. All calculations were made using the Allen (1949) correction. Tetrahydrocortisol was used as standard.

*Determination of 6-β-hydroxycortisol* 6-β-hydroxycortisol was determined using a specific radioimmunoassay (Park, 1978).

3. *Plan of the study*

After studying the function of the liver microsomal enzyme system 10 mg diazepam (Valium Roche®) was given orally to each volunteer after an overnight fast. Blood samples were taken at the following time intervals: 30, 60, 90, 120 min, 4, 6, 8, 24, 32, 48, 56 and 72 h. As it is known that dietary intake can produce an increase of diazepam plasma concentrations two standardized meals were given during the first 2 days of the study. In addition, the blood

samples were taken always before each meal or in the morning in a fasting state. After the measurement of diazepam elimination the induction period was started by giving 2 × 600 mg antipyrine daily to each volunteer for 14 days. Following the induction period the parameters of liver microsomal enzyme function were newly assessed and 36 h after the last dose of antipyrine the study for diazepam elimination was repeated.

### 4. *Analytical techniques*

In the plasma samples taken up to 72 h before and after the induction period the concentrations of diazepam and desmethyldiazepam, the main metabolite were measured by a specific sensitive gas-chromatographic procedure (de Silva, Bekersky, Puglisi, Brooks, & Weinfeld, 1976).

### 5. *Pharmacokinetic and statistical calculations*

The measured diazepam concentrations in the plasma were used to calculate the pharmacokinetic parameters according to a two compartment open model by nonlinear regression assuming first order absorption. For each subject the measured concentrations were used to calculate the disposition parameters of the concentration-time curve ($\alpha$- and $\beta$-phase), i.e. A, B, $\alpha$, $\beta$ according to the following formula

$$C_p(t) = -(A+B) \cdot e^{-k_a t} + A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (4)$$

by an iterative digital computer program. From the disposition constants $\alpha$ and $\beta$ the respective half-lives $T_{\frac{1}{2}}\alpha = \ln 2/\alpha$ and $T_{\frac{1}{2}}\beta = \ln 2/\beta$ were calculated. The areas under the concenration time curves (AUC) were estimated according to the trapezoidal rule (Wagner, 1975) and extrapolation to time infinity using the $\beta$-phase of diazepam, i.e.

$$\text{AUC}\,(t_{max} - \infty) = \frac{B \cdot e^{-\beta t_{max}}}{\beta} \quad (5)$$

The volume of distribution, $V_d$ was calculated according to the following formula:

$$V_d\,(\text{AUC}) = \frac{D \cdot f}{\beta \cdot \text{AUC}_{(\infty)}} \quad (6)$$

whereby D represents the dose of diazepam given and f the fraction absorbed in the gut, which was assumed 1 in the case of diazepam (Kaplan *et al.*, 1973). The total body clearance of diazepam was calculated in the same manner as antipyrine clearance according to formula 3 using the disposition constant $\beta$ instead of $K_e$.

The pharmacokinetic parameters of diazepam calculated in each volunteer were tested with the *t*-test for differences between pairs and in the case of nonhomogeneous variances by Wilcoxon's matched-pairs signed-rank-test.

From the volunteers participating in the present study, blood samples were only obtained up to 72 hours. During this time period a wide variation in desmethyldiazepam transformation and elimination was observed and therefore too few data points were available for the calculation of a disposition constant $\beta$ in each volunteer. On the other hand, comparing the plasma concentrations of desmethyldiazepam at the different time intervals before and after the induction period by a paired-*t*-test a significant difference was found at 56 and 72 h ($P<0.01$). Therefore, from the mean plasma concentration at 48, 56 and 72 h the mean elimination rate constant or half-life was calculated by means of linear regression using the method of least squares of errors. In addition, a mean area under the plasma concentration time curve for the experimental period $AUC_{t72}$) and extrapolated for the time infinity ($AUC_{t\infty}$) was estimated according to the formulas mentioned above. No statistical analysis was performed, as only mean pharmacokinetic parameters were calculated and compared.

## Results

The *in vivo* parameters of the enzymes of the endoplasmic reticulum measured before and after the induction period are shown in Table 1. The antipyrine half-life decreased significantly from 12.3 to 7.5 h with an increase in antipyrine clearance from 40.1 up to 62.9 ml/min showing no changes in the volume of distribution. The D-glucaric acid was excreted to a higher but not significant extent in the urine while the $\gamma$-glutamyl transpeptidase in the plasma was significantly increased. The urinary excretion of 6-$\beta$-hydroxycortisol increased from 277 to 512 µg and the ratio of 6-$\beta$-hydroxycortisol/17-hydroxycorticosteroids from 5.3 to 10.8%, whereby no changes in the total 17-hydroxycorticosteroid excretion occurred.

The elimination of diazepam before and after the induction by antipyrine is seen in Figure 1. The plasma concentration time curve up to 72 h showed a biexponential decline with no differences during the absorption and the $\alpha$-phase, but a faster elimination of diazepam was found after the induction period. The pharmacokinetic parameters of diazepam calculated are seen in Table 2. The maximal plasma concentration ($C_{max}$) measured was identical and was reached at the same time ($t_{max}$) under both experimental conditions. There were no significant differences in the absorption rate constant and in the $\alpha$-phase calculating identical rate constants or half-lives respectively. In contrast the $\beta$-phase or the respective half-life decreased significantly from 37.4 to 18.4 h combined with a doubling in diazepam total

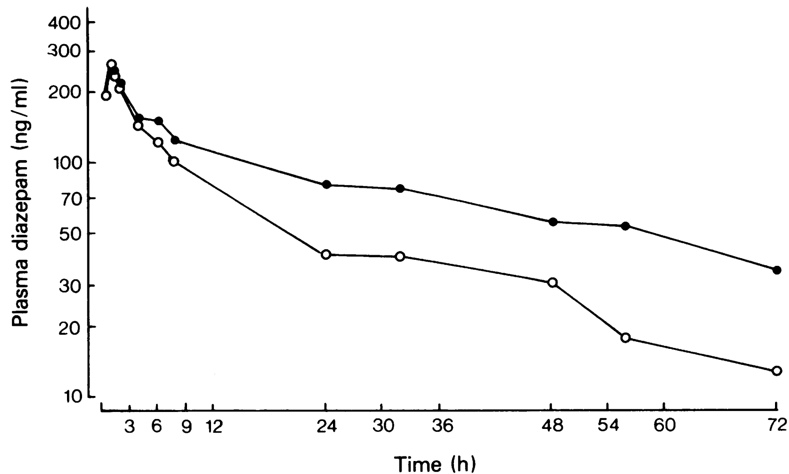


**Figure 1** The mean ($n = 7$) plasma concentration of diazepam is plotted *v* time before (●) and after (○) induction of the liver microsomal enzyme system by antipyrine.

**Table 1** Measurements of the *in vivo* parameters of liver microsomal enzyme activity before and after administration of 2 × 600 mg antipyrine for 14 days.

| | *Before* | *After* | *Significance* |
|---|---|---|---|
| Antipyrine $V_d$ (l) | 40.5 ± 8.9 | 38.4 ± 8.7 | NS |
| Antipyrine $k_e$ ($h^{-1}$) | 0.059 ± 0.013 | 0.099 ± 0.027 | $P<0.001$ |
| Antipyrine $T_{\frac{1}{2}}$ (h) | 12.3 ± 2.9 | 7.5 ± 2.1 | $P<0.001$ |
| Antipyrine clearance (ml/min) | 40.1 ± 12.2 | 62.9 ± 19.9 | $P<0.001$ |
| γ-GT (μmol $l^{-1}$ $min^{-1}$) | 9.9 ± 5.2 | 14.7 ± 5.5 | $P<0.001$ |
| D-glucaric acid (μmol/mg creatinine) | 0.067 ± 0.04 | 0.096 ± 0.06 | $P>0.05$ |
| 6-β-hydroxycortisol (μg/24 h) | 277 ± 183 | 512 ± 279 | $P<0.01$ |
| Ratio 6-β-OHC/17-OHC (%) | 5.3 ± 3.0 | 10.8 ± 3.5 | $P<0.01$ |

**Table 2** Calculated pharmacokinetic parameters of diazepam before and after administration of 2 × 600 mg antipyrine for 14 days

| | *Before* | *After* | *Significance* |
|---|---|---|---|
| $C_{max}$ (ng/ml) | 283 ± 42 | 269 ± 53 | NS |
| $t_{max}$ (h) | 1.1 ± 0.4 | 1.2 ± 0.5 | NS |
| Absorption rate constant ($h^{-1}$) | 1.42 ± 0.6 | 1.71 ± 0.6 | NS |
| α ($h^{-1}$) | 1.06 ± 0.4 | 1.42 ± 0.6 | NS |
| $T_{\frac{1}{2}}\alpha$ (h) | 0.75 ± 0.3 | 0.56 ± 0.2 | NS |
| β ($h^{-1}$) | 0.0199 ± 0.006 | 0.039 ± 0.008 | $P<0.001$ |
| $T_{\frac{1}{2}}\beta$ (h) | 37.4 ± 11.2 | 18.4 ± 3.5 | $P<0.001$ |
| $V_d$ (l) | 70.2 ± 15.5 | 70.1 ± 21.2 | NS |
| Cl (ml/min) | 22.7 ± 5.2 | 43.8 ± 8.4 | $P<0.001$ |

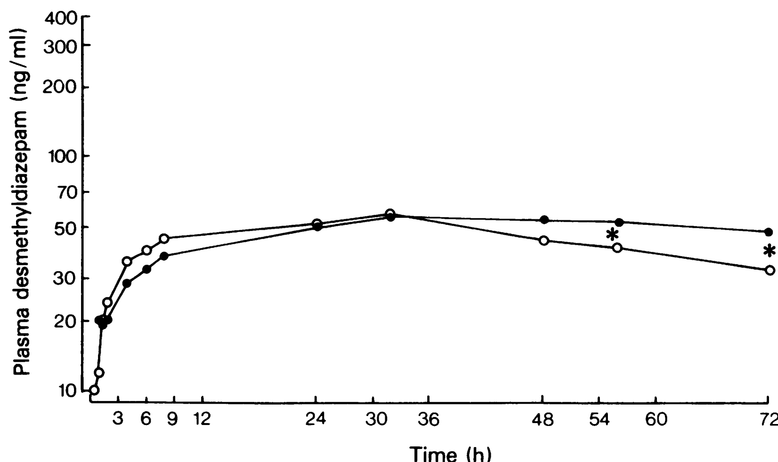


**Figure 2** The mean ($n = 7$) plasma concentration of desmethyldiazepam, the main metabolite of diazepam is plotted *v* time before (●) and after (○) induction. There is a significant difference in the mean plasma concentrations at 56 and 72 h ($P<0.01$).

body clearance from 22.7 to 43.8 ml/min while the volume of distribution remained unchanged showing a value of about 70 l.

The formation of the main metabolite desmethyldiazepam is seen in Figure 2 and no differences in plasma concentrations were found before and after the induction period up to 48 h. After 48 h differences in desmethyldiazepam plasma concentrations occurred whereby significant differences were found at 56 and 72 h. The mean pharmacokinetic parameters calculated from the mean plasma concentrations of desmethyldiazepam are listed in Table 3. The area under the plasma concentration curve up to 72 h is identical before and after induction by antipyrine. In contrast, calculating the area under the curve for the time infinity a much smaller area was found following antipyring administration. The mean elimination rate constant increased from 0.005 $h^{-1}$ to 0.012 $h^{-1}$ having calculated half-lives of about 139 h and 58 h respectively.

## Discussion

In the present study an induction of the liver microsomal enzyme system was found following antipyrine administration for a period of 14 days based on the changes in the *in vivo* parameters measured. The extent of enzyme induction was similar to that found in other studies using antipyrine as an inducing agent (Davies, Simmons, Dordoni & Williams, 1974; Whitfield, Moss, Neale, Orme & Breckenridge, 1973; Ohnhaus, Martin, Kinser & Colombo, 1977; Ohnhaus & Park, 1979); antipyrine clearance, $\gamma$-glutamyl-transpeptidase,, D-glucaric acid and 6-$\beta$-hydroxycortisol urinary excretion showed similar increases.

In addition, the pharmacokinetic parameters for the elimination of diazepam before the induction of the liver microsomal enzyme system were very close to values reported in healthy volunteers after a single dose of 10 mg diazepam orally (Klotz, Antonin & Bieck, 1976). Following antipyrine administration for a period of 14 days the elimination of a single dose of diazepam was significantly changed showing a decrease in $\beta$-half-life or an increase in total body clearance. Parallel to these changes the desmethyldiazepam plasma concentrations were significantly decreased at 56 and 72 h following the induction period. Therefore an increased elimination of this metabolite seems likely and the comparison of the pharmacokinetic parameters calculated from the mean plasma concentrations before and after enzyme induction supports this assumption.

In man diazepam is completely metabolized and its possible metabolic pathways (Schwartz, Koechlin, Postma, Palmer & Krol, 1965) are seen in Figure 3. There are two distinct oxidative pathways for diazepam, one to desmethyldiazepam, the major

**Table 3** Mean pharmacokinetic parameters of desmethyldiazepam calculated from the mean plasma concentrations before and after administration of 2 × 600 mg antipyrine

| | *Before* | *After* |
|---|---|---|
| $AUC_{t72}$ (ng $ml^{-1}$ h) | 3428 | 3204 |
| $AUC_{t\infty}$ (ng $ml^{-1}$ h) | 13248 | 6062 |
| $\beta$ ($h^{-1}$) | 0.0049 | 0.0119 |
| $T_{\frac{1}{2}}$ (h) | 138.9 | 58.3 |

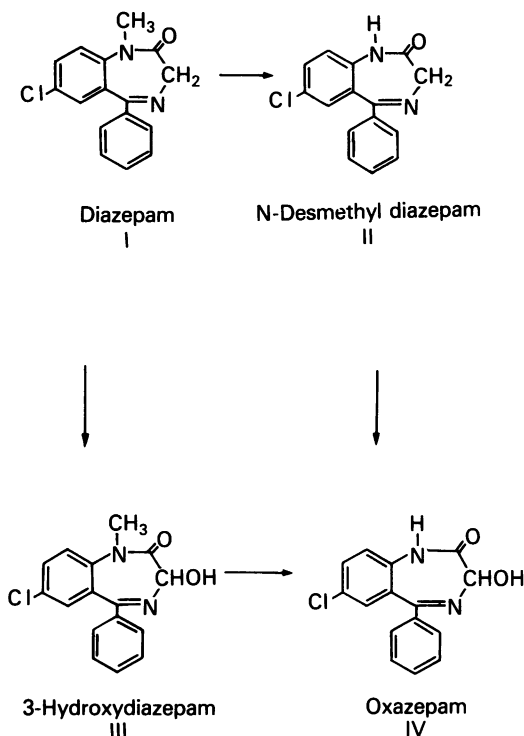


**Figure 3** The metabolic degradation of diazepam shows a major metabolic pathway into desmethyldiazepam and minor one into 3-hydroxydiazepam. Both metabolites are then converted into the final metabolite oxazepam.

metabolite, and other to 3-hydroxydiazepam. There after both metabolites are then hydroxylated to oxazepam the main metabolite found in the urine as a glucoronide. The increased elimination of diazepam following antipyrine administration found in the present study is probably based on an increase in desmethyldiazepam formation followed by an increased transformation into oxazepam. However, no experimental evidence for this combined induction towards these metabolic pathways can be presented as oxazepam was not estimated, but the identical desmethyldiazepam plasma concentrations up to 48 h and the significant differences afterwards support such a hypothesis. An additional induction on the 3-hydroxydiazepam metabolic pathway can not be confirmed or excluded from our data as this low concentration of metabolite was not measured. In experiments in animals similar results were found following chronic phenobarbitone administration whereby both N-demethylation and hydroxylation to oxazepam were increased in rats. In contrast, in mice and guinea pigs only one metabolic pathway either hydroxylation or N-demethylation was influenced by phenobarbitone administration (Marucci *et al.*, 1970).

In conclusion, in the present study a faster elimination of diazepam was found in healthy volunteers following treatment with antipyrine but other enzyme inducing drugs like phenobarbitone or rifampicin were not investigated. Therefore, the influence of other drugs known as enzyme inducing agents in man on diazepam elimination and possible induction of different metabolic pathways of diazepam metabolism as found in the animal experiments (Marucci *et al*.,, 1970) has to be further elucidated. However, based on the increased elimination of diazepam and desmethyldiazepam found in the present study a decreased pharmacodynamic effect seems possible in induced individuals, which might be sometimes of clinical importance.

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