The dual FXR/TGR5 agonist INT-767 counteracts nonalcoholic steatohepatitis in a rabbit model of high fat diet-induced metabolic syndrome

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Introduction and objectives

Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) are interesting pharmacological targets for the treatment of liver and metabolic diseases. FXR-deficient mice on a high-fat diet (HFD) exhibit massive hepatic steatosis, necro-inflammation and fibrogenesis. Moreover, **pharmacological activation of TGR5 in mice promotes protective mechanisms in biliary epithelial cells, inhibits hepatic and systemic inflammation**.

The aim of this study is to investigate the effect **of FXR/TGR5 dual agonists on** nonalcoholic steatohepatitis (**NASH**) in a rabbit model of high fat diet (HFD)-induced MetS.

Methods

We employed a recently established **animal model of high fat diet (HFD)-induced MetS**, characterized by insulin resistance, hypertension, atherogenic dyslipidemia, visceral adipose tissue accumulation and NASH (Filippi et al., 2009, Maneschi et al., 2013). **Subgroups of MetS rabbits were treated with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30mg/Kg, orally, daily, 5 days a week for 12 weeks)**. We studied the effects of HFD and in vivo INT-767 treatments on **liver function**. Liver was studied by immunohistochemistry and RT-PCR.

Results

- Treatment with increasing doses of the dual FXR/TGR5 agonist **INT-767** (3,10,30mg/Kg/day, 5 days a week for 12 weeks) in a rabbit model of HFD-induced MetS, characterized also by NASH, **dose-dependently reduced several MetS-associated alterations**, including hepatomegaly, insulin resistance, increase of ALT, glucose and cholesterol levels, while significantly increasing HDL levels. ALT was positively associated with all MetS parameters; however introducing all MetS factors in a multivariate analysis, only total cholesterol levels resulted positively associated with ALT level (Adj.r: 0.493, p=0.014). High macrophage M1pro-inflammatory/M2 anti-inflammatory ratio was observed in MetS-induced NASH, which was independently associated with serum ALT levels (Adj.r: 0.322, p=0.032). **HFD-induced increase in M1/M2 ratio was reduced by INT-767 treatment and M2 macrophage markers (IL-10,TGFβ) were increased**.

- Genes related to neutrophil apoptosis/apoptotic-neutrophil clearance (**lactoferrin**, **eNOS**, **RAGE**) and to extracellular matrix degradation (**MMP2**, **TIMP2**) were also **increased by INT-767** treatment. **INT-767** also **reduced** liver expression of **IL-6**, which preferentially skews the Th cell response towards a Th17-phenotype, while **increasing Foxp3** expression, a Treg cell marker (Fig. 1) . Thus these data indicate that INT-767 can promote the neutrophil- and macrophage-driven resolution phase of inflammation and fibrosis regression. In addition, **INT-767 increased** genes related to hepatic fatty acid metabolism (**PPARα**, **AR**, **CD36**) and lipid droplet formation (**SNAP23**, **VAMP4**,**syntaxin5**, **perilipin**) therefore suggesting that INT-767 counteracts excess fatty acid mediated lipotoxicity in the liver. Genes related to insulin signaling (**IRS1**, **SREBP1**, **G6Pase**, and **PEPCK**) were also **increased by INT-767** (Fig. 1).

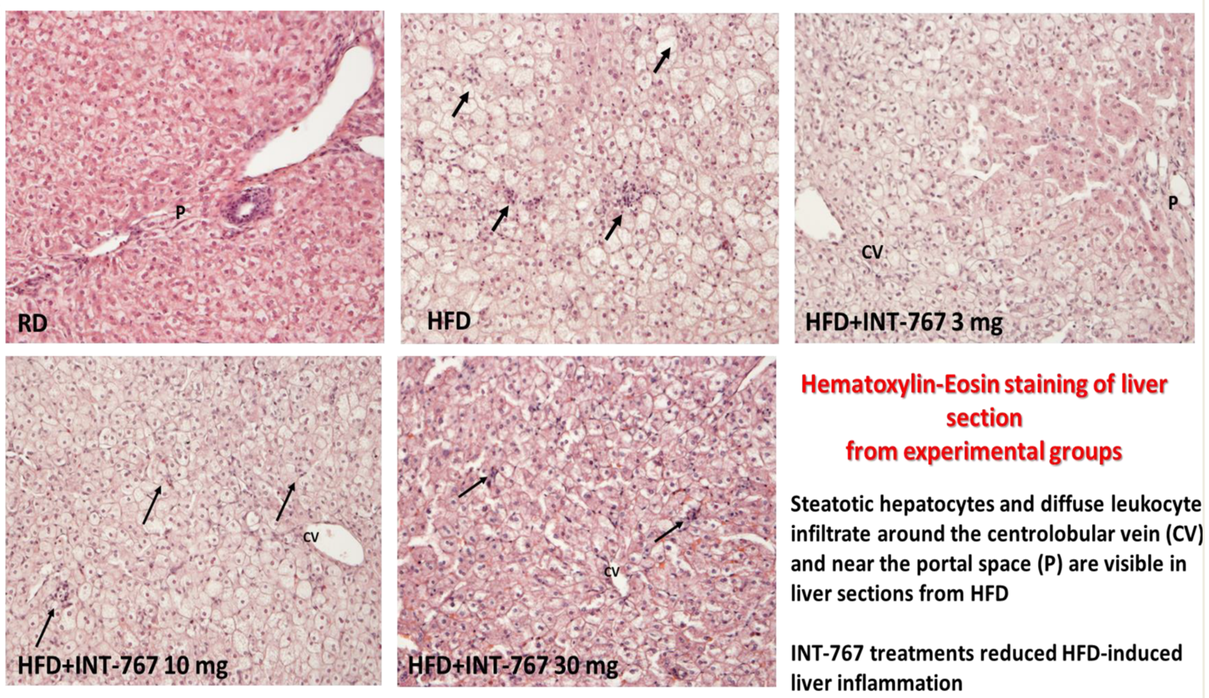
- Finally, immunohistochemical studies demonstrated that **INT-767** treatment significantly **reduced both HFD-induced liver inflammation** (Fig. 2) and **fibrosis**. (Fig. 3).

1)

****p<0.01, ***p<0.0001 vs. RD;°p<0.05, °°p<0.01,°°°p<0.0001 vs. HFD**

		RD	HFD	HFD+INT-767 3mg	HFD+INT-767 10mg	HFD+INT-767 30mg
	M1/M2 ratio	1.1±0.2	219.9±24.5***	62.6±9.4°°	34±16°°	27.1±12°°°
M2 macrophage markers	IL10	100±8.69	1218.28±143.19***	2779.2±560.50°°	2125.1±831.1°	2486.4±774.3°
	TGFβ	100±6.48	389.02±37.03***	1416.45±228.28°°°	693.3±204.5	1064.3±405.7°°
Neutrophil apoptosis markers	lactoferrin	100±10.58	83.09±23.29**	95.52±54.95	107.5±57.7	30.2±21.7
	eNOS	100±5.95	116.38±11.70	279.52±38°°	255.56±21.34°°	311±40.8°°°
	RAGE	100±19.21	434.93±63.55***	813.96±123.17°	379.7±36.7	514.4±105.3
Extracellular matrix degradation markers	MMP2	100±6.48	4095.79***	10264±2497.28°°	2348.4±872.2	2779.4±815.9
	TIMP2	100±7.09	954.77±118.80***	2021.2±140.55°°	1128.2±352.3	1336.8±307
Th17 cell marker	IL-6	100±13.33	215.36±33.77**	82.89±49.37	49.55±8.04°	105.5±51.5
Treg cell marker	Foxp3	100±13.41	1438.8±225.62	3188.2±1414.39°	766.4±544	332.1±103.7
Hepatic fatty acid Metabolism markers	PPARa	100±4.11	71.28±6.11***	173.33±11.89°°°	214.9±26.3°°°	193.4±44.1°°
	AR	100±4.67	118.78±11.85	211.39±13.88°	179.2±31.2	252.2±34.3°°
	CD36	100±5.03	98±10.50	313.21±32.91°°°	179.5±33.4°	156.3±43
Lipid droplet Formation markers	SNAP23	100±6.80	125.93±15.11	404.97±23.46°°	294.6±43.9°°	302±28.9°°°
	VAMP4	100±7.26	95.99±9.45	194.84±9.01°°	151.5±28.7	187.7±13.05°°
	Syntaxin 5	100±4.63	104.39±9.68	294.53±37.62°°	209.4±24.3°°	221.6±22.7°°°
	Perilipin	100±16.38	255.29±48.62***	568.76±63.83°	761.4±250.8	1557.3±625.9
Insulin signaling markers	IRS1	100±6.21	150.79±19.06**	268.60±33.07°	231.6±38.5	298.9±7°°°
	SREBP1	100±9.30	202.77±33.01***	557.49±80.17°°	310.5±108.7	306.1±104.6
	G6Pase	100±7.84	37.81±4.85***	259.07±58.26°°°	247.51±33.7°°°	184.4±61.1°°°
	PEPCK	100±4.62	53.71±5.18***	157.71±3.68°°°	158.24±12.1°°°	160±35.5°°°

2)



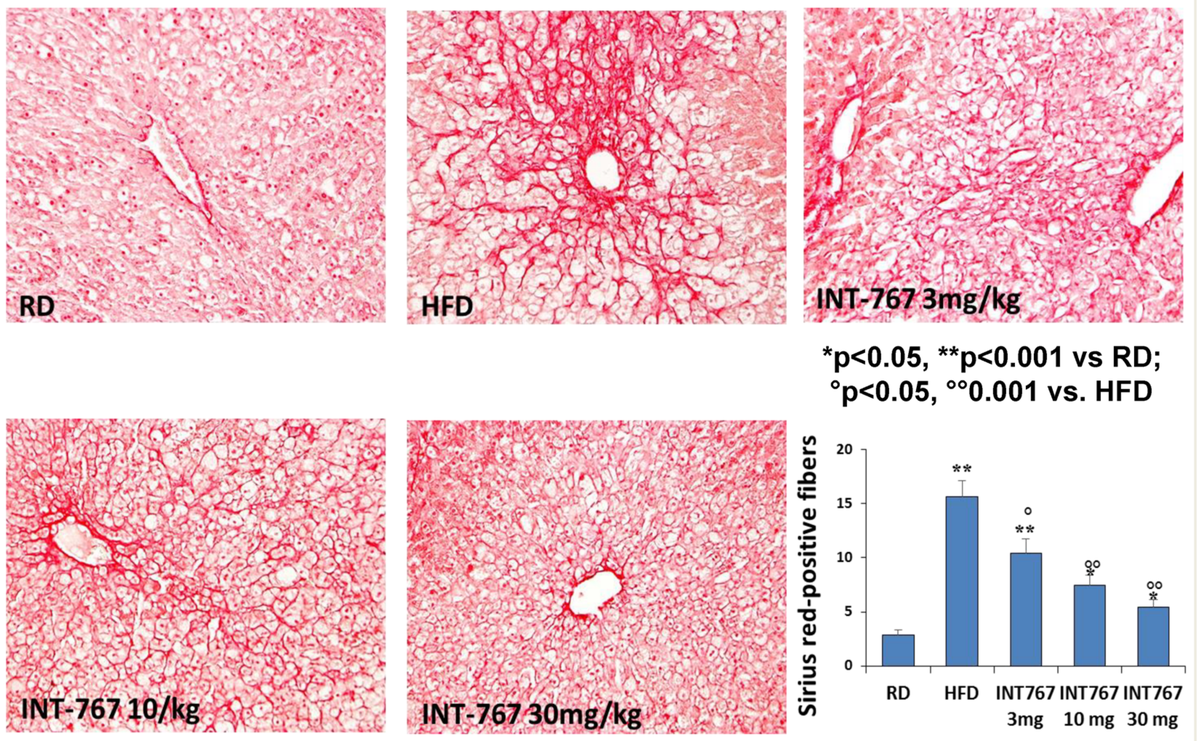
Hematoxylin-Eosin staining of liver section from experimental groups

Steatotic hepatocytes and diffuse leukocyte infiltrate around the centrolobular vein (CV) and near the portal space (P) are visible in liver sections from HFD

INT-767 treatments reduced HFD-induced liver inflammation

Magnification 10x

3)



***p<0.05, **p<0.001 vs RD; °p<0.05, °°0.001 vs. HFD**

Conclusions

In conclusion, **INT-767 treatment counteracts NASH in a rabbit model of HFD-induced MetS** by promoting insulin sensitivity, resolution of inflammation and fibrosis regression.

References

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