Cholesterol peaks found in MRS are a biomarker of Niemann-Pick disease progression

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Introduction

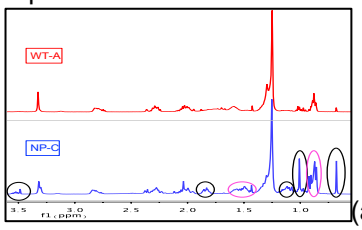
Niemann-Pick (NP) is a genetic and rare group of diseases that affect many body organs. NP patients have an abnormal lipid metabolism that causes an accumulation of lipids in liver, spleen, brain and bone marrow. NP patients develop cirrhosis very fast[1]. The purpose of this study is to characterize the composition of lipids in the liver using *in vitro* spectroscopy in a mice model of NP Type A and C and compare with wild-type (WT) mice with the aim of provide a non-invasive technique to follow-up these patients and provide new hypothesis about the bad progression of NAFLD in some patients.

Methods

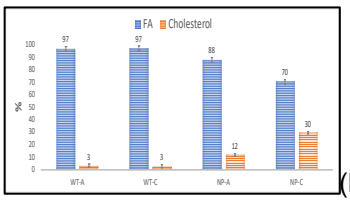
Genetic modifications were made to create mice with NP type A and C. Those mice were euthanized, and we obtained the livers from mice with NP type A (NP-A), NP type C (NP-C), wild-type mice with same age as NP-A (WT-A) and wild-type mice with the same age as NP-C (WT-C). Four samples of each group were analyzed. We made a lipid extraction from the liver[2], followed by the addition of $CDCl_4$ (internal reference), and then, we made a spectrum using a 9.4 T MRS (Bruker Ultra-shield). The acquisition parameters were: spectral width 8012.820 Hz, relaxation delay 1 s, number of scans 16, flip angle 30 to avoid T1 relaxation effects and total acquisition time 48.72 s.

Results

We identified seven metabolite peaks that correspond to the FAs and seven that corresponds to cholesterol by using the MRS. Figure 1a shows a comparison between the spectrum correspondent to a WT group (red) and a NP group (blue). The peaks in black are the cholesterol peaks, the ones in pink are a mixture of FA and cholesterol and the others (1.3 ppm, 2.0 ppm, 2.3 ppm, 2.8ppm) are only FA. The olefinic peak (5.3 ppm) is not showed in the figure. We calculated the area under the curve (AUC) of all the metabolites normalized by the AUC of the reference peak (Methylene alfa carboxyl, in 2.3 ppm) using MetreNova V10[3]. Figure 1b shows that mice group with NP has more peaks corresponded to cholesterol than the WT group. Figure 1c shows the percentage of FA participation.



(a)



(b)

	AUC Normalized (%)			
FA (ppm)	WT-A	WT-C	NP-A	NP-C
0.8	9.3±0.6	9.2±1.0	5.6±0.5	11.6±0.4
1.3	51.4±1.0	49.4±1.8	64.5±1.1	47.8±2.0
1.6	7.0±0.6	6.4±0.4	2.9±0.3	7.7±0.4
2.0	10.2±1.1	9.4±0.7	15.7±1.5	10.5±1.2
2.3	7.0±0.2	8.2±0.7	3.5±0.5	9.6±1.1
2.8	5.8±0.3	7.3±0.2	2.6±0.1	5.5±0.6
5.4	9.4±0.9	10.2±0.3	5.3±0.2	7.1±1.1

(c)

Figure1.

Discussion

Mice with NP-C have more cholesterol than the ones with NP-A because this genetic condition affects proteins related to cholesterol transportation and type A affects more the production of ceramides. FA profile also changes if we compare WT with NP. The value of the diallilic peak (2.8ppm) and olefenic (5.3 ppm) decreased significantly in NP-A when compared to the WT group.

Conclusion

Our results showed that the increase in the liver cholesterol concentration is the main difference of NPC liver manifestation and it has been associated with the bad liver prognosis. Also, there is a different pool of fatty acids storage in the NP-A and NP-C liver, and the different MR liver spectra would provide biomarkers for non-invasive follow-up of these patients. Maybe, the accumulation of cholesterol in the liver, the decrease in some FA could be a biomarker of bad prognosis in NAFLD and could be detected not invasively using MRS. To prove that theory, more mice with advanced stages of NAFLD should be studied.

References

[1] Beltroy e. et al. Hepatology 2005. [2] Folch et al. The Jour Bio Chem. 226(1) ,1957. [3] Salibi & Brown. Clinical MR spectroscopy, Wiley-Liss, 1998.

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