Published in final edited form as:

Hepatology. 2011 November ; 54(5): 1503–1505. doi:10.1002/hep.24696.

Should combination therapy be the paradigm for future NASH clinical trials?

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States and consists of a spectrum of disease ranging from relatively benign steatosis to more aggressive steatohepatitis (NASH) which can lead to cirrhosis and liver failure. Obesity, type 2 diabetes, and metabolic syndrome are closely associated with NAFLD and NASH. Adipose tissue insulin resistance is believed to be one of the fundamental mechanisms leading to an increased hepatic influx of non-esterified fatty acids (NEFA).^{1,2} In addition, increased *de novo* lipogenesis and dietary intake also contribute to the accumulation of hepatic lipids. An increased hepatic fatty acid load is thought to be lipotoxic, either through toxic lipid-intermediaries or by causing oxidative stress.³ Fatty acid oxidation by mitochondria, microsomes, and peroxisomes leads to oxidative stress and increased lipid peroxidation. Peripheral insulin resistance and oxidative stress have been the main therapeutic targets and previously clinical trials have almost exclusively investigated insulin sensitizers and antioxidants to treat NASH.

Lifestyle modification leading to sustained weight loss can be an effective treatment for NASH but is difficult to achieve and importantly more difficult to sustain. Thus, there has been significant interest in developing pharmacological agents to treat NASH, and insulin sensitizers such as metformin and thiazolidinediones (rosiglitazone and pioglitazone) and antioxidants such as vitamin E have been investigated to treat NASH. A recently published meta-analysis concluded that metformin is not effective in improving liver histology in adult with NASH³ and the recently published TONIC trial showed that metformin administered at a daily dose of 1 gram is of no benefit to children with NAFLD.⁴ However, pioglitazone and rosiglitazone have shown effectiveness in improving liver histology, primarily in non-diabetic adults with NASH.³ The meta-analysis by Musso et al., summarized that TZDs improve steatosis (OR 4.05, 95% CI 2.58 – 6.35) and inflammation (OR 3.53, 95% CI 2.21–5.64) but not fibrosis (OR 1.40, 95% CI 0.87–2.24).³ Two randomized controlled conducted by the NASH Clinical Research Network have shown that vitamin E administered at a daily dose of 800 IU improves liver histology both in adults and in children.^{4,5} These results are statistically attractive but are relatively small steps forward because vitamin E and TZDs improve liver histology in no more than 40–45% of patients and they do not improve fibrosis. Thus, there is a significant unmet need in terms of effective available treatments and this unmet need may be overcome by adopting alternate therapeutic strategies such as (a) employing different agents to improve insulin resistance (e.g., GLP-1 agonists) and oxidative stress (betaine, SAmE), (b) exploring agents affecting different targets such as

apoptosis (e.g, GS 9450) and FXR (e.g, obeticholic acid), or stellate cell activation (losartan), or (c) investigating combination of agents affecting different targets/pathways.

In this issue of *Hepatology*, Harrison's group report the results of their randomized controlled trial which investigated two different combination therapies to treat NASH.⁶ Angiotensin II receptors have been identified on hepatic stellate cells (HSC) and their activation leads to HSC proliferation. Angiotensin II receptor knockout mice when challenged with carbon-tetrachloride had significantly reduced hepatic fibrosis when compared to wild type mice, suggesting angiotensin receptor II is a novel target to improve hepatic fibrosis.⁷ Losartan, an angiotensin II receptor blocker (ARB), has been shown in mice models to have beneficial effects on liver fibrosis,^{8,9} and in a small study of consisting of 7 patients with NASH, Yokohama et al., suggested that losartan may improve necroinflammation and fibrosis.¹⁰

Torres et al., randomized one hundred thirty-seven subjects with biopsy proven NASH to a 48-week course of rosiglitazone 4mg twice daily alone, or in combination with either metformin 500mg twice daily or losartan 50mg daily.⁶ The primary endpoint was change in hepatic histology as evidenced by at least one point improvement in steatosis, inflammation, and fibrosis. Steatosis, inflammation, and fibrosis improved between baseline and end of the treatment within each treatment arm, but the changes in liver histology were statistically not different among three treatment groups.⁶

Randomized controlled trials of NASH with histological endpoints are expensive and difficult to conduct and thus Harrison's group must be applauded for undertaking this study and for their other important contributions to the field. However, we suggest caution in interpreting the results of this study because some of its limitations. This study was prematurely stopped due to severe restriction imposed by the Food and Drug Administration on rosiglitazone use in the United States, leading to an underpowered sample size. The presence of fibrosis was not a prerequisite at baseline and yet its improvement was required to achieve the primary outcome. If the expected benefit from losartan is to significantly improve fibrosis, then there must be sufficiently advanced fibrosis to begin with, or if the objective is to reduce the progression of fibrosis, then the study duration must be longer than 48 weeks. The rationale for adding metformin to rosiglitazone was to further improve the insulin sensitivity and to mitigate the weight gain caused by rosiglitazone. However, presumably due to insufficient metformin dose, these benefits were not evident in this trial. There was no systematic monitoring of alcohol consumption, nutrient and calorie intake, or physical activity during the trial. Notwithstanding these limitations, we find this study to be appealing not only because it represents the first serious attempt to use combination therapy to treat NASH but also is the first serious effort to investigate the role of losartan to improve fibrosis in NASH.

What is next for the NASH treatment trials? Since there are no approved treatments for NASH, we believe that placebo-controlled trials are ethical and should be encouraged. It is possible that recent findings from the PIVENS and TONIC clinical trials may lead to extensive use of vitamin E by patients and practitioners, and in such a scenario the newer treatments would need to be tested against vitamin E background. An important question is if a combination of pioglitazone and vitamin E is more effective in treating NASH than these two agents given separately. There is an ongoing study of vitamin E and pioglitazone combined in the United States veterans and its results are awaited. There are two ongoing multicenter, phase IIb/III, placebo-controlled, randomized clinical trials in the United States; one is comparing two different doses of ethyl eicosapentate (EPA-E) to placebo, and the other trial is comparing obeticholic acid (FXR agonist) to placebo. It is possible that one or both may yield positive results, but it is unlikely that either compound will prove to be the magic

bullet. We believe that combining agents that reduce hepatic influx/load of fatty acids (e.g., weight loss, insulin sensitizers) with agents that reduce lipotoxicity and cell injury (e.g., vitamin E, caspase inhibitors, pentoxifylline) is the best therapeutic strategy to investigate moving forward. Losartan and other proprietary anti-fibrotics compounds need to be tested, but more thought is needed with regards to the study population and the end points. Clinical trials with hard endpoints such as mortality, liver decompensation, and time to transplantation are difficult to conduct, and commonly used fibrosis endpoints are not sufficiently dynamic. One may have to consider alternative options such as elastography, enhanced liver fibrosis panel, or quantitative histological assessments that include markers of fibrogenesis and fibrosis. It is more logical to test anti-fibrotic compounds in combination with insulin sensitizers and/or antioxidants, rather than investigating them as sole agents. We encourage the academic and industry investigators to consider combination therapies and to incorporate recently published endpoints and clinical trial design for future clinical trials.¹¹

Acknowledgments

Dr. Chalasani serves currently or served within last 12 months as a paid consultant to Amylin and Mochida in the areas related to NAFLD and NASH. He serves as a paid consultant to several other entities in the area of drug safety. This work is in part supported by K24 DK 069290A to N.C.

Abbreviations

NASH	Nonalcoholic steatohepatitis
NEFA	Nonesterified fatty acids
TZD	Thiazolidinediones

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