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ENVIRONMENTAL FACTORS AS DISEASE ACCELERATORS DURING CHRONIC HEPATITIS C

Short title : HCV and environmental factors

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Summary

Progression of chronic hepatitis is highly variable among individuals, as the result of several host, viral and environmental factors. The latter have been extensively investigated in order to achieve their control and ameliorate hepatitis C outcome, particularly in difficult-to-treat patients.

Over the last decade, several studies have shown that combination of HCV infection and high levels of alcohol abuse results in synergistic acceleration of liver fibrogenesis. In addition, recent data indicate that light alcohol intake may also exacerbate fibrosis progression. It has also been suggested that cigarette smoke may enhance activity grade in patients with chronic hepatitis C, thereby increasing progression of fibrosis. This assumption mostly relies on epidemiological evidences in the absence of mechanistic studies. Finally, cannabis use is increasingly emerging as a novel co-morbidity in patients with chronic hepatitis C (CHC). Indeed, regular cannabis smoking is an independent predictor of both fibrosis and steatosis severity in infected patients. In addition, experimental studies have shown that cannabinoid CB1 receptors enhance liver fibrogenesis and steatogenesis by distinct mechanisms, therefore strongly supporting epidemiological findings.

Altogether, patients should be informed of the deleterious impact of alcohol, tobacco and cannabis use and should be offered appropriate support to achieve abstinence.

Key words: Alcohol, tobacco, hepatitis C virus, chronic hepatitis C, cannabis, CB1 receptor, CB2 receptor, cannabinoid

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Chronic hepatitis C (CHC) affects over 170 million of people worldwide and is a leading cause of cirrhosis and hepatocellular carcinoma (1). The disease severity is however highly variable among patients and over time, some individuals running a benign clinical course for decades and others, rapidly progressing to end-stage liver disease. Several factors have been linked to fibrosis progression (table 1) or a to a reduced rate of antiviral response and among those, comorbidities amenable to therapeutic intervention require special attention, particularly in difficult-to-treat patients such as non responders to standard therapy. Not surprisingly, cofibrogenic effects of alcohol intake have been largely described over the last decade. More recently, tobacco and cannabis use have emerged as novel cofactors of fibrosis enhancement in patients with CHC. The scope of this manuscript is to review current data on the impact of alcohol intake, tobacco and cannabis use on CHC outcome.

1. Alcohol and hepatitis C

1.1 Epidemiological data

Defining the impact of alcohol use on hepatitis C has been the focus of intensive efforts during the last decade, given the frequent coexistence of alcoholism and HCV infection (2). Hence, the third US National Health and Nutrition Examination Survey reported that 20 % of patients with CHC consume 2 or more drinks daily. Similarly, HCV infection is highly prevalent among alcohol abusers, with HCV antibody seroprevalence ranging between 4.6 and 55.5 % (2). The reasons for these high rates of HCV infection in alcohol abusers remain unclear and may be related to both enhanced exposure to HCV and decreased spontaneous viral clearance in excessive drinkers. Although intravenous drug users are frequently alcohol abusers (3), high rates of HCV seropositivity have been found in alcohol users with no

identified risk for viral hepatitis (4), suggesting an impact of alcohol on HCV persistence. This assumption was recently supported in a study comparing two groups of veterans with recovered or ongoing HCV infection. By multivariate analysis, a history of past or present alcohol abuse was significantly associated with chronic HCV infection (5).

1.2 Impact of alcohol on CHC outcome

Several lines of evidence indicate that alcohol affects survival of patients with CHC. In a study of a US national sample of hospitalizations, alcohol abuse was associated with a 40 % increased odds of death in patients with CHC (6). In addition, retrospective analysis of 6354 consecutive admissions related to alcohol dependence or abuse in a single hospital found a trend for an increased inpatient mortality in individuals with HCV infection (7).

An overwhelming number of studies has shown that high levels of alcohol intake accelerate fibrosis progression in patients with CHC. In a retrospective analysis of 1574, Poynard et al. showed that heavy alcohol consumption (≥50g/d) was an independent predictor of fibrosis progression rate (8). Similarly, several reports identified heavy alcohol intake (≥50g/d) as a major cofactor of fibrosis progression (8-12) or cirrhotic outcome (table 2) (9, 12-21). These studies were somewhat limited by their predominant cross-sectional or retrospective design and displayed significant variability in the definition of alcohol abuse in terms of level, duration and recording, but nevertheless yielded consistent results. In addition, longitudinal follow-up of patients with CHC also found that alcohol use predicts fibrosis progression (10), cirrhotic decompensation (18) and increases the rate of liver-related deaths (20). Finally, a recent meta-analysis including 15 positive and 5 negative studies in a total of 13706 patients estimated the pooled relative risk of cirrhosis or decompensated cirrhosis in alcohol abusers to be 2.33 (95 % CI 1.67 – 3.26) (22).

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Fewer studies have evaluated the impact of low to moderate alcohol intake on fibrosis progression (9, 11, 18, 23, 24). Hezode et al. prospectively evaluated predictors of clinically significant fibrosis (≥F2 according to Metavir scoring system) in 260 patients with a daily alcohol intake ≤50 g during the 6 months preceding liver biopsy (24). By multivariate analysis, daily alcohol consumption ranging between 31 and 50 g was a strong predictor of fibrosis stage ≥F2 (OR = 4.3; CI: 1.2 – 16.0). A retrospective analysis of 78 patients with paired biopsies and a daily alcohol consumption below 40 g found that. progression of fibrosis was associated with a higher drinking frequency and a higher level of alcohol intake per occasion (11). Alcohol intake below 30 g/day were specifically investigated in two studies that found a non significant trend for worsening of liver fibrosis (18, 20). Finally, Monto et al analysed the average lifetime alcohol intake in 800 HCV patients and found a stepwise increase in mean fibrosis stage between nondrinkers, light (≤20 g), moderate (>20-50 g) and heavy (≥50 g) drinkers, although differences did not reach statistical significance (23). Overall, these results suggest that there is no safe level of alcohol intake.

1.3 Impact of alcohol on treatment outcome

Past and ongoing alcohol abuse has been reported to dose-dependently decrease the response to standard interferon treatment (2). However, studies were fraught with several limitations such as the low number of patients included, the retrospective design or the use of standard interferon monotherapy. Nevertheless, these observations suggested that alcohol might reduce the sensitivity to interferon and/or adherence to treatment. These issues were addressed in a prospective multicenter study of 726 patients receiving a combination of standard interferon and weight based ribavirin (25). Adherence to treatment and sustained viral response rates were similar in non drinkers and past alcohol users, as defined by abstinence within the year

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preceding treatment. In contrast, recent alcohol use was associated with a significant increase in treatment discontinuation rate (40 % vs 26 %) and a corresponding reduction in sustained viral response (14% vs 20 %). Subgroup analysis excluding patients with early drop-out indicated that the rate of viral eradication was similar in alcohol users undergoing a full course of treatment, compared to non drinkers. These results strongly suggest that alcohol reduces therapeutic response by decreasing patient adherence rather than by reducing sensitivity to interferon. Therefore, alcohol users should not be deferred from treatment but should rather be offered specific support to achieve abstinence and improve adherence. In this respect, it has been suggested that detoxification programs might be more successful in alcohol abusers with concurrent HCV infection compared to excessive drinkers without HCV infection (26).

1.4 Molecular mechanisms of interactions between alcohol and HCV

Despite strong epidemiological evidence linking alcohol use to acceleration of liver injury in CHC, little is known about the combined effects of ethanol and HCV on the pathogenesis of liver disease. Proposed mechanisms of interaction include enhancement of viral replication, increased oxidative stress and cytotoxicity, as well as impairment of immune response.

Several studies have shown modest increases in serum viral load in alcohol abusers compared to non drinkers (2), suggesting that alcohol may enhance viral replication. In support of this assumption, alcohol metabolites were shown to potentiate expression of viral proteins in an experimental replicon system (27). However, other studies failed to demonstrate an impact of of alcohol on serum HCV titers (2) and a recent meta-analysis from 9 available studies concluded that there were no significant differences in serum HCV viral titers of alcohol abusers and non drinkers (25).

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Both HCV and alcohol are known stimuli of hepatic oxidative stress and lipid peroxidation, suggesting that coexistence of these factors might enhance these pathways (28), thereby leading to increased activation of liver fibrogenic cells and subsequent acceleration of fibrogenesis. Thus, chronic administration of alcohol to HCV-core transgenic mice results in additive hepatic lipid peroxidation, synergistic induction of the profibrogenic cytokine TGF-β1 and activation of hepatic stellate cells (29). In addition, indirect clinical evidence suggest that enhanced oxidative stress may contribute to increased severity of CHC in alcohol users: in a series of 145 patients with CHC, the frequency of serum antibodies to lipid peroxide adducts was significantly increased in alcohol users compared to non users. Moreover, there was a significant correlation between the titers of lipid-peroxidation related antibodies and the severity of inflammation and fibrosis (30).

Experimental data suggest that alcohol-induced impairment of immunity may account for the high rates of persistent viral infection reported in excessive drinkers (5). Mice chronically exposed to ethanol show a reduced cellular immune response to HCV core and non structural proteins, following alteration in dendritic cell maturation leading to a propensity to generate Th2-immune response (31). In keeping with these data, plasmacytoid and myeloid dendritic cells of patients with CHC display a reduced allostimulatory potential which is further impaired in the presence of alcohol (32).

1.5 Summary

Coexistence of HCV infection and alcohol intake is a frequent finding. Assessment of the interaction between alcohol and HCV is inherently difficult, due to the inaccuracy in alcohol consumption recording. Nonetheless, available studies indicate that alcohol intake is a cofactor of worsened outcome and no safe threshold may be defined at the present time.

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2. Impact of cigarette smoke on the course of CHC

Whereas morbidity and mortality of cardiopulmonary diseases associated to tobacco use have been extensively depicted, available clinical evidence suggest that cigarette smoking does not induce chronic liver injury in healthy individuals. Likewise, experimental data documenting hepatotoxicity and/or liver directed fibrogenic effects of tobacco components are scarce (33).

That tobacco use might negatively impact chronic liver injury was initially suggested by two retrospective studies indicating a deleterious effect of cigarette smoking on prevalence and/or severity of alcoholic (34) and HBV-related cirrhosis (35). More recently, a history of smoking has been incriminated as a predisposing cofactor of primary biliary cirrhosis (36). Moreover, tobacco use was identified as an independent predictor of advanced fibrosis at presentation in a retrospective study of patients with primary biliary fibrosis (37). Finally, several reports suggest that cigarette smoking is associated with an increased incidence of hepatocellular carcinoma associated with cirrhosis (35, 38-42).

Table 3 summarizes main data regarding the impact of cigarette smoking on fibrosis progression in CHC. Two retrospective studies showed that tobacco use was an independent predictor of fibrosis stage (43, 44). However, in the study of Pessione et al., further investigation indicated that this relationship was lost when activity grade was included in the multivariate analysis, suggesting that cigarette smoke may indirectly enhance fibrosis severity, by increasing necroinflammatory grade (43). Hezode et al. subsequently investigated this hypothesis in a prospective study and found that recent tobacco use predicted activity grade, irrespective of alcohol intake (45); in contrast, whereas activity grade was an independent cofactor of fibrosis severity, there was no relationship between tobacco use and

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fibrosis stage by multivariate analysis (45). The impact of tobacco use was also investigated in a large-scale population study, using ALT levels as a surrogate marker of necroinflammation. In this survey of 886 patients with positive HCV antibodies, alcohol intake and cigarette smoking were independent predictors of elevated alanine aminotransferase levels (46). Altogether, these data suggest that cigarette smoking may aggravate necroinflammation associated with CHC and thereby accelerate fibrogenesis.

Pathways underlying cigarette smoke-induced progression of CHC or other chronic liver diseases remain elusive in the absence of experimental data. Studies in other tissues indicate that tobacco use enhances several pathways of the wound healing response, such as oxidative stress (47, 48) or the production of proinflammatory cytokines (49), leading to accumulation of fibrogenic cells and enhancement of extracellular matrix protein synthesis (47). Moreover, nicotin has also been shown to impair immune response (50). Finally, tobacco use also generates insulin resistance (51, 52), a known cofactor of fibrosis severity during CHC (53, 54).

Altogether, available studies, although mostly retrospective, suggest that cigarette smoke may enhance necroinflammation in patients with CHC, thereby accelerating progression of fibrosis. Additional prospective investigations are warranted in order to confirm these observations and experimental studies are needed to support these findings.

3. Cannabis and the endocannabinoid system

Cannabis Sativa has been used for medicinal purposes over millennia. During the 19th century, the plant was increasingly recommended for its analgesic, muscle relaxant, orexigenic and anticonvulsivant properties in a variety of diseases, ranging from epilepsy,

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tetanus, rheumatism to gastroenterological symptoms, until growing concern about the dangers of abuse led to banning from the pharmacopeia in the 1930's (55-57). Recreational use of marijuana progressively expanded worldwide thereafter. Albeit cannabis use primarily occurs in teen-agers and young adults and is usually self-limited, continued consumption for prolonged periods has been described, predominantly in frequent users. (58, 59)

3.1 Cannabis and the endocannabinoid system

Δ9-tetrahydrocannabinol (THC) was identified in 1964 as the compound responsible for psychoactive effects of cannabis, and further studies unravelled the concurrent presence of over 60 bioactive phytocannabinoids (55-57, 60). Subsequent research efforts led to the characterisation of a cannabinoid system, comprising specific binding sites (CB1 and CB2), their endogenous lipid ligands known as endocannabinoids, and a machinery dedicated to endocannabinoid and synthesis and degradation (55, 57, 60, 61).

CB1 and CB2 belong to the superfamily of G-protein coupled receptors and display similar affinity for THC (57, 60-62). CB1 receptor is highly expressed in the central nervous system and accounts for the psychoactive effects of cannabis (55, 57, 62). CB2 receptor displays lower levels of expression and is primarily found in immune cells (57, 61). Anandamide and 2-arachidonoylglycerol are currently the best known endocannabinoids. (62, 63). Both compounds derive from membrane fatty acids on demand and undergo intracellular uptake and degradation by specific pathways, following receptor binding. Anandamide preferentially binds CB1 receptors, whereas 2-arachidonoylglycerol displays similar affinity for CB1 and CB2 receptors (56, 57).

3.2 Cannabinoids and liver fibrogenesis: experimental data

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Over the last decade, accumulating report have shown that distribution of CB1 and CB2 receptors is far less restricted than initially thought and accordingly, the cannabinoid system has been implicated in a wide variety of physiological and pathological conditions (56, 57). We recently showed that CB1 and CB2 receptors are highly upregulated in cirrhotic human surgical liver samples, predominating in liver fibrogenic cells (64, 65). Moreover, marked elevations in circulating levels of anandamide and hepatic concentrations of 2-AG were described in cirrhotic patients and in rodent models of liver fibrosis (66-68). These results strongly suggested a role of the endocannabinoid system during chronic liver disease. We therefore delineated the respective roles of CB1 and CB2 receptors in liver fibrogenesis, owing to the use of mice genetically deficient for CB1 or CB2 receptors or treated with the CB1 antagonist rimonabant.

CB1 receptors were identified as potent enhancers of liver fibrogenesis, based on the finding that administration of the CB1 antagonist rimonabant or genetic inactivation of CB1 receptors reduces the density of liver fibrogenic cells and inhibits fibrosis progression in three models of chronic liver injury (carbon tetrachloride, thioacetamide or bile duct ligation) (65). In addition, culture studies demonstrated that CB1 enhances proliferation of liver fibrogenic cells and and enhances their survival (65). Similar findings were subsequently reported by others in mice treated with the CB1 antagonist AM 251 (69). In contrast, experiments in CB2 KO mice chronically exposed to carbon-tetrachloride indicated that CB2 receptors exert antifibrogenic properties, related to enhanced apoptosis and reduced proliferation of hepatic myofibroblasts (64). In aggregate, these findings unraveled opposite effects of CB1 and CB2 receptors on liver fibrogenesis and suggested that cannabis use may alter fibrosis progression in patients with ongoing chronic liver injury.

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3.3 Impact of cannabis use on fibrosis severity during CHC

We therefore investigated the impact of cannabis smoking on fibrosis severity in a cross-sectional study of 270 patients with untreated CHC of known duration (70). Data were recorded at the time of liver biopsy, including epidemiological details, lifetime histories of alcohol tobacco and cannabis use, body mass index, metabolic parameters and viral genotype. Patients were categorized according to cannabis smoking over the span of HCV disease as nonusers, occasional (<1 joint weekly) or daily users (at least 1 daily joint during the course of the disease). Logistic regression analysis identified daily cannabis use as a strong predictor of the severity of liver fibrosis, as assessed by fibrosis stage or fibrosis progression rate (table 1). A subsequent independent study reported similar findings (71), supporting our recommendation that patients with ongoing CHC should abstain from regular cannabis use.

3.4 Cannabis and steatosis in patients with chronic hepatitis C

Steatosis, a common histologic finding in patients with CHC, is associated with higher rates of fibrosis progression and decreased sensitivity to antiviral treatment (53, 54, 70, 72). Factors contributing to steatogenesis during CHC include HCV genotype 3, the presence of a metabolic syndrome and alcohol abuse (73, 74).

Recent experimental studies suggest a central role of CB1 and CB2 receptors in the pathogenesis of metabolic steatosis. Osei Hyiaman et al. unravelled an increased CB1-dependent cannabinoid tone in the liver and the hypothalamus of obese mice with fatty liver and demonstrated that CB1 receptors promote liver steatogenesis via central orexigenic properties and peripheral lipogenic effects in hepatocytes (75). In line with these observations, administration of the CB1 receptor antagonist rimonabant to genetically obese fa/fa rats

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prevented the development of steatosis and improved parameters of the metabolic syndrome (76). We recently investigated the impact of CB2 receptors on metabolic steatosis in CB2KO mice submitted to a high fat diet and showed that CB2 receptors promote steatosis, by enhancing inflammation in the adipose tissue (preliminary results, (77)).

Collectively, these data identify CB1 and CB2 receptors as key players in metabolic steatogenesis and suggest that the cannabinoid system may play a significant role in the development of steatosis associated to CHC. We therefore took advantage of the high prevalence of steatosis in patients with chronic hepatitis C, and investigated the impact of recent (6 months) cannabis use on the severity of steatosis in 277 untreated patients. By logistic regression analysis, daily cannabis use was identified as a predictor of severe steatosis, irrespective of genotype, activity grade, body mass index, the presence or diabetes or viral load (78).

3.6 Future prospects

Altogether, our results indicate that regular cannabis use is strongly associated with enhanced steatosis and worsening of fibrosis in patients with CHC. These findings are supported by our experimental studies demonstrating that CB1 receptors promote liver fibrogenesis and steatosis and that CB2 receptors display antifibrogenic effects and enhance steatosis. We therefore recommend that management of patients with CHC should routinely include evaluation of cannabis use history and incentive to abstain from continued consumption. Whether the deleterious impact of cannabis use also holds true in other chronic liver diseases remains to be investigated.

Rimonabant, the first generation of CB1 antagonists, has been approved by the European Agency for the Evaluation of Medicinal products in 2006 for the treatment of

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obesity/overweight and associated cardio-metabolic risk factors, and other CB1 antagonists are undergoing clinical development (79). Therefore, the identification of profibrogenic and steatogenic properties of CB1 receptors might find therapeutic applications for patients with CHC in the coming years. Future clinical trials should evaluate the antifibrosing properties of CB1 antagonists in patients with advanced fibrosis non responders to antiviral therapy, or in patients with a contraindication to ribavirin or interferon. CB1 antagonism might also open a new therapeutic approach in the management of insulin resistance associated to chronic hepatitis C. Indeed, recent data indicate that insulin resistance reduces sensitivity to interferon-based treatment and clinical trials are under way in order to evaluate the impact of insulin-sensitizers on the rate of viral eradication following combined antiviral therapy (80).

4. Conclusion

Physicians are largely aware of the deleterious effects of alcohol abuse in patients with chronic hepatitis C. Recent data suggest that there is no safe level of alcohol intake in this setting, and that abstinence should be recommended. In addition, abstinence appears to enhance the rate of antiviral response. Accordingly, patients should be offered appropriate support, including deaddiction programs if needed. Awareness of the harmful effects of tobacco and cannabis use on fibrosis progression is more recent. Additional studies should further document the impact of tobacco. Evaluation of cannabis exposure should be part of the routine evaluation of patients with chronic hepatitis C and patients should strongly be advised to refrain from regular cannabis use.

Table 1: Factors associated with fibrosis progression in patients with chronic hepatitis C

Host	Older age at contamination
	Male gender
Virus	Duration of infection
Co-morbidities	HIV co-infection
	Immunosupression
	High BMI
	Insulin resistance, diabetes
Environmental	Alcohol intake
	Cigarette smoke
	Cannabis use
Others	Fibrosis stage
	Activity grade

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Table 3: Impact of tobacco smoking on severity of CHC

Authors, year	P/R	Patients (n)	Definition of tobacco use	Impact on ALT level elevation[1]	Impact on activity grade[1]	Impact on fibrosis stage[1]	Other independent cofactors of fibrosis [1]
Pessione, 2001 (43)	R	310	None ≤15 pack-years >15 pack-years	-	Increase in mean activity grade P=0.04	OR=1 OR=1.2 (0.6-2.2) [2] OR=1.9 (1.1-3.6) [2]	Age at biopsy Male gender Alcohol intake > 40g/d
Wang, 2002 (46)	P	880	Yes vs No	OR=1.8 (1.1-2.7)	-	-	Frequent alcohol use Age >50
Hezode, 2003 (45)	P	244	None ≤15 cig/day >15 cig/day	-	OR=1 OR=1.2 (0.5-2.8) OR=3.6 (1.5-8.8)	NS	Age at biopsy Male gender Alcohol intake > 30g/d Necroinflammatory grade
Dev, 2006 (44)	R	170	Number of cigarettes smoked/day at presentation	-	-	OR= 1.3 (1.0-1.8)[3]	HCV genotype 1, Serum VEGF-D

P: prospective; R: retrospective; NS: not significant

[1] By multivariate analysis; [2] Only if necroinflammatory grade omitted in the model; [3] No data on necroinflammatory grade.

Table 4: Factors independently related to rapid fibrosis progression rate* in 267 patients with untreated CHC of known duration (from (70))

	FPR >0.074 U/yr (%)	OR	95% CI	p
Disease-time cannabis use				
None	39.7	1		
Occasional	42.5	1.3	0.5-3.3	0.57
Daily	68.5	3.4	1.5-7.4	0.005
Age at contamination				
≤ 20 years	41.4	1		
21-40 years	52.9	2.4	1.2-4.8	0.01
> 40 years	70	10.5	3.0-37.1	<0.001
Metavir activity grade				
< A2	25.9	1		
≥ A2	67.5	5.4	2.9-10.3	<0.001
HCV genotype				
1	42.0	1		
2	35.0	1.0	0.3-3.1	0.95
3	74.2	3.4	1.5-7.7	0.005
4/5	45.8	1.2	0.4-3.6	0.69
Disease-time alcohol intake				
< 30 g/day	42.1	1	1	
≥30 g/day	69.3	2.2	1.1-4.5	0.03
Steatosis				
Absent-mild	40.7	1		
Moderate-severe	72.4	2.0	1.0-4.1	0.05

* as defined by fibrosis progression rate >0.076 Metavir Units/year (median value of the cohort)

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Table 2: Impact of alcohol on progression to cirrhosis in patients with chronic hepatitis C

Author, country, year	Patients (% cirrhosis)	Definition of alcohol intake	Impact of alcohol on cirrhotic outcome	Other cofactors of fibrosis
Roudot-Thoraval, France, 1997 (12)	5789 CHC (21 %)	> 50 g/d (F), > 60 g/d (M), at least 1 yr	OR = 3.38 (2.82- 4.05)	Age, route of transmission, duration of infection, HBV coinfection
Wiley, US, 1998 (15)	176 CHC (39 %)	> 40 g/d (F), > 60 g/d (M), at least 5 yrs	Higher rate of cirrhosis : 58% vs 10% in the 2nd decade of the disease (p<0.01)	-
Ostapowicz, Australia, 1998 (9)	234 CHC (21 %)	Per 100.000 g lifetime intake	OR = 1.16 (1.02 – 1.31)	Age
Pol, France, 1998 (19)	553 CHC (12.5 %)	> 80 g/d at least 2 yrs	RR = 2.9 (1.6-5.4)	Age at contamiantion, duration of infection, HIV status
Corrao, Italy, 1998 (14)	285 cirrhosis 417 controls with acute diseases	quantitative lifetime : 0, 25, 50, 75, 100, ≥125 g/d	Additive for > 50 g/d alcohol Synergistic for alcohol >125 g/d	Age, gender
Bellentani, Italy, 1999 (21)	General population survey 162 HCV (12 %)	< 30 g/d for 10 years > 30 g/d for 10 years	NS OR = 3.8 (1.2 – 7.4)	Male gender, age, genotype 1b
Thomas, US, 2000 (18)	Follow-up of 1667 IDU with CHC (2.3% cirrhosis at 8.8 yrs	90 – 260 g/week at entry > 260 g/week at entry	RR = 1.57 (0.65 -3.79) RR = 3.6 (1.73 – 7.52)	Age at enrollment
Harris, US, 2001 (13)	Follow up of post transfusion cohort; 206 CHC (17.0%) and 535 controls (3.2%)	> 80 g/d	OR = 4.0 (2.1 – 7.7)	-

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Harris, UK, 2002 (20)	Follow up of post transfusion cohort; 924 CHC vs 475 controls (9.5%)	>260 g/week	RR= 2.84 (1. 09-7.41)	Male gender
Delarocque, 2005 (17)	3404 VHC; first referral in 26 french reference centers (11.5 %)	>30 g/d (F), > 40 g/d (M)	2.6 (1.9 – 3.5)	Male gender, age >39 at referral, HIV, duration, ag HBs status, risk factors,
Stroffolini, 2006 (16)	5632 CHC referred to 79 italian hospitals (18.9 %)	10 – 40 g/d ≥ 40/d	0.8 (0.4 – 1.4) 2.2 (1.3 – 4.0)	Age, gender

IDU : intravenous drug user; RR : risk ratio; OR: odds ratio;

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Figure legends

Figure 1: Cannabis and chronic hepatitis C: impact and experimental mechanisms

CCl4: carbon tetrachloride; TAA: thioacetamide.

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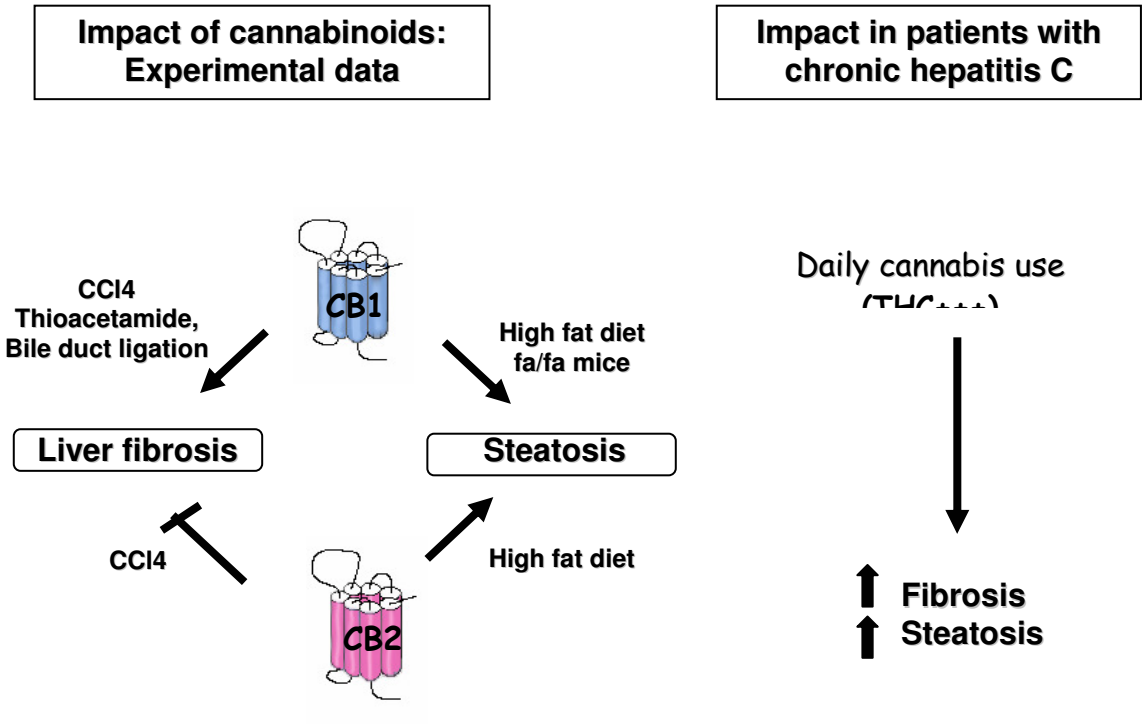

Figure 1