Mediford Corporation

Development of Non-Alcoholic Steatohepatitis (NASH) Model Using a Chimeric Mouse with Humanized Livers (PXB-Mouse®) and Evaluation of Efficacy of Pioglitazone

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Objective

Non-alcoholic steatohepatitis (NASH) is characterized by accumulation of fat (steatosis), inflammation, and fibrosis in the liver without alcohol consumption. It is serious threat to health. However, currently, there are no approved therapeutic agents for NASH, and it is considered there is no animal model that appropriately represents the complexity of human NASH disease. Chimeric mice with humanized livers (PXB-Mouse®) could show potential for developing new drugs for human liver diseases.

There are research reports which highlights potential benefits of Pioglitazone (PTZ) in NASH.

The aim of this study is to develop a NASH model using PXB-Mouse®, which could be promising tool for investigating therapeutic agents for NASH, and evaluate the potential therapeutic benefits of PTZ.

Summary in Japanese

現在、非アルコール性脂肪肝炎（NASH）の治療薬がなく，さらにヒトのNASH病態の複雑さを模倣する適切な動物モデルがない．そこで，私達はヒト肝キメラマウス（PXB-Mouse®）を用いてNASHモデルを作製し，ピオグリタゾン（PTZ）の作用を検討した．PXB-Mouse®に独自に開発したコリン不含・L-アミノ酸規定（CDAA）飼料を84日間自由摂取させることでNASHモデルを作製した．給餌期間中，PTZを1日1回経口投与した．NASHモデル群では血漿ALT，T-Cho量，血清サイトカインおよび肝臓中のヒドロキシプロリン（HYP）量が増加し，病理組織学的検査で肝臓の線維化が認められた．一方，PTZ投与群では血漿ALT，T-Cho量，血清サイトカインおよび肝臓中のHYP量および線維化面積が減少したことから，PTZはNASHの病態を改善すると考えられた．

Materials and Methods

● Animal

PXB-mouse® (PhoenixBio Co., Ltd.), ♂, 18-22 weeks old

● Diet

CRF-1: Oriental Yeast Co., Ltd.
AS: Oriental Yeast Co., Ltd.
CDAA: The choline-deficient L-amino acid-defined 50kcal% fat containing diet (patented by Mediford Corporation, Patent no.: JP6211035B2).

● Drug

Pioglitazone HCl:
FUJIFILM Wako Pure Chemical Corporation,
PPAR-γ agonist.

● Measurement parameters

Serum Human & Mouse Cytokine and Chemokine:
Bio-Plex Pro Human Cytokine 27-Plex Panel, Mouse Cytokine 23-Plex Panel (Bio-Rad Laboratories, Inc.), at terminal point.

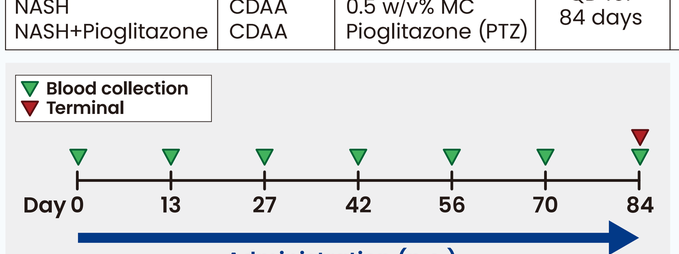
Plasma ALT, T-Cho:
DRI-CHEM FDC7000iZ (Fujifilm Chemical Co., Ltd.), ALT measurement was performed per two weeks, T-Cho measurement was performed at terminal point.

Blood h-Alb: Biochemistry Automatic Analyzer (7180 Clinical Analyzer, Hitachi High-tech Corporation), per two weeks.

Liver Hydroxyproline (HYP):
Measurement was performed at terminal point.

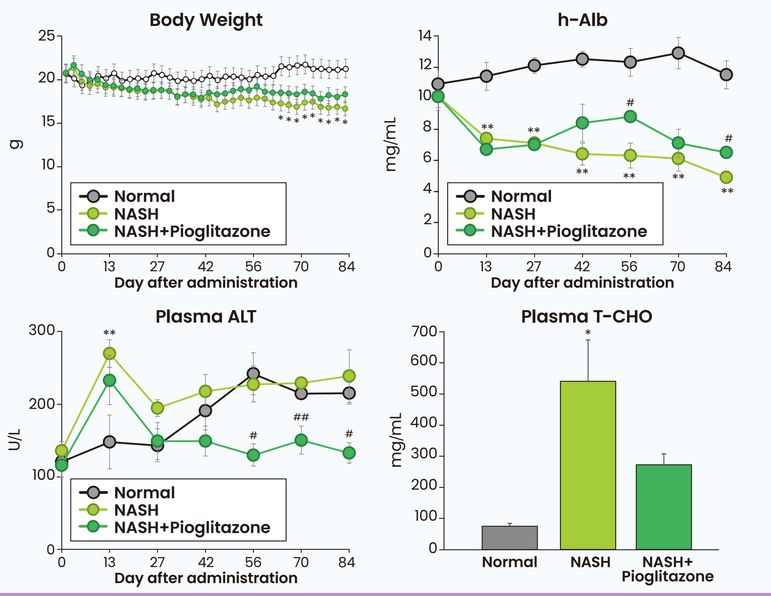
Histopathological observation:
Hematoxylin and Eosin stain (HE), Sirius Red stain (SR), Anti-human Lamin stain (Lamin)

Test group	Diet	Administration	Frequency & Period	Dose (mg/kg)	N
Normal	CRF-1, AS	0.5 w/v% MC	QD for 84 days	0	3
NASH	CDAA	0.5 w/v% MC		0	5
NASH+Pioglitazone	CDAA	Pioglitazone (PTZ)		20	4

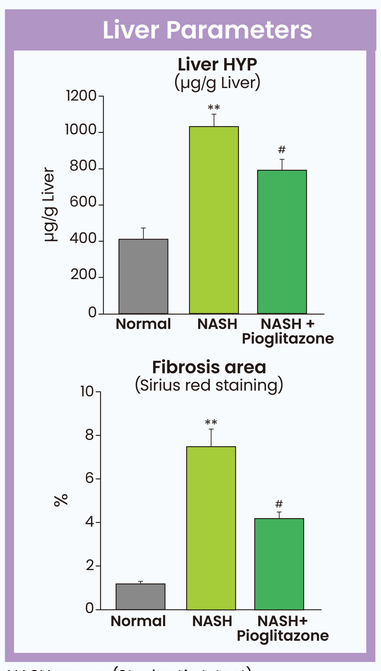


Results

Body Weight & Plasma Parameters

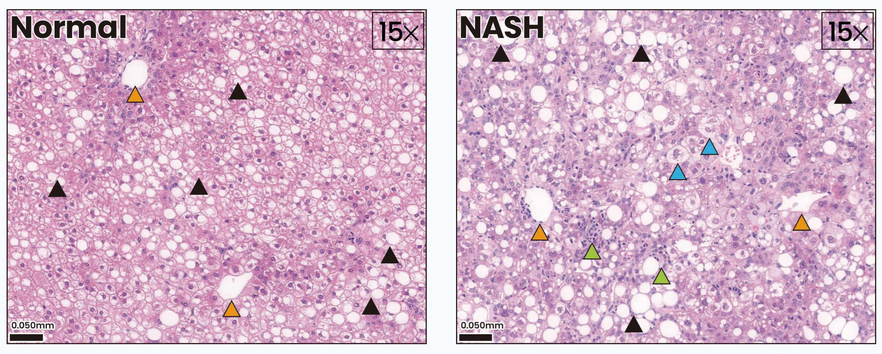


Liver Parameters



*: P<0.05, **:P<0.01; Normal group vs NASH group (Student's t-test)
#: P<0.05, ##:P<0.01; NASH group vs NASH+Pioglitazone group (Student's t-test)
Each value represents the mean±S.E. (N=3-5)

HE



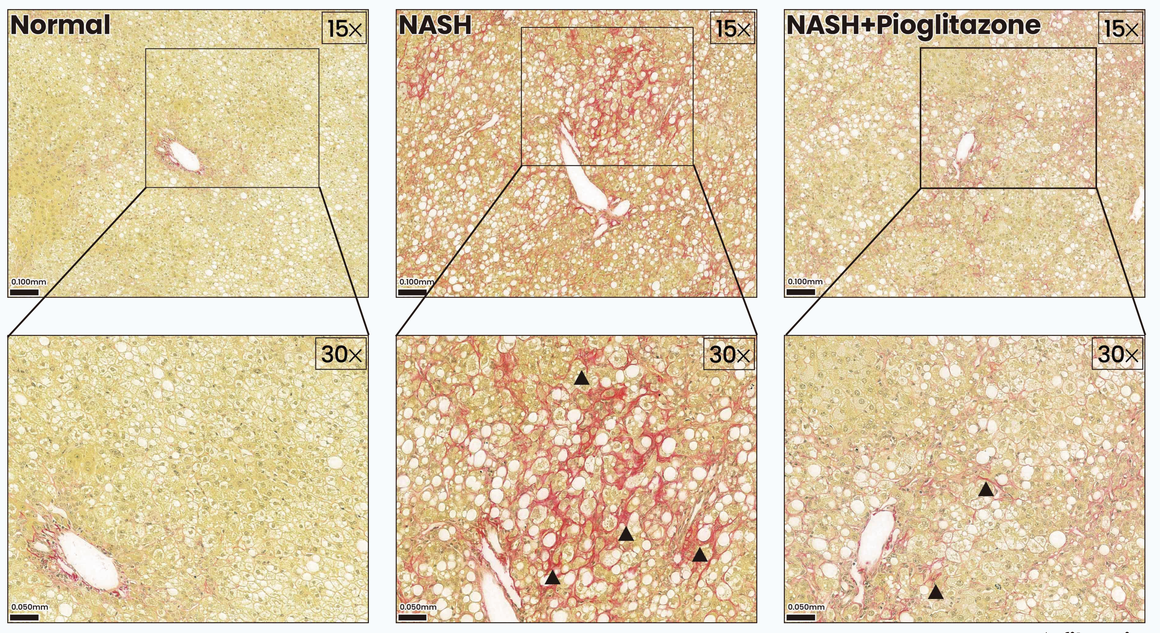
▲ hepatocytes with large sized lipid droplets
▲ Ballooning hepatocytes and Mallory–Denk-body-like aggregates
▲ inflammatory cell
▲ central vein

● Microscopic Examination (HE stain)

Group		Steatosis	Lobular Inflammation	Ballooning hepatocyte
Normal	(3)	2.3 ± 0.3	0.7 ± 0.3	0.0 ± 0.0
NASH	(5)	3.0 ± 0.0	0.6 ± 0.2	1.0 ± 0.0
NASH+Pioglitazone	(4)	2.8 ± 0.3	0.5 ± 0.3	1.0 ± 0.0

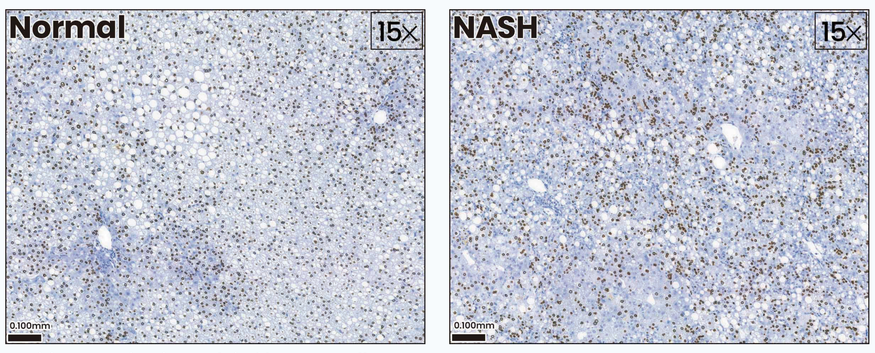
Grade sign: 0, none; 1, minimal; 2, mild; 3, moderate; 4, marked.
Each value represents the mean±S.E. of the histopathology score.
Figures in parentheses are number of animals with tissues examined histopathologically.

SR



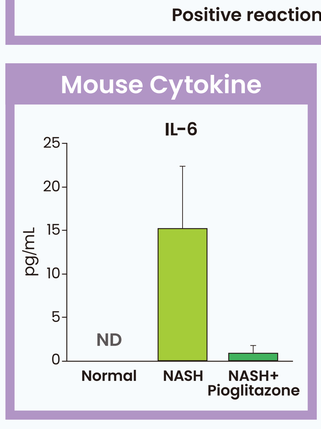
▲ fibrosis

Lamin

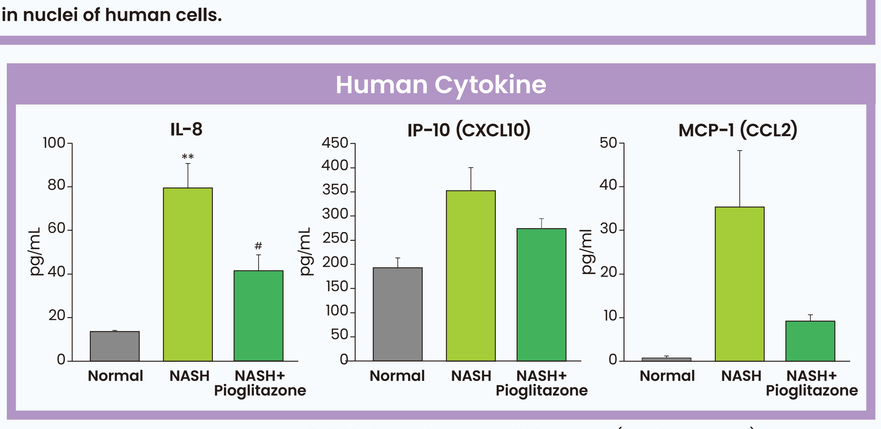


Positive reaction in nuclei of human cells.

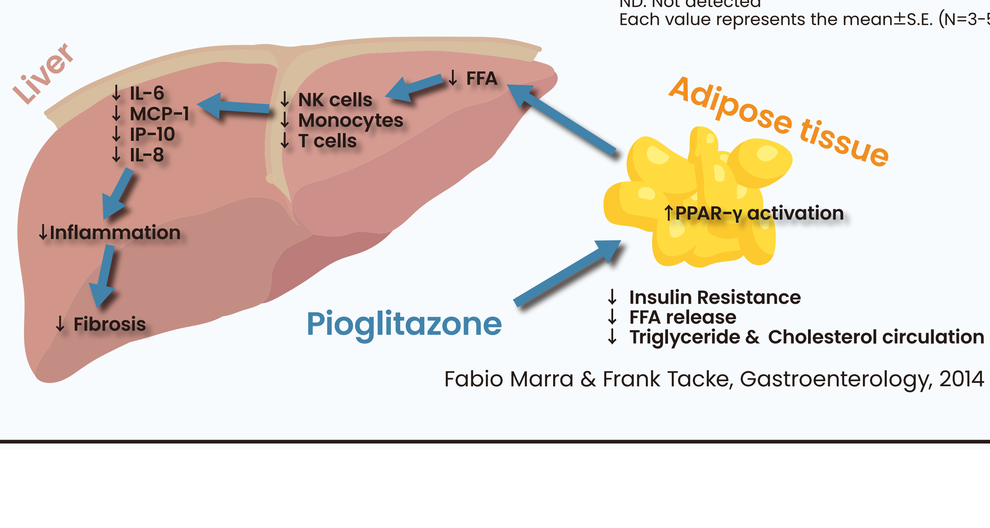
Mouse Cytokine



Human Cytokine



**:P<0.01; Normal group vs NASH group (Student's t-test)
#: P<0.05; NASH group vs NASH+Pioglitazone group (Student's t-test)
ND: Not detected
Each value represents the mean±S.E. (N=3-5)



Fabio Marra & Frank Tacke, Gastroenterology, 2014

Conclusion

In the present study, the NASH model mice (PXB-Mouse®) showed a variety of parameter changes that resemble human pathological conditions, such as elevated ALT, T-Cho, liver HYP, proinflammatory cytokines and chemokines. In addition, the enhanced hepatic fibrosis was confirmed by SR staining in NASH model animals. Higher steatosis and ballooning hepatocyte grade were also confirmed. These findings suggest that the NASH model mice using PXB-Mouse® could develop a human NASH-like pathology better than the previous animal models.

Pioglitazone (PTZ) decreased the lipid accumulation in the liver, reflected by lower T-Cho level, as well as lower levels of IL-8, IP-10, MCP-1 and IL-6, and lower hepatic fibrosis area.

PTZ's action mechanism in the NASH is considered as follows. PTZ activates PPAR-γ in the adipose tissue, and it decreases the insulin resistance. In addition, it regulates free fatty acid (FFA) release and triglyceride (TG) circulation. As the result of FFA regulation, the FFA and lipid droplets levels decrease in the liver. From these changes, the liver cells are exposed to less stress or damage, which leads to decrease in attraction of monocyte, NK cells etc., and lower level of proinflammatory markers like MCP-1, IL-6, IL-8, IP-10. Resultantly, PTZ contribute to a reduction of inflammation within the liver.

As a summary, NASH model mice using PXB-Mouse® could be helpful for new drug development.

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