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# THE EFFECT OF CARBON TETRACHLORIDE ($CCl_4$) INDUCED LIVER DAMAGE ON THE VOLUME OF DISTRIBUTION, THE ELIMINATION HALF-LIFE AND BODY CLEARANCE OF ANTIPYRINE AND WARFARIN IN RABBITS*

By

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LADEFOGED, O.: *The effect of carbon tetrachloride ($CCl_4$) induced liver damage on the volume of distribution, the elimination half-life and body clearance of antipyrine and warfarin in rabbits.* Acta vet. scand. 1979, *20*, 429—437. — The pharmacokinetics of antipyrine and warfarin were investigated in rabbits with carbon tetrachloride ($CCl_4$) induced liver damage. The volume of distribution of antipyrine was slightly increased whereas it was markedly reduced for warfarin when estimated 24 h after the $CCl_4$ injection. Twenty-four h after the $CCl_4$ injection, the elimination rate constant, the half-life and the body clearance were significantly changed for both compounds. The effect of $CCl_4$ on the pharmacokinetic parameters of antipyrine persisted 10 days after the $CCl_4$ injection, whereas the pharmacokinetic parameters of warfarin were normalized at that time. The clinical importance of the changes of drug pharmacokinetics in liver diseases is mentioned, and it is concluded that each drug may behave differently, so that a drug has to be investigated separately in every disease for which the drug is prescribed.

carbon tetrachloride; liver damage; antipyrine; warfarin; pharmacokinetics; rabbits.

The pharmacokinetics of drugs which are metabolized by the liver may change during liver disease (*Wilkinson & Schenker* 1975, *Kato* 1977). Some of these changes may be predicted by knowledge of the pathophysiology of the disease (*Shand* 1977). However, drugs behave differently pharmacokinetically (*Klotz*

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1976 a) and no single liver test is able to characterize the functional status of the liver. In acute liver disease, changes in the drug metabolizing capacity may not be closely related to the degree of cellular necroses and may vary from drug to drug (*Willson & Hart* 1977).

Carbon tetrachloride ($CCl_4$) has been used for years to induce experimental liver damage, but only few investigations have been carried out on the pharmacokinetics of various drugs in experimentally induced liver diseases (*Alfieri-Rolla & Segre* 1968).

The aim of the present study was to measure the pharmacokinetic parameters of antipyrine and warfarin during experimental $CCl_4$ intoxication in rabbits. Antipyrine was chosen as a drug with one-compartment pharmacokinetics and warfarin as one fitting a two-compartment model (*Ladefoged* 1978).

## MATERIAL AND METHODS

Twenty-two clinically healthy male rabbits weighing 3—4 kg were used in the experiments. In a control experiment, antipyrine 100 mg/kg b. wt. or warfarin 3 mg/kg b. wt. was injected. In order to calculate the pharmacokinetics of the drugs, blood samples (1 ml) were taken from a vein in the right ear in the time range of 5 min to 400—600 min after the drug administration. One week later $CCl_4$ was injected intraperitoneally. Two doses of $CCl_4$ were used (0.4 ml and 0.2 ml/kg b. wt.) in the antipyrine experiment. Only 0.4 ml $CCl_4$ was used in the experiment where warfarin was injected. Twenty-four h after the $CCl_4$ injection and also 10 days later, the pharmacokinetic parameters were determined after a new intravenous injection of the drugs.

Antipyrine was determined in plasma according to the method of *Brodie et al.* (1949). Warfarin plasma concentration was determined by a spectrofluorometric method described by *Corn & Berberich* (1967).

The nonlinear iterative curve fitting computer program, which has been described previously (*Ladefoged* 1975), was used for the calculation of the pharmacokinetic parameters from the plasma disappearance curves. The equations:

$C_t = A.e^{-k_e \cdot t}$ for the one-compartment model

and

$C_t = A.e^{-\alpha t} + B.e^{-\beta t}$ for the two-compartment model were used.

The volume of distribution ($V_d$) for the one-compartment model was calculated by the equation:

$$V_d = \frac{D}{A}; \; D = \text{dose (mg/kg)}.$$

In the two-compartment model the volume of distribution of the central compartment ($V_1$), the peripheral compartment ($V_2$) and the volume of distribution at steady state ($Vd_{ss}$) were calculated by the equations:

$$V_1 = \frac{D}{A + B}; \; D = \text{dose (mg/kg)},$$

$$V_2 = \frac{V_1 \cdot k_{12}}{k_{21}}; \; k_{12} \text{ and } k_{21} = \text{distribution rate constants},$$

and

$$Vd_{ss} = V_1 + V_2.$$

The body clearance was calculated by the equation:

$Cl_{body} = V_d \cdot k_e$; $k_e$ = elimination rate constant (one-compartment model)

or

$Cl_{body} = V_1 \cdot k_e$, (two-compartment model).

The half-life of the drugs was calculated as:

$$t_{\frac{1}{2}\,el} = \frac{0.693}{k_e}.$$

Paired t-tests were used to evaluate differences between the parameters in the control groups and the $CCl_4$ treated groups.

## RESULTS

Previous injection with antipyrine increased the hepatotoxicity of $CCl_4$ since seven out of 10 animals injected with antipyrine died of a dose of $CCl_4$ (0.4 ml/kg b. wt.) which is not lethal to other rabbits (own experiments). Three rabbits died less than 24 h after the $CCl_4$ injection, and four others died three-four days after the $CCl_4$ injection. No rabbits died in the group pretreated with antipyrine and injected with 0.2 ml/kg b. wt. of $CCl_4$ or in the group pretreated with warfarin and injected with 0.4 ml/kg b. wt. of $CCl_4$.

The averages ± s.e.m. of the pharmacokinetic parameters of antipyrine in the control experiment and in the experiment 24 h after injection of $CCl_4$ 0.4 ml/kg b. wt. are shown in Fig. 1. The

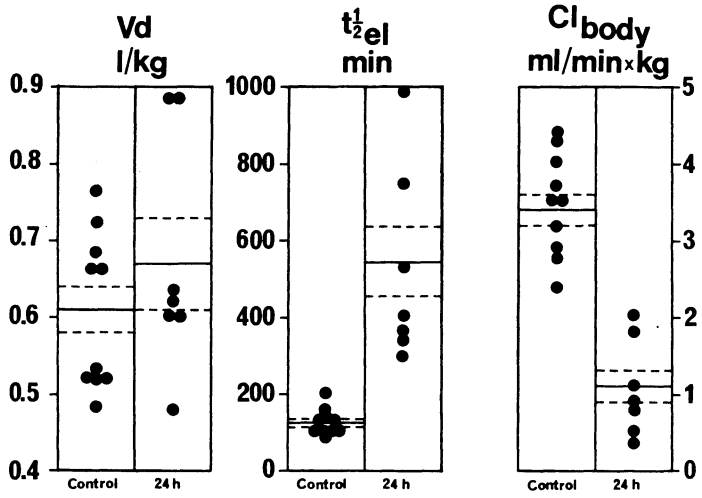


Figure 1. The effect of $CCl_4$ induced liver damage on the volume of distribution, elimination half-life and body clearance of antipyrine 100 mg/kg b. wt. i.v. in rabbits. The results shown are the parameters in the control experiment and the parameters 24 h after injection of $CCl_4$ 0.4 ml/kg b.wt. i.p.

elimination of antipyrine was remarkably reduced after $CCl_4$ pretreatment as manifested by changes in $k_e$, $t_{\frac{1}{2}el}$ and $Cl_{body}$, whereas the volume of distribution showed no statistically significant changes. The results from the experiment in rabbits injected with antipyrine and 0.2 ml/kg b. wt. of $CCl_4$ are shown in Table 1. In these animals the volume of distribution increased from 0.64 to 0.76 l/kg after the $CCl_4$ injection, a change which was still persistent 10 days later. The elimination of antipyrine was reduced to about one third of the control value 24 h after the injection, and significant changes in $k_e$, $t_{\frac{1}{2}}$ and $Cl_{body}$ were still present 10 days later.

Table 2 shows the pharmacokinetic parameters of warfarin before and following $CCl_4$ intoxication. The volume of distribution of the central compartment as well as of the peripheral compartment for warfarin were reduced in rabbits 24 h after injection of $CCl_4$, but no statistically significant changes were present 10 days later. The elimination of warfarin was reduced 24 h after the $CCl_4$ injection but not after 10 days.

Table 1. Mean and s.e.m. values for the pharmacokinetic parameters of antipyrine in six rabbits before and following $CCl_4$ intoxication (0.2 ml/kg b.wt. i.p.).

| Parameter | Control | 24 h after $CCl_4$ | 10 days after $CCl_4$ |
|---|---|---|---|
| Volume of distribution l/kg, $V_d$ | 0.64 ± 0.01 | 0.76 ± 0.05<br>$P < 0.05$ | 0.76 ± 0.04<br>$P < 0.05$ |
| Elimination rate constant $min^{-1}$, $k_e \times 10^{-2}$ | 0.59 ± 0.05 | 0.18 ± 0.01<br>$P < 0.001$ | 0.43 ± 0.04<br>$P < 0.01$ |
| Half-life min. $t_{\frac{1}{2}el}$ | 124 ± 12 | 407 ± 30<br>$P < 0.001$ | 168 ± 12<br>$P < 0.01$ |
| Body clearance ml/min × kg, $Cl_{body}$ | 3.7 ± 0.3 | 1.3 ± 0.1<br>$P < 0.001$ | 3.2 ± 0.3<br>$P < 0.05$ |

Table 2. Mean and s.e.m. values for the pharmacokinetic parameters of warfarin in six rabbits before and following $CCl_4$ intoxication (0.4 ml/kg b.wt. i.p.).

| Parameter | Control | 24 h after $CCl_4$ | | 10 days after $CCl_4$ | |
|---|---|---|---|---|---|
| Volume of distribution $V_d$, l/kg | | | | | |
| $V_1$ | 0.15 ± 0.01 | 0.11 ± 0.01 | $P < 0.01$ | 0.16 ± 0.01 | NS |
| $V_2$ | 0.07 ± 0.01 | 0.04 ± 0.01 | $P \pm 0.01$ | 0.06 ± 0.01 | NS |
| $Vd_{ss}$ | 0.22 ± 0.01 | 0.15 ± 0.01 | $P < 0.01$ | 0.22 ± 0.01 | NS |
| Elimination rate constant $min^{-1}$, $k_e \times 10^{-2}$ | 0.30 ± 0.03 | 0.13 ± 0.02 | $P < 0.01$ | 0.23 ± 0.03 | NS |
| Half-life min. $t_{\frac{1}{2}el}$ | 247 ± 25 | 601 ± 98 | $P < 0.01$ | 350 ± 56 | NS |
| Body clearance ml/min × kg, $Cl_{body}$ | 0.42 ± 0.02 | 0.10 ± 0.04 | $P < 0.001$ | 0.34 ± 0.03 | NS |

All the P values for differences between the pharmacokinetic parameters in control and $CCl_4$ injected rabbits are summarized for both antipyrine and warfarin in Table 3.

Table 3. Changes in pharmacokinetic parameters of antipyrine and warfarin following experimental $CCl_4$ intoxication in rabbits.

| | Antipyrine 100 mg/kg b.wt. i.v. | | Warfarin 3 mg/kg b.wt. i.v. | |
|---|---|---|---|---|
| Parameter | 24 h after $CCl_4$ | 10 days after $CCl_4$ | 24 h after $CCl_4$ | 10 days after $CCl_4$ |
| Volume of distribution, l/kg | | | | |
| $V_1$ | — | — | decreased $P < 0.01$ | unchanged |
| $V_2$ | — | — | decreased $P < 0.01$ | unchanged |
| $V_d/Vd_{ss}$ | increased $P < 0.05$ | increased $P < 0.05$ | decreased $P < 0.01$ | unchanged |
| Elimination rate constant | | | | |
| $min^{-1}$, $k_e$ | decreased $P < 0.001$ | decreased $P < 0.01$ | decreased $P < 0.01$ | unchanged |
| Half-life | | | | |
| min. $t_{\frac{1}{2}el}$ | increased $P < 0.001$ | increased $P < 0.01$ | increased $P < 0.01$ | unchanged |
| Body clearance | | | | |
| ml/min × kg, $Cl_{body}$ | decreased $P < 0.001$ | decreased $P < 0.05$ | decreased $P < 0.001$ | unchanged |

## DISCUSSION

The volume of distribution of a drug is a useful pharmacokinetic parameter as it relates the drug concentration in plasma with the total amount of drug in the body. In man with liver disease it has been shown that the volume of distribution for the same drug may increase, decrease or be unchanged compared to the volume of distribution in control individuals (*Pessayre et al.* 1977). A part of the explanation for this inconsistence in the alteration may be that it is usual to observe greater intersubject variability in the pharmacokinetic parameters in man with liver diseases compared with normal individuals (*Wilkinson &*

*Schenker* 1976) and that the selection and characterization of patients for this type of experiment are difficult. In the present investigation, problems of this kind are avoided by using an experimentally induced liver disease and by using the animals as their own controls.

As seen from Fig. 1 and Tables 1 and 2 there is a trend towards a greater variability in some of the pharmacokinetic parameters in the $CCl_4$ induced liver disease.

Several factors are involved in experimental or disease induced changes in drug distribution volume (*Klotz* 1976 b). The reason for the slight increase in volume of distribution of antipyrine and the reduction of the volume of distribution of warfarin is not obvious. In $CCl_4$ induced liver damage a complex change in physiological and biochemical parameters appears, and the change in volume of distribution might be the result of more than one of these changes.

The results of investigation concerning the effect of liver disease on the elimination rate constant, half-life and body clearance in humans indicate that two types of drugs exist. In one type of drug the elimination is determined by the blood flow to the liver. In the other type of drug the elimination is limited by the enzyme capacity of the liver cells (*Wilkinson & Schenker* 1975). The marked change in elimination rate constant, half-life and body clearance of antipyrine and warfarin in the present experiment may likewise be explained by changes in either blood flow to the liver or changes in enzyme capacity, but unfortunately very little is known about the liver blood flow after $CCl_4$ induced liver damage, and the reports on the metabolism of drugs in experimentally induced liver diseases are controversial too (*Kunii et al.* 1975, *Siegers et al.* 1978).

In the present experiment, the body clearance of both compounds was reduced to about one third of the value in the control animals. Such marked change in body clearance would have therapeutic implication for the clinical use of drugs. In domestic animals almost nothing is known about the effect of liver disease on drug pharmacokinetics. The $CCl_4$ induced liver damage might be a useful model for the further investigation of the therapeutic implication of the liver disease in the veterinary clinic.

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SAMMENDRAG

*Virkningen af tetraklorkulstof ($CCl_4$) induceret leverbeskadigelse på fordelingsvolumen, halveringstid og body clearance af antipyrin og warfarin hos kaniner.*

Fordelingsvolumen, eliminationskonstant, halveringstid og body clearance for antipyrin og warfarin blev bestemt hos normale kaniner og hos de samme kaniner 24 timer og 10 dage efter injektion af tetraklorkulstof ($CCl_4$) 0,4 ml/kg lgv. eller 0,2 ml/kg lgv. Fordelingsvolumenet for antipyrin var større hos kaninerne 24 timer efter $CCl_4$ injektionen, og ændringen kunne stadig påvises 10 dage efter $CCl_4$ injektionen. Warfarins fordelingsvolumen var signifikant mindre hos kaninerne 24 timer efter $CCl_4$ injektionen sammenlignet med kontrolværdien, men 10 dage efter $CCl_4$ infektionen var fordelingsvolumenet igen normaliseret. Eliminationskonstanten, halveringstiden og body clearance for både antipyrin og warfarin ændredes signifikant 24 timer efter $CCl_4$ injektionen. Kun for antipyrin kunne der påvises ændringer i de tre parametre 10 dage efter injektionen.

Den kliniske betydning af ændringer i lægemidlers farmakokinetik ved leversygdomme påpeges. Mulighederne for at anvende $CCl_4$ inducerede leverskader som model ved studier af farmakokinetiske konstanter ved leversygdomme i veterinærmedicinen fremhæves.

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