# Review of experimental models for inducing hepatic cirrhosis by bile duct ligation and carbon tetrachloride injection[1]

## Revisão de modelos experimentais de cirrose hepática induzida por ligadura do ducto biliar e por injeção de tetracloreto de carbono

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**ABSTRACT**

**PURPOSE**: To present a review about a comparative study of bile duct ligation versus carbon tetrachloride Injection for inducing experimental liver cirrhosis.

**METHODS**: This research was made through Medline/PubMed and SciELO web sites looking for papers on the content "induction of liver cirrhosis in rats". We have found 107 articles but only 30 were selected from 2004 to 2011.

**RESULTS**: The most common methods used for inducing liver cirrhosis in the rat were administration of carbon tetrachloride (CCl4) and bile duct ligation (BDL). CCl4 has induced cirrhosis from 36 hours to 18 weeks after injection and BDL from seven days to four weeks after surgery.

**CONCLUSION**: For a safer inducing cirrhosis method BDL is better than CCl4 because of the absence of toxicity for researches and shorter time for achieving it.

**Key words**: Liver Cirrhosis. Drugs. Carbon Tetrachloride. Common Bile Duct. Ratos.

**RESUMO**

**OBJETIVO**: Apresentar revisão sobre estudo comparativo da indução de cirrose hepática (CH) experimental com a injeção de tetracloreto de carbono (CCl4) comparado à ligadura do ducto biliar (BDL).

**MÉTODOS:** A pesquisa foi realizada nas bases de dados do Medline/PubMed e SciELO procurando trabalhos com as palavras indução de CH e ratos. Foram encontrados 107 artigos, mas somente 30 foram selecionados no período de 2004 à 2011.

**RESULTADOS:** Os procedimentos mais comum para indução de CH em ratos foram a injeção de CCl4 e a BDL. O CCl4 induzia CH no período de 36 horas após a injeção e a DBL de sete dias à quatro semanas após a cirurgia.

**CONCLUSÃO**: A BDL é o método mais seguro para indução de CH quando comparado a injeção de CCl4 pela ausência de toxicidade para os pesquisadores e o menor tempo para se obter a lesão hepática.

**Descritores**: Cirrose Hepática. Drogas. Tetracloreto de Carbono. Ducto Colédoco. Ratos.

## Introduction

Liver cirrhosis (LC) is considered a public health, according to World Health Organization, about 800 thousand people die from LC every year. Only in United States LC is responsible for around 27 thousand deaths per year, representing a mortality rate of 9.2 per 100,000, placing it as the 12th overall cause of death[1-3].

Nowadays major research centers focus on studying LC, its mechanisms and its behavior, complications and possible treatments. For that reason it has been developed, efficient experimental models of induction of LC in rats. The two most common methods used for experimental LC are the administration of carbon tetrachloride (CCl4) and the bile duct ligation (BDL)[7-9]. Our aim is to present a comparative study of bile duct ligation versus carbon tetrachloride injection for inducing experimental liver cirrhosis

## Methods

This research was made through Medline/PubMed and SciELO web sites looking for papers on the content "induction of liver cirrhosis in rats". We found 107 articles but only 30 from 2004 to 2011 were selected. The inclusion criteria were: only rats; bile duct ligation and injection of carbon tetrachloride. Histopathologic examination for confirming cirrhosis; Time of inducing cirrhosis. The exclusion criteria were: large animals cirrhosis; others rodent animals; drug inducing cirrhosis such as dimethylnitrosamine; thiocetamide; butylhydroperoxide. Others drugs association methods: buprenorphine with reduction of portal inflow over a stent inserted in the right renal artery; Others methods: unrestricted flow using an aortic-portal segment; orthotopic liver transplantation with unrestricted portal arterialisation. Thioacetamide associated with partial hepatectomy.

## Results

The main methods of induction of LC in rats are the administration of carbon tetrachloride and bile duct ligation. Below are shown induction of cirrhosis by CCl4, dosage of the drug, time for inducing cirrhosis, main test assessment with the method and their results (Table 1) and for bile duct ligation (Table 2).

**Table 1 - Induction of cirrhosis in rats by carbon tetrachloride (CCl4)**

| Author/ Year | Dosage of CCl4 | Number of Rats | Time do induce cirrhosis | Test | Results |
|---|---|---|---|---|---|
| Maya-Mendoza et al; 2004 [7] | ND | ND | 8 weeks | Explore genes with differential activity or position in the nuclear matrix | Changes in the relative position of specific genes to the nuclear matrix occur during the chronic administration of CCl4 |
| Peng et al; 2005 [11] | ND | 60 | ND | Expression of bFGF and HSC | bFGF regulates liver fibrogenesis through regulating metabolism of extracellular matrix |
| Fiorucci et al; 2005 [12] | 100µ l/100 g | 22 | 4 weeks | Farnesoid X receptor | FXR promotes the development of a quiescent phenotype and increases apoptosis of HSCs |
| Lewis et.al; 2005 [14] | 1,5 mL/kg | 8 | 36 hours | CYP2E1 | The CYP2E1 can induce endoplasmatic reticulum protein damage and stress via its catalytic activation of pro-oxidants |
| Oria et al; 2006 [16] | 20µL/kg/week | 41 | 8 – 14 weeks | Propofol | Reproduce functional abnormalities of the central motor tract |
| Kotsiou et al; 2006 [17] | ND | 40 | 6 weeks | G1:propranolol; G2:propranolol + lidocaine; G3: Propanolol + CCl4; G4: Propanolol + Lidocaine + CCl4 | Propranolol dosage should be reduced when lidocaine is co-administered |
| Li et al; 2006 [18] | 2mg/kg | ND | 6 weeks | Small interfering RNA | Prevent liver fibrosis |
| Tsui et al; 2006 [19] | 0,2 ml/kg/week | ND | 9 weeks | Heme oxygenase | Suppresses the development of cirrhosis |
| Cheung et al; 2006 [20] | ND | 42 | 8 weeks | WeiJia | Reduces liver fibrosis and improves liver funtion |
| Liu et al; 2006 [23] | 1.5 mL/kg in liquid parafin 1:1 twice a week for 8 weeks | 70 | 8 weeks | Ginkgo Biloba Extract | Inhibits the HSC |
| Xue et al; 2007 [25] | 5 or 3 ml/kg in 400 ml olive oil twice a week | ND | 11 weeks | Hemina | Liver protection |
| Shen et al; 2007 [26] | 0.4 ml/kg in corn oil 1:1 once time | ND | ND | ND | Increases of Smad1 expression |
| Abe et al; 2007 [28] | 1ml/kg twice a week | ND | 7 weeks | Dalteparin sodium | Enhances hepatic regeneration and minimizes hepatic fibrogenesis |
| Yuan et al; 2008 [31] | 50% in Olive Oil | 40 | 18 weeks | *Bidens bipinnata L.* | Decrease hepatic desease |
| Borkham-Kamphorst et al; 2008 [32] | 1 ml/kg in mineral oil same volume | 30 | 12 weeks | ND | PDGF expression is relationed with liver regeneration |
| Tiberio et al; 2008 [34] | 0.02 mL/100g once a week | ND | 15 weeks | IL-6 | Liver regeneration |
| Tsai et al; 2008 [35] | 2.5 ml/kg twice a week in corn oil 1:5 | 20 | 8 weeks | Silymarin | Regeneration (decrease AST, ALT, FA) |
| Kim et al; 2009 [40] | ND | ND | 2 weeks | Betaine | Decrease cirrhosis |

**ND** - Not Described; **CCl4** - Carbon Tetrachloride; **bFGF** - Basic Fibroblast Growth Factor ; **FXR** - Farnesoid X receptor; **HSC** - Hepatic Stellate Cell; **CYP2E1** - Cytochrome P450 2E1; **Smad1** - Gene Mothers Against Decapentaplegic 1; **PDGF** - Platelet-Derived Growth Factor; IL-6 - Interleukin 6; **AST** - Aspartate Transaminase; **ALT** - Alanine Aminotransferase; **FA** - Phosphatase Alcaline .

**Table 2 - Induction of cirrhosis in rats by using bile duct ligation (BDL)**

| Author/ Year | Number of rats | Time do induce cirrhosis | Test | Results |
|---|---|---|---|---|
| Antoine et al; 2005 [9] | ND | 2 weeks | Pleiotrophin | Pleiotrophin will increase the HSC expression |
| Hsu et al; 2006 [10] | ND | 3 weeks | Tet and silymarin | They reduced the fibrosis scores and hepatic collagen content of BDL rats |
| Sztrymf et al; 2005 [13] | 65 | 5 weeks | Bacterial translocation | Bacterial translocation have a role in the pathogenesis of hepatopulmonary syndrome by inducing pulmonary intravascular macrophages through TNF-alpha upregulation |
| Peretz et al; 2006 [21] | 40 | 2 weeks | Phlebotomy before or after sham operation or BDL | Lowered hepatic iron concentration. After BDL: body weight increase, lower hepatic weight, less portal hypertension, less periportal necrosis, less portal inflammation, lower hepatic activity index score and higher albumin levels |
| Anan et al; 2006 [22] | ND | 7 days | Bortezomib and MG132 | Inducing HSC apoptosis and inhibiting liver fibrogenesis |
| Mikami et al; 2007 [24] | ND | 4 weeks | L-carnosine, zinc sulfate, and zinc L-carnosine | Protected portal hypertensive gastricmucosa with increased HSP72 expression |
| Tieppo et al; 2007 [27] | 28 | 28 days | Quercetin | Quercetin-treated cirrhotic rats showed reduced DNA damage in lung and liver tissues as compared to untreated cirrhotic rats |
| Lee et al; 2007 [29] | ND | 27 days | YCHT (Yin-Chen-Hao-Tang) | Hepatic hydroxyproline accumulation and hepatic collagen levels can be decreased |
| Thomsen et al; 2008 [30] | ND | 1 month | LPS e IGF-1 (infection simulation) | Accelerated tissue loss during infection |
| Langer et al; 2008 [33] | ND | 4 weeks | ND | Nitric Oxide induce HSC apoptosis |
| Vercelino et al; 2008 [36] | 24 | 2 weeks | N-Acetilcisteína | Protective effects in cirrhotic rats with hepatopulmonary syndrome |
| Hagens et al; 2008 [37] | ND | 10 days, 3 weeks | ND | A new HSC type was found |

**ND** - Not Described; **BDL** - Bile Duct Ligation; **HSP72** - 72-kDa Heat Shock Protein; **HSC** - Hepatic Stellate Cell; **LPS** - Lipopolysaccharide; **IGF-1** - Insulin-like Growth Factor 1.

## Discussion

The main methods of induction of LC in rats are the administration of carbon tetrachloride (CCl4)[4] and bile duct ligation (BDL)[11]. CCl4 is one of the most used methods nowadays however it is considered an extremely toxic method[5]. Several important basic mechanisms of tissue damages induced by CCl4 have emerged, involving metabolic activation, reactive free radical metabolites, lipid peroxidation, covalent binding and disturbance of calcium homeostasis.

The CCl4 administration results in hepatocyte damage, necrosis, inflammation, and fibrosis, which spreads to link the vascular structures that feed into and drain the hepatic sinusoid (the portal tract and central vein radicle, respectively)[3,39]. It activates the hepatic stellate cell (HSC) inducing hepatocyte apoptosis and zone III necrosis[40]. Continuous administration of CCl4 can provide moderate cell necrosis and fatty infiltration in four weeks.

The CCl4 is excreted from the body within the first 24 hour by conjugation reaction mediated by phase. In the literature, three mechanisms have been proposed as the possible explanations for progression of injury: (1) Contribution of inflammatory cells; (2) Production of free radicals; and (3) Leakage of degradative enzymes from the dying and injured cells. Activated resident Kupffer cells and the neutrophils recruited at the site of parenchymal liver injury are considered as the primary culprits in damaging surrounding healthy cells as the result of nonspecific action. However, evidence suggests that the contribution of the inflammatory cells does not or is not sufficient to mediate progression of injury.

The second theory regarding progression of injury is production of free radicals and oxidative stress, and subsequent lipid peroxidation that propagates injury. Though the antioxidants prevent/delay the tissue damage partially, progression of injury still occurs. The inhibition of lipid peroxidation by antioxidants only decreases the initial injury of CCl4, blocking lipid peroxidation fails to prevent progression of injury and subsequent lethality[45]. By 8 weeks, a micronodular cirrhosis takes place [31]. It has been used mainly by intraperitoneal injection[12] or oral administration[20], the dosage 0,2 to 5ml/kg and LC is achieved between 36 hours to 18 weeks[15,20,11,24].

BDL is a safer method comparing to the CCl4. When the BDL is done, it provides an acute obstructive jaundice in two weeks, and progression to cirrhosis in 4 or 6 weeks[9,10]. BDL stimulates the proliferation of biliary epithelial cells and oval cells (which are hepatocyte progenitors), resulting in proliferating bile ductules with an accompanying portal inflammation and fibrosis[43]. Cholangiocyte proliferation started after BDL at the edge of the portal tract. During the first week from BDL the hepatic microcirculation did not show any alterations with respect to the normal liver[46]. Using this method LC is achieved between seven days to four weeks[17,26].

## Conclusions

Based in our broadly review we concluded that LC can be induced by CCl4 and BDL, however the manipulation of CCl4 can be dangerous for the researcher with risk of inducing liver tumor. BDL is a safer method than CCl4 and cirrhosis could be induced in rats in average mean time of two weeks.

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