

EDITORIAL

How Does Coffee Prevent Liver Fibrosis? Biological Plausibility for Recent Epidemiological Observations

The published epidemiological data demonstrating an inverse relationship between coffee (and potentially other caffeinated beverage) consumption and liver fibrosis and its downstream complications are weighty and rapidly accumulating. Several excellent recent reviews examine this evidence in great detail,¹⁻³ and the overwhelming conclusion is that this inverse relationship is real—coffee drinking reduces liver fibrosis. Among the strongest studies to support this observation are the findings that, after adjustment for confounders, individuals in the highest quintile of caffeine consumption had less than one third the risk of alanine aminotransferase (ALT) elevation of those in the lowest quintile (odds ratio [OR]: 0.31; 95% confidence interval [CI]: 0.16-0.61)⁴ and, perhaps more important, advanced liver fibrosis from chronic liver diseases (CLDs) of various etiologies is associated with reduced coffee and total caffeine consumption,⁵ with one study showing that the odds of having cirrhosis decreased with increasing daily consumption of coffee in a step-wise manner from an OR of 0.47 (95% CI: 0.20-1.10) for patients consuming one cup of coffee per day to an OR of 0.16 (95% CI: 0.05-0.50) for patients consuming four cups per day, compared to lifetime abstainers as the reference (OR, 1.0).⁶ Demonstrating the clinical significance of coffee consumption, Freedman et al. found that among patients with advanced fibrosis, those who consumed no coffee had a risk of hepatic decompensation or

hepatocellular carcinoma (HCC) of 11.1 per 100 patient-years, compared to just 6.3 per 100 patient-years in those consuming at least three cups of coffee per day, with no beneficial effect noted with tea or other sources of caffeine.⁷ Coffee consumption has also been shown to be associated with a lower risk of fatty liver disease (FLD),⁸ metabolic syndrome,⁹ and, ultimately, HCC.¹⁰ As a clinician or scientist interested in the pathogenesis of liver fibrosis, one may very well ask whether these findings are of great value.

Biological plausibility is the concept that an observed epidemiological association is “consistent with existing biological and medical knowledge”.¹¹ This concept has long been considered a cornerstone in attempts to move epidemiological associations, even those that have been replicated on multiple occasions, to a high likelihood of causality (e.g., the now overwhelmingly accepted concept that tobacco smoking causes lung disease).¹² Here, we provide one of potentially several mechanisms by which coffee/caffeine consumption blocks liver fibrosis—that caffeine inhibits adenosinergic signaling in liver myofibroblasts—with strong hopes of providing biological plausibility for the observed epidemiological associations. We acknowledge fully that other potential mechanisms, such as antioxidant and -inflammatory properties of coffee constituents, are of possible importance; however, these concepts are not sufficiently developed at the level of observed science.

The beneficial effects of coffee and caffeine extract against liver fibrosis have been demonstrated by several studies using standard rodent models of experimental liver fibrosis induced by intoxication with dimethylnitrosamine (DMN), CCl₄, or thioacetamide (TAA).¹³⁻¹⁸ In almost every study, ingestion of coffee blocked toxin-induced liver fibrosis and cirrhosis. Of note, conventional filtered coffee is the form generally used in most of the published studies supporting its protective role. In contrast to the above-cited studies, one report showed that “Turkish style” unfiltered coffee consumption not only lacks any protective effect against CCl₄-induced liver fibrosis, but rather aggravates CCl₄-induced hepatotoxicity with significant aspartate aminotransferase and ALT elevation.¹⁹ Of

Abbreviations: ALT, alanine aminotransferase; ARs, adenosine receptors; cAMP, cyclic adenosine monophosphate; CI, confidence interval; CLD, chronic liver disease; DMN, dimethylnitrosamine; FLD, fatty liver disease; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; OR, odds ratio; α -SMA, alpha-smooth muscle actin; TAA, thioacetamide; TGF- β , transforming growth factor beta.

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note, the mechanism(s) underlying these differences was not studied, so more-definitive animal experiments are highly warranted.

One mechanism by which coffee may protect against liver fibrosis is through alterations of liver signaling or inflammation. Transforming growth factor beta (TGF- β) is a major liver-regulatory cytokine secreted in large quantities in standard rodent liver fibrosis models.²⁰ TGF- β levels are reduced by coffee and caffeine administration to rats subjected to CCl₄-, DMN-, and TAA-induced liver fibrosis.¹³⁻¹⁸ One of the most significant downstream effects of TGF- β signaling is the activation of hepatic stellate cells (HSCs).²¹ In normal liver, HSCs are vitamin A-rich, lipid-storing cells present in the space of Disse.²²⁻²⁴ In fibrosing liver, HSCs undergo myofibroblastic differentiation and markedly up-regulate secretion of extracellular matrix proteins, a process commonly known as HSC activation.²⁴ When liver fibrosis models are performed on rodents exposed to coffee, total liver collagen contents are decreased.^{13-15,18}

Activated HSCs also secrete matrix metalloproteinases (MMPs), whose activity is essential to maintain the balance between tissue repair and scar formation in fibrotic livers.²⁵ Total liver MMP secretion and activity are decreased by coffee consumption.^{13,14} Expression of alpha-smooth muscle actin (α -SMA) protein is commonly used as a marker of HSC activation in the fibrotic liver.²⁴ In the presence of coffee and caffeine, α -SMA total liver expression is diminished,^{13,16,18} potentially being indicative of reduced activation of HSCs and disease progression. Altogether, the *in vivo* studies reviewed here show that the antifibrotic properties of coffee/caffeine converge at a point in which HSC activation is diminished, providing biologic plausibility for the human studies cited above.

As noted above, coffee contains myriad chemical substances that could potentially be antifibrotic. A number of studies using experimental liver models have specifically addressed this question by administration of decaffeinated coffee or caffeine solution to animals.^{13,16,19} Noncoffee caffeine was shown to protect liver against fibrosis in both TAA- and CCl₄-induced liver fibrosis in rats.^{16,19,26} On the other hand, several studies demonstrate that decaffeinated coffee is also protective, but to a lower extent than caffeinated coffee in experimental animals.^{13,19} Taken together, it appears that there are noteworthy holes in the animal liver fibrosis literature; there are simply not enough data to make firm conclusions about the relative importance of coffee caffeine content. At present, though it is

premature to assume that the major effect of coffee is mediated by caffeine, the preponderance of evidence would suggest that this is the case.

Caffeine and other xanthines, including theophylline, have several known biological targets. These molecules have been characterized as nonselective antagonists of adenosine receptors (ARs), inhibitors of phosphodiesterases, antagonists of the gamma-aminobutyric acid type A receptor, and stimulators of intracellular calcium release.²⁷ Though each of these effects is relevant to multiple biological processes, this section focuses on the antagonistic effects of caffeine on ARs because this biological effect is relevant to the pathogenesis of liver fibrosis and cirrhosis.

Extracellular adenosine acts through four G-protein-coupled receptors, known as A₁, A_{2a}, A_{2b}, and A₃ ARs, to induce downstream effects, (as recently reviewed^{28,29}). A₁AR, A_{2a}AR, and A₃AR are high-affinity receptors that respond to low concentrations (>10 nM) of extracellular adenosine, whereas A_{2b}AR is a low-affinity receptor (>1 μ M) thought to be selectively activated in pathological conditions.³⁰ A₁AR and A₃AR are coupled to G proteins of the G_{i/o} type, leading to down-regulation of cyclic adenosine monophosphate (cAMP)-dependent signaling pathways. In contrast, A_{2a}AR and A_{2b}AR increase the intracellular concentration of cAMP through G_s coupling. Interestingly, A_{2b}AR can also be coupled with the G_q subunit to mobilize intracellular calcium (Ca²⁺).

Experimental evidence of the antagonist effects of caffeine on ARs was first reported 40 years ago in the heart³¹ and in the brain.³² Caffeine is a nonspecific antagonist of all ARs. Specific synthetic agonists and antagonists derived from caffeine and other xanthine compounds have been developed for each AR and are now used as research tools in the studies of their functions, as well as potential therapeutic drugs.²⁷ This is relevant because specific antagonists of A_{2a}AR inhibit experimental liver fibrosis.^{26,33} In contrast, administration of A₁AR-, A_{2b}AR-, and A₃AR-specific antagonists does not significantly affect liver fibrosis progression.²⁶ Thus, the antifibrotic effect of caffeine seems to be modulated by its antagonism of A_{2a}AR. In addition, mice lacking A_{2a}AR expression are protected against liver fibrosis induced by CCl₄ and TAA.²⁶ A potential role of A₁AR in liver fibrosis is more controversial, because A₁AR-deficient mice are also protected against CCl₄-induced liver fibrosis,³⁴ but administration of the A₁AR-specific antagonist, *8-cyclopentyl-1,3-dipropyl-xanthine*, has no effect.²⁶

HSCs are well established as primary effector cells during liver fibrosis. Interestingly, human HSCs express

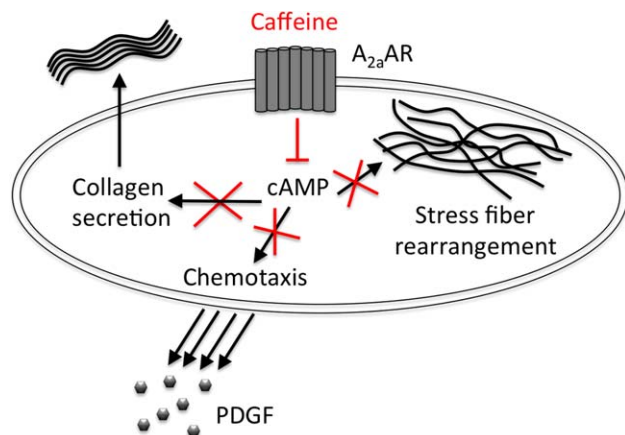


Fig. 1. Proposed mechanism for the antifibrotic effect of caffeine in CLD. Caffeine is a known antagonist of the A_{2a} adenosinergic receptor expressed on activated HSCs and other liver myofibroblasts. Stimulation of A_{2a}AR has several downstream profibrogenic effects, including rearrangement of stress fibers, chemotaxis in response to PDGF and other stimuli, and secretion of fibrillar collagen, all of which may be inhibited by caffeine.

messenger RNA for all four adenosine receptors (as previously reported³⁵ and Dranoff, unpublished data), among which A_{2a}AR is the most studied as a regulator of HSC function. Mouse HSCs express all but A₃AR receptors.³⁵ Thus, HSCs represent a highly plausible cellular target mediating the antifibrotic effect of coffee and caffeine acting through AR antagonism. Indeed, activation of HSC A_{2a}AR by extracellular adenosine markedly up-regulates collagen secretion.^{26,35,36} Adenosinergic signaling, through A_{2a}AR activation, redistributes stress fibers and contractile capacity in HSCs,³⁷ likely providing a mechanism for a “stop” signal after cell migration, as evidenced by the observation that A_{2a}AR activation blocks chemotaxis of HSCs in response to platelet-derived growth factor (PDGF).³⁵ Finally, A_{2a}AR activation increased HSC TGF- β secretion³⁵ and decreased MMP expression.²⁶ Because all of the mechanisms listed can be blocked by caffeine, blockade of profibrotic adenosinergic signaling in HSCs is a reasonable explanation for the antifibrotic effects of coffee.

According to the literature presented here, coffee consumption provides protection against liver fibrosis induced by well-established chemical models. The protective mechanism seems to be mediated primarily by the action of caffeine on HSC A_{2a}AR. However, there are holes in the literature that will need to be closed. First, because CCl₄ and other profibrotic chemical agents require inflammation to induce fibrosis and cirrhosis, and multiple inflammatory cell types express adenosine receptors,^{38,39} the observed effects may be mediated by changes in inflammatory cell function,

rather than those on HSC function. Second, the animal studies performed have taken only a cursory look at the relative importance of noncaffeine coffee constituents, in part because of methodological limitations. Last, animal models of fibrosis are themselves analogs of human fibrosis-to-cirrhosis progression, but they are not identical. Thus, it is very possible that animal models and studies in isolated HSCs will prove useful to identify biological mechanisms, but the relevance to human health will be best tested in studies of human patients.

Progression of liver injury to fibrosis to cirrhosis is a slow, but deadly, process. The number of North American and European patients with CLD is increasing, primarily as a result of steady levels of hepatitis C infection, but rapidly expanding levels of FLD (primarily nonalcoholic). Thus, identification of simple measures that can slow fibrosis and prevent cirrhosis in at-risk patients is critical. Because coffee consumption appears to have salutary effects on human health overall, coffee is an attractive lifestyle measure that patients can take.

Are we ready to “write a prescription for coffee,” as asked by Torres and Harrison in a recent commentary article?¹ Most likely, the answer is yes. Our rationale is as follows. First, there is sufficient evidence to provide biological plausibility for coffee as an antifibrotic. Second, coffee (for most individuals) is a pleasant addition to the diet, without profound adverse effects and, possibly, some other health benefits (again for most individuals). Last, other antifibrotic treatments are simply lacking; they are in the pipeline, but not yet available clinically.

However, we must face caveats as well. The human studies cited suggest that the most potent observed effects of coffee require the equivalent of four or more cups per day. We are not convinced that most individuals would easily tolerate this. Moreover, if we assume that the antifibrotic effects of coffee are mediated by caffeine, then should patients also be offered equivalent “doses” of tea, caffeinated soft drinks, or even caffeine pills? The latter two do not seem to be consistent with contemporary health practice and probably for good reason. Thus, at present, we would suggest that any recommendations be limited to coffee (and for reasons cited above, limited to brewed coffee).

Hopefully, the most important effect gained by the observations reviewed here is not the use of coffee as a drug, but rather the generation of testable hypotheses as to the pathogenesis, prevention, and treatment of liver fibrosis and cirrhosis.

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