Colchicine Intervention Abolishes Resiliency of Aged Fischer 344 Rats Against Chlordecone-Amplified Carbon Tetrachloride Hepatotoxicity

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ABSTRACT

Aged Fischer 344 (F344) rats are resilient to chlordecone (CD) amplified CCl_4 hepatotoxicity due to prompt, and robust tissue repair response (Murali B. et al., 2004). If this is the underlying mechanism for the resiliency, then antimitotic intervention after infliction of CCl_4-induced liver injury should render those aged F344 rats susceptible to CD + CCl_4 interactive toxicity and mortality. After a non-toxic dietary regimen of CD (10 ppm) or normal powdered diet for 15 days, rats received a single non-toxic dose of CCl_4 (100 µl/kg, ip, 1:4 in corn oil) or corn oil (500 µl/kg, ip) alone on day 16. Thirty h later one group of rats received single dose of colchicine (CLC, 1mg/kg, i.p.) and the other group received distilled water as a vehicle. Liver injury was assessed by plasma ALT, AST, and histopathology during a time course of 0 to 48 h. Liver tissue repair was measured by [^{3}H-CH_3]-Thymidine incorporation assay and PCNA immunohistochemistry. Exposure to CLC alone neither caused liver injury nor mortality. CLC administration to CD + CCl_4 group killed 2 out of 3 rats by 48 h, whereas none of the ND + CCl_4 rats receiving CLC died. Liver injury was further increased in CD + CCl_4 + CLC group at 36 h indicating that blocking cell division led to further progression of liver injury, and death. These findings further confirm that it is the stimulation of prompt and robust tissue repair rescued the 14-month old F344 rats from the lethal effect of the CD + CCl_4 combination.

INTRODUCTION

Previous studies demonstrated that chemical-induced hepatic injury and disease could either increase or decrease with age (1). Recent studies have revealed that neonates and young rats are resilient to a wide variety of structurally and mechanistically dissimilar hepatotoxicants such as CCl_4 (2, 3), allyl alcohol (2), galactosamine (4) and acetaminophen (5). Newborn and young developing rats are resilient to the chlordeone (CD) + CCl_4 combination, known to be lethal in adult rats (6,7).

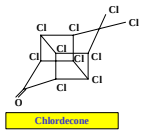
Aged (14 and 24 months) male F344 rats are resilient to deadly combination of CD + CCl_4 toxicity due to prompt and robust tissue repair response (7). If tissue repair response is the critical response for the resiliency of aged rats, then antimitotic intervention (ie, Colchicine administration) should abolish this tissue repair response. Present study was designed to test this hypothesis. We report here that colchicine antimitotic intervention inhibited the tissue repair response and led to animal death from an ordinarily nonlethal combination of CD + CCl_4 in the aged F344 rats. These findings confirm the critical importance of timely and adequate tissue repair response in the ultimate outcome of CD + CCl_4 toxicity in aged F344 rats.

OBJECTIVE

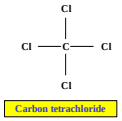
To test the importance of timely and adequate tissue repair in the final outcome of CD + CCl_4 toxicity in aged rats.

HYPOTHESES

CLC intervention will abolish the robust tissue repair response, leading to death of aged rats exposed to an ordinary nonlethal combination of CD + CCl_4.



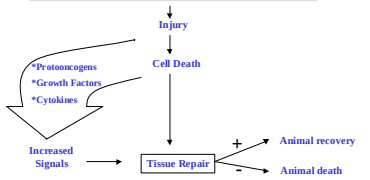
Chlordecone



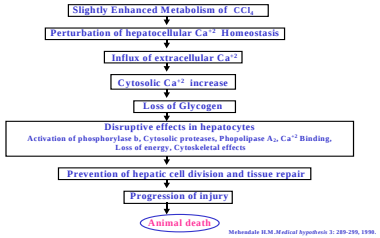
Carbon tetrachloride

Mechanisms of Toxicity

* Free radicals
* Reactive intermediate
* Lipid peroxidation
* Blocked of endogenous pathways



Mechanism for Chlordecone + CCl_4 Toxicity



Mehendale H.M. *Medical hypothesis* 3: 289-299, 1990.

Table 1: Effect of age on CD+CCl_4-induced mortality in F344 rats

Groups	% Mortality
CD + CCl_4 (3 Mon.)	100
CD + CCl_4 (14 Mon.)	0
CD + CCl_4 (24 Mon.)	0

CD = Chlordecone (10 ppm diet for 15 days)
CCl_4 = Carbon tetrachloride (100 µl/kg)

Murali et al., Mech. Ageing Dev. 125:421-435, 2004.

Comparison of Liver Injury

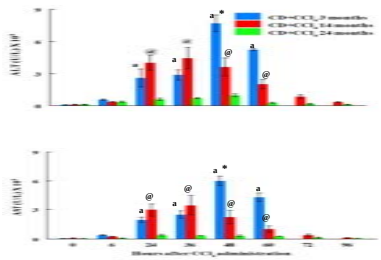
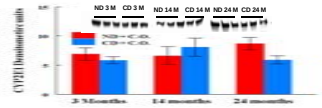


Figure 1: Result: Injury progresses in 3-month rats leading to death of all the rats by 72 h.

CYP2E1 Enzyme Concentration



CYP2E1 Enzyme Activity

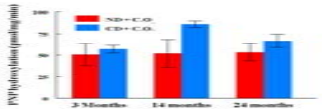


Figure 2: Result: Neither CYP2E1 protein nor enzyme activity change in any age groups by exposure to CD.

Covalent binding of $^{14}CCl_4$ to macromolecules

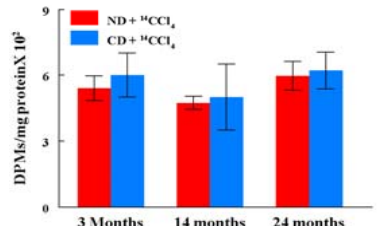


Figure 3: Result: Neither age difference nor exposure to CD diet was found.

Comparison of S-phase DNA Synthesis

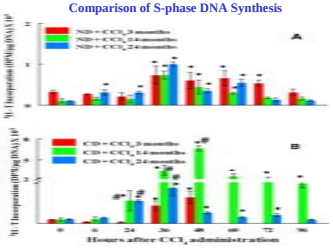


Figure 4: Result: Inhibited liver tissue repair in 3 month rats. There is an early onset and marked increase in tissue repair in 14 month rats compared to young adult and 24 month rats.

EXPERIMENTAL PROTOCOL

Animals: Male F344 rats of 14 months were purchased from Harlan Sprague Dawley/NIA, Chicago. Rats had free access to commercial rat chow (Teklad diet #. 7001, Indianapolis, IN) and water.

Chemicals: Chemicals were purchased from Sigma Chemical Co. and [^{3}H-CH_3] - Thymidine (^{3}H-T) from Moravek Biochemicals Inc. (Brea, CA).

Treatment: After an acclimation, the rats were maintained on the standard rodent powder diet with or without 10 ppm CD for 15 days. On day16, rats were challenged with single injection of CCl_4 (100 µl/kg, ip, 1:4 solution in corn oil) or corn oil (500 µl/kg) alone. Thirty h later one group of rats received single dose of CLC (1 mg/kg, ip) and the other group received distilled water as a vehicle. Blood samples were collected at 0, 6, 24, 36 & , 48 h after CCl_4 or corn oil treatment.

Liver injury: Plasma ALT and AST were measured using the reagent kits.

Regeneration studies: Measured by ^{3}H-T incorporation into hepatonuclear DNA (8)), and DNA content by diphenylamine reaction (9).

Proliferating cell nuclear antigen assay (PCNA): Assay was as described by Greenwell et al. (10).

Statistical analysis: Data were analyzed by ANOVA followed by Duncan's multiple range tests. p< 0.05

Table 2: Effect of colchicine on CD + CCl_4 toxicity in 14-Month male F344 rats

Groups	% Mortality
ND + CCl_4 + DW	0
ND + CCl_4 + CLC	0
CD + CCl_4 + DW	0
CD + CCl_4 + CLC	90

CD = Chlordecone (10 ppm diet for 15 days)
CCl_4 = Carbon tetrachloride (100 µl/kg)
CLC = Colchicine (1 mg/kg, ip)
DW = Distilled water

Result: CLC abolished the age advantage !

Comparison of Liver Injury

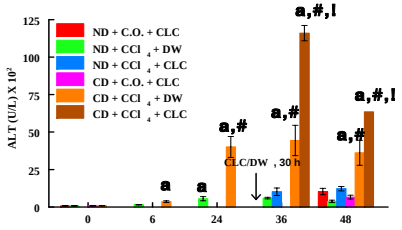


Figure 5: Result: CLC intervention led to progression of liver injury in 14-month rats leading to death by 48 h.

Comparison of Liver Injury

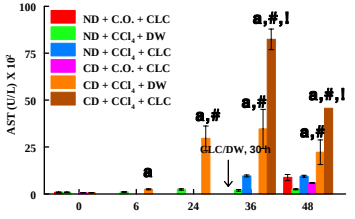


Figure 6: Result: CLC intervention led to progression of liver injury in 14-month rats leading to death by 48 h.

Comparison of S-phase DNA Synthesis

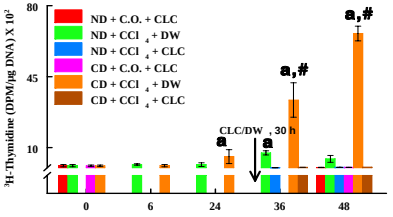


Figure 7: Result: As expected, CLC intervention inhibited markedly increased tissue repair response by 36 h.

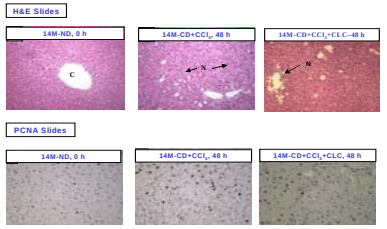


Figure 8: Representative photomicrographs of H&E-stained liver sections and proliferating cell nuclear antigen (PCNA) immunohistochemistry.

RESULTS & SUMMARY

1) CLC intervention led to progression of CD + CCl_4 toxicity culminating in death of 14-month rats by 48 h.
2) Further escalated liver injury culminating to death of aged rats, otherwise resilient to CD + CCl_4 toxicity.
3) CLC intervention inhibited prompt and robust tissue repair response.

CONCLUSIONS

1) CLC intervention abolishes resiliency of 14-month old rats to CD + CCl_4 toxicity.
2) Prompt and robust tissue repair is responsible for stopping the progression of injury, and restoration of liver structure & function, recovery and animal survival.

Future Studies

Molecular mechanisms of enhanced tissue repair.

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