

EFFECT OF CERNILTON ON THE HEPATOTOXICITY OF CARBON TETRACHLORIDE [CCl₄] in rats

JERZY WÓJCICKI, LEONIDAS SAMOCHOWIEC

Laboratory of Clinical Pharmacology, Department of Pharmacology, Medical Academy, Szczecin

Cernitin correspond to microbiologically fermented pollen extracts [AB Cernelle, Vegeholm, Sweden]. Cernitin T60 contains mainly water soluble, while Cernitin GBX mainly fat soluble substances.

There are many components isolated from pollen and playing an important and fundamental role in the biological processes and cellular metabolism: essential amino acids, vitamins, enzymes, coenzymes, steroids, minerals and trace elements such as calcium, potassium, magnesium, iron, copper, zinc, manganese, titanium, molybdenum, silicon, sulfur, phosphorus, boron [1, 7, 8, 9, 10].

Composition of pollen extracts could raise the possibility, that Cernitins would be useful in preventing and management of liver injury. In this work we preliminary investigate this possibility.

Material and methods

Male Wistar rats weighing 170-200 g fed on a standard laboratory chow were administered CCl₄/0.25 ml per 100 g body weight/diluted with an equal volume of liquid paraffin by a stomach tube.

Cernitins were given 30 minutes prior and 4 h after CCl₄ application.

Seventy two animals were divided into six equal groups:

- group 1 — controls,
- group 2 — received CCl₄,
- group 3 — rats were given CCl₄ and Cernitin T60 50 mg/kg by a stomach tube [p.o.],
- group 4 — animals were administered CCl₄ and Cernitin T60 50 mg/kg intraperitoneally [i.p.],
- group 5 — received CCl₄ and Cernitin GBX 50 mg/kg by a stomach tube [p.o.],
- group 6 — rats were given CCl₄ and Cernitin GBX 50 mg/kg i.p.

The animals were fasted for 16 h prior to autopsy. After 24 h from CCl₄ administration the blood was collected and the liver was rapidly removed, weighed and homogenized.

The following biochemical parameters were determined: serum glutamic pyruvic transaminase [SGPT] activity according to the method of Reitman and Frankel [12], serum alkaline phosphatase [SAP] activity according to the method of Bodansky [5], bilirubin level in the blood serum by the method of Malloy and Evelyn [5] and serum total protein level according to the procedure described by Gornall et al. [4]. Triglycerides concentration in the liver homogenate was estimated using Eggstein and Kreutz method [3].

Specimens for histopathological studies were always taken from the same place of the liver; for routine microscopic investigations they were stained with haematoxylin and eosin, and for the lipids presence with oil red.

The results were analysed by Student's t-test.

Results

Treatment with CCl_4 caused a huge increase in SGPT activity and a marked increase in SAP activity [Table 1]. Intraperitoneal Cernitin T60 application was associated with a drop of SGPT activity by 52 per cent [$p < 0.01$] and SAP activity by 40 per cent [$p < 0.001$] in rats gavaged with CCl_4 [group 4] as compared with group 2. Diminution of these enzymes activity was observed also in animals receiving Cernitin T60 orally, but this diminution was less than that noted in group 4. Nevertheless the difference was statistically significant.

Rats given CCl_4 showed a bilirubin level that was increased to 1.93 $\mu\text{mol/l}$ in comparison with the control value 0.03 $\mu\text{mol/l}$ [Table 2]. Cernitin T60 reduced bilirubin level, especially when the drug was administered intraperitoneally, from 1.93 $\mu\text{mol/l}$ [group 2] to 0.10 $\mu\text{mol/l}$ [group 4]. The reduction of the bilirubin concentration occurred in rats receiving Cernitin GBX orally, as well.

Total protein level was practically unchanged in all the examined groups [Table 3]. Treatment with CCl_4 caused the expected rise in liver triglycerides by 245 per cent [Table 4]. In animals that were administered Cernitins, there was no decrease in the liver triglycerides concentration observed.

The mean relative liver weight was significantly higher by 77 per cent in rats given CCl_4 in relation to group 1 [Table 4]. Animals of group 4 and group 5 revealed statistically significant decrease in the relative liver weight as compared with group 2.

Histopathological examination showed marked fatty infiltration [Table 5] and remarkable centrilobular necrosis in all the rats of group 2. The characteristic centrilobular changes consisted of degeneration and necrosis of parenchymal cells around central veins, while peripheral part of the lobules contained a lot of cells revealing balloon degeneration.

In animals receiving Cernitin T60 intraperitoneally [group 4] fatty infiltration of the liver cells was to some degree diminished [Table 5], and necrotic changes were less severe in 6 of 10 rats. In 4 of the 6 mentioned rats necrosis of the liver parenchymal cells was even not shown, however balloon degeneration occurred. The necrosis was also less severe in rats given Cernitin T60 orally [group 3]. In 2 rats of this group necrosis disappeared almost completely.

Discussion

Various forms of treatment have been suggested for hepatic lesions: cysteine, glutathione, methionine, choline, vitamins, hormones and organ extracts. Although there are many drugs used in treatment of liver diseases, their effectiveness is very often insufficient and questioned. Search for new drugs and methods of pharmacotherapy of liver damage requires therefore further perpetual attention.

Severe injury of liver cells has been evoked by CCl_4 in our study. Remarkable centrilobular necrosis and ballooning, as well as fatty infiltration of liver cells was observed. This was accompanied by marked and significant elevation of serum en-

zymes activity and bilirubin concentration. Such a model of liver damage [6] can be useful for evaluation of potentially protective and therapeutic agents. Such a strong destruction of liver cell would require a special and strong drug, that could be able to remove completely all the alterations appearing in the form of histopathological a biochemical abnormalities. So potent and effective a drug has not existed, in our opinion, until now.

Our results can be assumed as promising. Cernitin T60 administered intraperitoneally and in less degree given orally possesses benefit effect on the liver of animals treated with CCl_4 . Serum glutamic pyruvic transaminase, accepted as a sensitive parameter in detecting structural abnormalities [2], and alkaline phosphatase activities were distinctly and significantly decreased in animals receiving Cernitin. Marked lowering of bilirubin level in the blood serum, as well as diminution of liver weight was also stated. These results were confirmed by the histopathological studies of the liver. Although triglycerides concentration per 1 g of liver homogenate was unchanged, nevertheless it was decreased when calculated per total organ.

Significance of our observations should be proved by using another models of liver cell damage, and in human beings suffering from acute or chronic liver injury and its consequences.

Cernitin could be applied alone or in combination with other substances known as liver protecting agents.

Conclusion

Significance of Cernitin as liver protecting agent should be considered.

References

1. Bolinder A.: Unpublished data.
2. Cutler M.G.: *Toxicol. Appl. Pharmacol.* 1974, 28, 349.
3. Eggstein M., Kreutz F.H.: *Klin. Wschr.* 1966, 44, 262.
4. Gornall A.G., Bardanill C.J., David M.M.: *J. Biol. Chem.* 1949, 177, 751.
5. Krawczyński J., Osiński T.: *Laboratoryjne Metody Diagnostyczne*. PZWL, Warszawa 1967.
6. Marchand C., McLean S., Plaa G.L., Traiger G.: *Biochem. Pharmacol.* 1971, 20, 869.
7. Nielsen, N., Grömmer J., Lunden R.: *Acta Chem. Scand.* 1955, 9, 1100.
8. Nielsen N.: *Acta Chem. Scand.* 1956, 10, 332.
9. Nilsson M.: *Acta Chem. Scand.* 1956, 10, 1.
10. Nilsson M., Pyhaga R., von Sydow E.: *Acta Chem. Scand.* 1957, 11, 634.
11. Papacharalampous N.X.: *Acta Hepato-Splen.* 1964, 11, 27.
12. Reitman S., Fränkel S.: *Am. J. Clin. Pathol.* 1957, 28, 56.

Address of authors: Laboratory of Clinical Pharmacology
Powstańców Wlkp. 72
70-111 Szczecin

Table 1. Serum glutamic pyruvic transaminase [SGPT] and serum alkaline phosphatase [SAP] activity in rats receiving Cernitin[50 mg/kg] T60 and GBX [mean±SE]

| Group | Treatment | SGPT [units] | SAP [units/l] |
|-------|-----------------------------|--------------|---------------|
| 1 | — | 38± 3 | 100± 4 |
| 2 | CCl ₄ | 9900± 946 | 380±28 |
| 3 | CCl ₄ + T60 p.o. | 6251± 429 | 286±27 |
| 4 | CCl ₄ + T60 i.p. | 4775±1036 | 230±15 |
| 5 | CCl ₄ + GBX p.o. | 8214± 615 | 310±21 |
| 6 | CCl ₄ + GBX i.p. | 10509± 340 | 252±15 |
| P | 1/2 | <0.001 | <0.001 |
| | 2/3 | <0.01 | <0.05 |
| | 2/4 | <0.01 | <0.001 |
| | 2/5 | >0.1 | >0.2 |
| | 2/6 | >0.5 | <0.001 |

Table 2. Effect of Cernitin [50 mg/kg] T60 and GBX on serum bilirubin level [μ mol/l] in rats receiving carbon tetrachloride

| Group | Treatment | Mean ± SE |
|-------|-----------------------------|------------|
| 1 | — | 0.03±0.001 |
| 2 | CCl ₄ | 1.93±0.21 |
| 3 | CCl ₄ + T60 p.o. | 1.13±0.28 |
| 4 | CCl ₄ + T60 i.p. | 0.10±0.02 |
| 5 | CCl ₄ + GBX p.o. | 0.38±0.06 |
| 6 | CCl ₄ + GBX i.p. | 1.76±0.31 |
| P | 1/2 | <0.001 |
| | 2/3 | <0.05 |
| | 2/4 | <0.001 |
| | 2/5 | <0.001 |
| | 2/6 | >0.5 |

Table 3. Total protein level [g/100 m³] in the blood of animals treated with Cernitin [50 mg/kg] T60 and GBX

| Group | Treatment | Mean ± SE |
|-------|----------------------------|-----------|
| 1 | — | 6.7±0.12 |
| 2 | CCl ₄ | 6.6±0.11 |
| 3 | CCl ₄ +T60 p.o. | 6.1±1.67 |
| 4 | CCl ₄ +T60 i.p. | 6.1±0.10 |
| 5 | CCl ₄ +GBX p.o. | 6.4±0.10 |
| 6 | CCl ₄ +GBX i.p. | 6.0±0.2 |
| P | 1/2 | >0.5 |
| | 2/3 | >0.5 |
| | 2/4 | <0.001 |
| | 2/5 | >0.3 |
| | 2/6 | <0.01 |

Table 4. Triglycerides concentration in the liver homogenate [mmol/g] and liver weight calculated in g per 100 g body weight of animals receiving Cernitin [50 mg/kg] T60 and GBX [mean±SE]

| Group | Treatment | Triglycerides | Liver weight |
|-------|----------------------------|---------------|--------------|
| 1 | — | 0.20±0.02 | 2.83±0.06 |
| 2 | CCl ₄ | 0.69±0.07 | 5.01±0.12 |
| 3 | CCl ₄ +T60 p.o. | 0.66±0.08 | 4.50±0.25 |
| 4 | CCl ₄ +T60 i.p. | 0.66±0.08 | 4.04±0.12 |
| 5 | CCl ₄ +GBX p.o. | 0.87±0.05 | 4.17±0.11 |
| 6 | CCl ₄ +GBX i.p. | 0.60±0.09 | 3.98±0.44 |
| P | 1/2 | <0.001 | <0.001 |
| | 2/3 | >0.5 | >0.05 |
| | 2/4 | >0.5 | <0.001 |
| | 2/5 | <0.05 | <0.001 |
| | 2/6 | >0.4 | >0.1 |

Table 5. Fatty infiltration of the liver cell examined histologically in rats receiving Cernitin [50 mg/kg] T60 and GBX

| Group | Treatment | Rat nr | | | | | | | | | |
|-------|-----------------------------|--------|------|------|------|------|------|------|------|------|------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | — | — | + | + | — | — | — | — | — | + | ++ |
| 2 | CCl ₄ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| 3 | CCl ₄ + T60 p.o. | ++++ | ++++ | +++ | ++++ | +++ | ++++ | ++++ | ++ | ++ | +++ |
| 4 | CCl ₄ + T60 i.p. | ++++ | — | ++++ | ++ | ++++ | ++++ | ++ | ++++ | +++ | ++ |
| 5 | CCl ₄ + GBX p.o. | ++++ | ++ | ++++ | — | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| 6 | CCl ₄ + GBX i.p. | ++++ | ++++ | +++ | ++++ | ++ | ++++ | ++++ | ++++ | ++++ | ++++ |

Infiltration was graded: — none
 + mild
 ++ moderate
 +++ marked
 ++++ severe

Wpływ Cernitynów na hepatotoksyczność czterochlorku węgla [CCl₄] u szczurów

Streszczenie

Cernitin T60 i GBX podawano dozodatkowo i dootrzewnowo szczurom otrzymującym CCl₄. Badano aktywność aminotransferazy glutaminowo-pirogronowej i fosfatazy zasadowej oraz oznaczano poziom białka i bilirubiny w surowicy krwi. W homogenacie wątroby określano zawartość trójglicerydów. Wątroby poddano również ocenie histopatologicznej.

Aktywność enzymów była znacznie i znamienne zmniejszona a poziom bilirubiny obniżony u zwierząt otrzymujących Cernitin T60 dootrzewnowo i w mniejszym stopniu doustnie, w porównaniu z wynikami uzyskanymi u szczurów otrzymujących jedynie CCl₄. Badania histopatologiczne wątroby w pewnym stopniu potwierdziły możliwość działania ochronnego Cernitynu w uszkodzeniu wątroby wywołanym zastosowaniem CCl₄.

ВЛИЯНИЕ ПРЕПАРАТОВ ЦЕРНИТИН НА ГЕПАТОКСИЧЕСКИЙ ЭФФЕКТ ЧЕТЫРЕХХЛОРИСТОГО УГЛЕРОДА (CCl₄)

Краткое содержание

Цернитин Т60 и GBX вводили в желудок и внутривентриально крысам, получавшим CCl₄. Исследовали активность глутамино-пируватной трансферазы и щелочной фосфатазы и определяли содержание белка и билирубина в сыворотке крови. В гомогенате печени определяли содержание триглицеридов. Исследовали также гистопатологические изменения печени.

По сравнению с крысами, получавшими только CCl₄, у животных, получавших также через брюшину Цернитин Т60, активность ферментов была значительно и характерно снижена, уровень билирубина был также снижен, в меньшей степени этот эффект наблюдался при введении препарата через желудок. Гистопатологические исследования печени свидетельствуют в пользу возможного защитного действия Цернитина при поражении печени, вызванном CCl₄.

Effekten av vissa Cernitinpreparat på CCl₄-orsakad hepatotoxicitet hos möss

Sammanfattning

Möss som hade tillförts CCl₄ behandlades peroralt och intraperitonealt med Cernitin T60 och GBX. Aktiviteten hos glutaminpyruvataminotransferasen och den alkaliska fosfatasen undersöktes, och halten av protein och bilirubin i blodserum fastställdes. Triglyceridhalten i homogeniserad levervävnad bestämdes. En histopatologisk bedömning av levern gjordes också.

Jämfört med de resultat som visades ifråga om möss som enbart tillförts CCl₄ var enzymhalten väsentligt och signifikativt lägre, och bilirubinhalten också lägre, hos djur som behandlades med Cernitin intraperitonealt och i mindre grad även peroralt. De histopatologiska undersökningarna av levern bekräftade i viss mån möjligheten att Cernitin har skyddande verkan vid leverskada framkallad av användningen av CCl₄.