Therapeutic potential of curcumin in non-alcoholic steatohepatitis

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Non-alcoholic steatohepatitis (NASH) may be associated with a number of clinical conditions, but it occurs most commonly in patients with insulin resistance. There is as yet no established disease-modifying treatment, and a safe and broadly available agent that targets hepatic steatosis, insulin resistance, inflammation and fibrosis is necessary. The polyphenolic compound curcumin exhibits antioxidant and anti-inflammatory properties, inhibits NF-κB and activates PPAR-γ. In rodents, curcumin prevents dietary-induced hepatic steatosis, hepatic stellate cell activation and production of fibrotic proteins, and ameliorates steatohepatitis induced by the intake of alcohol or a methionine–choline-deficient diet. Indirect evidence suggests that curcumin may improve insulin sensitivity in diabetes and inflammatory states. The present paper reviews the numerous cellular and animal studies indicating that curcumin attenuates many of the pathophysiological processes involved in the development and progression of NASH. It is suggested that basic and clinical studies on curcumin in the development and progression of NASH are indicated.

Introduction

Non-alcoholic steatohepatitis (NASH), like alcoholic steatohepatitis (ASH), is a liver disease characterised by diffuse fatty infiltration and inflammation, but is seen in patients with minimal alcohol consumption. Since this common and often clinically silent disorder can lead to cirrhosis, there is growing interest in understanding its pathophysiology and in developing an appropriate treatment (Angulo, 2002). The pathogenesis of NASH is not well established, but insulin resistance (IR) is considered a primary mediator of hepatic steatosis, the ‘first hit’ of the disease. In many patients, steatosis sensitises the liver to inflammation, oxidative stress, mitochondrial dysfunction and fibrosis, the ‘second hit’ (Neuschwander-Tetri & Caldwell, 2003). The prevalence of both simple hepatic steatosis and NASH is thought to be increasing in parallel to the diabesity epidemic (Sass et al., 2005). Less often NASH may develop secondarily into nutritional, metabolic, intestinal and post-interventional disorders (for example, abetalipoproteinaemia, lipodystrophy, ileo-jejunal bypass and total parenteral nutrition) or drug-induced hepatotoxicity (Neuschwander-Tetri & Caldwell, 2003).

The therapeutic potential of interventions able to target one or more of these inter-related processes is being evaluated in animal models as well as in patients with NASH (Angulo, 2002; Neuschwander-Tetri & Caldwell, 2003). Lifestyle changes may be beneficial and PPAR-γ agonists, betaine, vitamin E and pentoxifylline were found to be effective in clinical trials (Neuschwander-Tetri & Caldwell, 2003; Satapathy et al. 2004; Sass et al., 2005). But, no long-term studies have been performed and there is at present no established treatment that is both safe and that modifies the natural history of NASH (Sass et al., 2005). The polyphenolic substance diferuloylmethane, commonly known as curcumin (CUR), is a yellow water-insoluble pigment extracted from turmeric, the rhizome of Curcuma longa. The other two curcuminoids isolated from turmeric are demethoxycurcumin and bisdemethoxycurcumin, but CUR is considered the more important mediator of turmeric’s biological activity. Turmeric is extensively used as a spice, food preservative and medicinal plant in the Far and Middle East (for an important review, see Joe et al. 2004). CUR beneficially modulates the multiple processes involved in carcinogenesis and is being evaluated as a dietary chemopreventive agent (Aggarwal et al., 2003). It is a potent antioxidant and NF-κB-inhibitor, protects cells from injury- and inflammatory-induced necrosis and apoptosis, and enhances wound healing (Miquel et al., 2002; Aggarwal et al., 2003; Joe et al., 2004). CUR has been shown to be

Abbreviations: ASH, alcoholic steatohepatitis; CUR, curcumin; HO-1, haeme-oxygenase-1; HSC, hepatic stellate cells; IKK, inhibitory κB kinase; IR, insulin resistance; JNK, c-Jun N terminus protein kinase; LPS, lipopolysaccharide; MCD, methionine–choline-deficient; MMP, matrix metalloproteinase; NASH, non-alcoholic steatohepatitis.

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protective in animal models of toxic (Venkatesan, 1998; Venkatesan et al. 2000; Gukovsky et al. 2003), inflammatory (Madan & Ghosh, 2003) and ischaemic injury (Shoskes, 1998; Ghoneim et al. 2002) to different organs.

In rodents, pharmacologically active levels of CUR are found in the liver following its ingestion (Sharma et al. 2001a), and it has been shown to ameliorate many forms of hepatic insult (Reddy & Lokesh, 1996; Quiles et al. 1998; Chuang et al. 2000a,b; Park et al. 2000; Watanabe & Fukui, 2000; Asai & Miyazawa, 2001; Morikawa et al. 2002; Nanji et al. 2003; Shukla & Arora, 2003; Leclercq et al. 2004), including steatohepatitis due to a methionine–choline-deficient (MCD) diet (Leclercq et al. 2004). CUR (Nanji et al. 2003) and dietary-induced hepatic steatosis (Asai & Miyazawa, 2001). CUR also inhibits hepatic stellate cell (HSC) activation and type I collagen production (Kang et al. 2002; Xu et al. 2003). CUR’s ability to prevent hyperglycaemia in a mice model of type 2 diabetes (Nishiyama et al. 2005), and to inhibit inhibitory κB kinase (IKK; Joe et al. 2004) and c-Jun N terminus protein kinase (JNK; Chen & Tan, 1998), which interfere with insulin signal transduction, suggests that it may also ameliorate IR. The aim of the present article is to present some of the deleterious biochemical and cellular processes underlying NASH, and to review the evidence of their prevention by CUR.

Hepatic steatosis

Hepatic steatosis develops in a number of experimental and clinical settings, and may be the result of an increased uptake and synthesis of fatty acids by hepatocytes, insufficient fatty acid oxidation and/or defective VLDL export (Browning & Horton, 2004), CUR ameliorates biochemical and histological indices of hepatic steatosis in a number of models of metabolic and dietary-induced steatosis and steatohepatitis (Table 1). It attenuated the rise in hepatic and plasma total and VLDL triacylglycerols in normal rats fed a moderately high-fat (15 %) diet (Asai & Miyazawa, 2001), a moderately high-fat (15 % sunflower-seed oil) and ethanol diet (Rukkumani et al. 2002) and in streptozocin-induced diabetic rats fed a 10 % fat diet (Babu & Srinivasan, 1997). The hepatic NEFA content was also shown to be reduced by CUR in the ethanol model (Rukkumani et al. 2003). CUR also reduced plasma triacylglycerol concentrations in Wistar rats fed a high-fat (30 %) diet, although hepatic triacylglycerols were not measured in that study (Kempaiah & Srinivasan, 2004). A significantly lower histopathological index of steatosis was evident in Wistar rats fed CUR in ethanol and fish oil-induced liver injury (Nanji et al. 2003). In those of the above studies that included weight measurement, CUR administration was not associated with a significant change in weight. CUR also prevented the increase in epididymal adipose tissue weight in rats fed a moderately high-fat diet (Asai & Miyazawa, 2001). The finding that CUR reduces both hepatic and non-hepatic fat suggests that it lowers the fatty acid synthesis:oxidation ratio. CUR activates a key fatty acid oxidising enzyme, acyl-CoA oxidase (Asai & Miyazawa, 2001), a deficiency of which can lead to hepatic steatosis (Yeon et al. 2004). This might be one way CUR prevents lipid accumulation.

CUR inhibited the inflammatory, but not the steatotic, component of steatohepatitis in mice fed an MCD diet (Leclercq et al. 2004). In addition, acyl-CoA oxidase expression was not increased by CUR. The reason for the discrepancy in CUR’s effect on steatosis in different models is not clear, but may be related to the finding that MCD-fed mice do not exhibit IR (Rinella & Green, 2004), which appears to be necessary for the development of human NASH and which may be a target in CUR’s anti-steatotic effect.

Insulin resistance

The majority of patients with fatty liver and NASH exhibit IR, i.e. a reduced responsiveness to endogenous and exogenous insulin, as well as compensatory hyperinsulinaemia. IR is also strongly associated with type 2 diabetes mellitus, obesity and the metabolic syndrome, and a large body of evidence supports the causative role of IR in human hepatic steatosis and NASH (Angulo, 2002; Neuschwander-Tetri & Caldwell, 2003; Choudhury & Sanyal, 2004). Adipocyte IR increases liver fatty stores by disinhibition of lipolysis, thereby increasing NEFA efflux. Skeletal muscle and hepatocyte IR results in hyperglycaemia, caused by reduced peripheral uptake and increased hepatic production of glucose, respectively. Hyperglycaemia leads to a compensatory hyperinsulinaemia, which increases fatty acid synthesis and impairs hepatocyte export of VLDL (Choudhry & Sanyal, 2004). Elevated hepatocyte glucose levels may augment carbohydrate-mediated stimulation of lipogenesis via the carbohydrate response element binding protein (Browning & Horton, 2004), TNF-α, NEFA and oxidative stress inhibit insulin signal transduction by activating IKK, JNK and certain protein kinase C isoforms, which phosphorylate serine residues on insulin receptor substrates (Choudhury & Sanyal, 2004; Diehl, 2004).

CUR’s effect on target-organ or whole-body insulin sensitivity has not been assessed. However, both a turmeric extract and a purified CUR extract reduced hyperglycaemia in a mouse model of type 2 diabetes mellitus (Nishiyama et al. 2005). CUR also activated PPAR-γ in adipocytes in vitro and the authors attributed CUR’s hypoglycaemic effect to this mechanism. HSC and Moser cell PPAR-γ were also activated by CUR in vitro (Xu et al. 2003; Zheng & Chen, 2004; Chen & Xu, 2005). CUR’s ability to activate PPAR-γ and to reduce oxidative stress may result in the attenuation of IR (Choudhury & Sanyal, 2004; Oghihara et al. 2004). In addition, CUR may minimise IR under conditions of increased production of TNF-α, since it inhibits both the production and the action of the latter by inhibiting IKK activation and DNA binding of NF-κB (Chan, 1995; Singh & Agarwal, 1995; Xu et al. 1997–98; Joe et al. 2004). CUR has also been shown to inhibit JNK signalling (Chen & Tan, 1998). It is not known whether the ingestion of CUR results in significant pharmacological effects in fatty tissue and skeletal muscle. Even if this is not the case, dietary CUR produces pharmacodynamic effects in the liver (Sharma et al. 2001a), where it may ameliorate IR by local inhibition of TNF-α, JNK and oxidative stress (Choudhury & Sanyal, 2004; Nakatani et al. 2004). Steatosis impairs hepatocyte sensitivity to and the ability to clear insulin (Medina et al. 2004).
<table>
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<th>Model</th>
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<th>Diet: fat type and content; +/− ethanol; duration of diet</th>
<th>Curcumin: form of supplementation and dose</th>
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<td>Hepatic triacylglycerols content; Acyl-CoA oxidase activity</td>
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<td>Curcuminoids exerted a dose-dependent response, also 0.2 g % curcuminoids had a beneficial effect, but not always statistically significant</td>
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<td>High-fat diets in female Wistar rats</td>
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<td>10 g % groundnut oil for 8 weeks</td>
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<td>Alcoholic steatohepatitis (sunflower-seed oil + ethanol diet) in male and female Wistar rats</td>
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<td>Curcumin analogues prepared by photo-irradiation or ortho-hydroxy substitution were more effective than curcumin</td>
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<td>Plasma ALT, TBARS and nitrotyrosine</td>
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<td>Endotoxin-induced activation of Kupffer cell NF-κB activation and expression of TNF-α, IL-12, MCP-1, MIF-2, COX-2, iNOS</td>
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Curcuminoids exerted a dose-dependent response, also 0.2 g % curcuminoids failed to elevate acyl-CoA oxidase activity, expression or to reduce hepatic steatosis (hepatic lipid content and histopathological index of steatosis), TBARS levels and expression of CINC.

Importantly, CUR reduced membranal and intracellular lipid peroxide levels and induced antioxidant activity in hepatocytes and erythrocytes of rats on a high-fat diet (Kempaiah & Srinivasan, 2004; Table 1). Lipid peroxidation of liver microsomes and mitochondria induced by Fe (Reddy & Lokesh, 1994) and an atherosclerotic diet (Quiles et al., 1998) was reduced by CUR. In addition, CUR boosts the activity of a number of hepatic antioxidant enzymes, including catalase, superoxide dismutase and the glutathione system, both under normal and pathological conditions (Sharma et al. 2001a; Miquel et al. 2002; Leu & Maa, 2002; Iqbal et al. 2003; Joe et al. 2004). Importantly, CUR reduced membranal and intracellular lipid peroxide levels and induced antioxidant activity in hepatocytes and erythrocytes of rats on a high-fat diet (Kempaiah & Srinivasan, 2004; Table 1). Haeme-oxygenase-1 (HO-1) is an important cytoprotector (Zuckerbraun & Billiar, 2003) that is induced by CUR (Scapagnini et al. 2001). CUR induces HO-1 expression via modulation of the antioxidant response element (Balogun et al. 2003), thereby increasing cellular antioxidant capacity (Motterlini et al. 2000). Under normal conditions, CUR is a weak inhibitor of CYP2E1 activity (Oetari et al. 1996). But, by preventing the intracellular accumulation of fatty acids, which are both substrates and inducers of CYP2E1 (Browning & Horton, 2004), CUR may reduce this enzyme’s production of lipoperoxides. Finally, CUR exhibited a sparing effect on coenzyme Q10 and α--

Oxidative stress

Increased formation of reactive oxygen species and lipoperoxides by hepatocytes, Kupffer and recruited inflammatory cells appears to be necessary for the progression of steatosis to inflammation and fibrosis. Hepatocyte mitochondrial and peroxisomal β-oxidative enzymes, and microsomal CYP2E1, are important sources of lipoperoxides in NASH (Angulo, 2002; Neuschwander-Tetri & Caldwell, 2003; Choudhury & Sanyal, 2004). The reduced expression and activity of endogenous antioxidant systems might also contribute to the development and progression of NASH in man (Choudhury & Sanyal, 2004).

CUR reduces reactive oxygen species levels by a number of mechanisms; for instance it can inhibit formation, increase dismutation and directly scavenge the superoxide anion (Joe & Lokesh, 1994; Joe et al. 2004; Mishra et al. 2004). It attenuates in vivo formation of hepatic lipoperoxides in rats fed a high-fat or ethanol + fat diet (Nanjk et al. 2003; Rakkumani et al. 2003, 2004b; Kempaiah & Srinivasan, 2004; Table 1). Lipid peroxidation of liver microsomes and mitochondria induced by Fe (Reddy & Lokesh, 1994) and an atherosclerotic diet (Quiles et al. 1998) was reduced by CUR. In addition, CUR boosts the activity of a number of hepatic antioxidant enzymes, including catalase, superoxide dismutase and the glutathione system, both under normal and pathological conditions (Sharma et al. 2001a; Miquel et al. 2002; Leu & Maa, 2002; Iqbal et al. 2003; Joe et al. 2004). 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tocopherol in dietary-induced oxidative stress (Quiles et al. 2002), the latter antioxidant showing some therapeutic potential in NASH (Neuschwander-Tetri & Caldwell, 2003).

It is conceivable that CUR, due to its pleiotropic antioxidant activities, reduces the formation of lipid peroxides despite induction of acyl-CoA oxidase. These combined effects should minimise oxidative stress and attenuate the progression of steatosis to NASH.

### Inflammation, tumour necrosis factor-α, nuclear factor κB and lipopolysaccharide

TNF-α is both induced by and an activator of NF-κB and can lead to a partially self-perpetuating inflammatory process (Choudhury & Sanyal, 2004). Elevated TNF-α levels are related to the inflammation, necrosis and fibrosis characteristic of NASH (Angulo, 2002). Studies in animal models suggest that an augmented Kupffer cell inflamma-

**Fig. 1.** Curcumin may prevent hepatic steatosis in dietary, diabetic and inflammatory states by targeting different biochemical processes. Hepatocytes accumulate pathological amounts of fat due to increased fatty efflux, resistance to insulin's lipid-sparing effects and/or increase in fatty acid (FA) synthesis due to hyperinsulinaemia and an increased supply of substrates. Curcumin reduces hyperglycaemia in type 2 diabetes mellitus, reduces dietary-induced elevations in circulating lipids, inhibits inhibitory κB kinase (IKK) and c-Jun N terminus protein kinase (JNK) that are implicated in insulin resistance and activates acyl-CoA oxidase. Curcumin also inhibits the production of TNF and free radicals, inducers of insulin resistance. ROS, reactive oxygen species; LPO, lipoperoxide; →, activates; leads to; ─, inhibits; ┼, blocked or inhibited by curcumin.
tory response to lipopolysaccharide (LPS) may also be involved in obesity-related liver damage and that inhibition of LPS or of the TNF-α–IKKβ–NF-κB pathway could be therapeutic in NASH (Diehl, 2004). The TNF-α-induced phosphorylation of IKKβ, NF-κB activation and binding of DNA, and the resultant transcription of pro-inflammatory molecules, are inhibited by CUR (Chan, 1995; Singh & Aggarwal, 1995; Pendurthi et al. 1997; Bierhaus et al. 1997; Xu et al. 1997–98; Kumar et al. 1998; Plummer et al. 1999; Chun et al. 2003; Lee et al. 2003; Joe et al. 2004), as is the ex vivo LPS-induced production of TNF-α by macrophages (Abé et al. 1999; Punithavathi et al. 2003). CUR inhibited in vivo liver NF-κB activation and TNF-α expression, and LPS-induced production of TNF-α by Kupffer cells in rats with ASH (Nanji et al. 2003; Table 1). Activation of NF-κB by osteopontin, which may play a role in NASH (Sahai et al. 2004), is also inhibited by CUR (Philip & Kundu, 2003).

CUR administration also attenuates the phagocytic activity of Kupffer cells and leucocyte adherence to liver sinusoids following intravenous injection of LPS in mice (Lukita-Atmadja et al. 2002). Thus, CUR may minimise the deleterious inflammatory response of Kupffer cells and infiltrating monocytes by blocking the multiple pathways converging on NF-κB.

Since NF-κB activity appears to be necessary to prevent apoptosis and facilitate hepatocyte regeneration (Heyninck et al. 2003), it is conceivable a priori that its inhibition by CUR would be detrimental in NASH. However, in vitro studies show that CUR does not inhibit constitutive NF-κB activity (Gao et al. 2004), and that it induces apoptosis in activated HSC (Xu et al. 2003; Zheng & Chen, 2004) and in transformed hepatocytes (Syng–Ai et al. 2004), but not in normal hepatocytes (Syng–Ai et al. 2004).

Fibrosis

Activation of HSC is the central event in hepatic fibrosis. Locally synthesised lipoperoxides, matrix metalloproteinases (MMP)-9, MMP-2, transforming growth factor-β1 and monocyte chemotactic protein-1 may mediate the transformation of the quiescent HSC into a proliferative, fibrogenic and contractile myofibroblast (Friedman, 2000). Activated HSC deposit numerous scar proteins, ultimately leading to vascular and tissue contraction. HSC inhibition may prevent or even reverse hepatic fibrosis resulting from diverse disease states (Albanis et al. 2003). CUR has been shown to reduce the induced production of MMP-9 (Shishodia et al. 2003), MMP-2 (Yao et al. 2004) and transforming growth factor-β1 (Gaedeke et al. 2004). It also reduces lipoperoxide levels, as discussed above. CUR inhibited the expression of type I collagen and that of other markers of pulmonary fibrosis in rats after intratracheal instillation of amiodarone (Punithavathi et al. 2003), an agent that can also cause steatohepatitis (Stravitz & Sanyal, 2003). The synthesis of liver monocyte chemotactic protein-1 was limited by CUR in rodent ASH (Nanji et al. 2003) and MCD-diet-induced steatohepatitis (Leclercq et al. 2004). CUR normalised MMP-2 and MMP-9 activity in rats fed either ethanol, oxidised sunflower-seed oil or both hepatotoxins (Aggarwal, 2004). HSC proliferation and expression of collagen-α1, fibronectin and α-smooth muscle actin mRNA were all reduced by CUR, whereas HSC apoptosis was induced (Kang et al. 2002; Xu et al. 2003). HSC inhibition by CUR can be partially explained by the latter’s induction and activation of PPAR-γ, and inhibition of NF-κB (Kang et al. 2002; Zheng & Chen, 2003). PPAR-γ agonists were recently shown to have therapeutic effects in NASH (Choudhury & Sanyal, 2004). CUR also inhibited platelet-derived growth factor-induced proliferation of human hepatic myofibroblasts (Park et al. 2005). Finally, HO-1, which is induced by CUR, may have anti-fibrotic properties in activated HSC (Li et al. 2003). A simplified and schematic overview of how CUR may prevent the progression of hepatic steatosis to NASH is presented in Fig. 2. We recently established that CUR administration to rats attenuates the development of thioacetamide-induced hepatic cirrhosis (R Bruck, M Ashkenazi, H Shapiro, O Genia and M Pines, unpublished results.).

Safety, bioavailability and clinical trials

Turmeric is added to food as a natural colorant, food preservative or spice (Joe et al. 2004). As an additive, the WHO has defined an intake of up to 1 mg/kg per d as safe (World Health Organization, 2000).

CUR, like other food-derived polyphenolic substances, is only partially absorbed by rodents and man, undergoes extensive intestinal conjugation and reduction and is further metabolised in the liver (Joe et al. 2004; Manach et al. 2004). Although populations with a high intake of CUR have a lower incidence of Alzheimer’s disease and colon cancer (Chandra et al. 2001; Joe et al. 2004), and CUR has therapeutic effects in pre-clinical models of both diseases (Lim et al. 2001; Aggarwal et al. 2003), evidence of a causative link between dietary CUR and a reduced incidence of disease is lacking. Phase I studies of CUR in the prevention and treatment of cancer have used different forms of turmeric extracts and synthesised CUR at doses that may be considered pharmacological. These studies show that ingestion of up to 8 g CUR is not significantly toxic, with infrequent diarrheaea being the major side effect (Cheng et al. 2001; Aggarwal et al. 2003; Sharma et al. 2001b, 2004). Phase II clinical trials of CUR treatment for mild to moderate Alzheimer’s disease and advanced pancreatic cancer are presently enrolling patients (National Institutes of Health, 2004a,b).

Despite its low bioavailability, ingestion of pharmacological doses of CUR by human subjects can produce systemic pharmacological effects, as evident by a > 50 % reduction in ex vivo LPS-induced production of prostaglandin E2 by leucocytes. This anti-inflammatory effect is presumably attributable to inhibition of NF-κB-mediated expression of cyclo-oxygenase-2 by CUR (Sharma et al. 2004). In addition, ingestion of 20–80 mg of a highly concentrated CUR preparation by twelve healthy adults induced a powerful, dose-dependent increase in gallbladder contractility in two double-blind, ultrasonographic studies (Rasyid & Lelo, 1999; Rasyid et al. 2002). Thus, CUR can produce pharmacological effects in the liver. Attempts are being made to develop more powerful and potent CUR analogues.
The structure of CUR’s benzene rings, its hydrogen substitutions and their position determine its antioxidant ability (Joe et al. 2004). A CUR analogue with ortho-hydroxyl substitution, for instance, has an enhanced ability to neutralise free radicals (Rukkumani et al. 2004b). Administration of CUR analogues prepared by ortho-hydroxyl substitution or photo-irradiation indeed resulted in greater hepatoprotection compared with that of CUR in a rat model of ASH (Rukkumani et al. 2004b; Table 1). It is not known how modulation of CUR’s chemical structure affects its interaction with its protein targets, such as the TNF-α–IKK–NF-κB pathway and PPAR-γ. A number of CUR analogues that display in vitro anti-neoplastic activity that is superior to their mother compound have also been developed (Venkatesan & Rao, 2000; Ishida et al. 2002; Robinson et al. 2003). Assessment of CUR’s effects vs. those of its analogues should be tried out in animal models of NASH that are similar to the human disease in order to help detect more effective treatment. In addition, a comparison of the in vitro effect of CUR and its analogues on acyl-CoA oxidase, the TNF-α–IKK–NF-κB pathway, PPAR-γ and other modulators of the disease process could reveal the relative importance of the different mechanisms underlying NASH.

In conclusion, CUR inhibits many serial and parallel pathways leading to hepatic steatosis, inflammation and fibrosis. Since CUR has a good safety profile, its role in the prevention and treatment of NASH merits further investigation.

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Therapeutic potential of curcumin

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