Original Paper

Liver Fatty Acid Binding Protein And Hemojuvelin - Potential Biomarkers For Liver Function in Rat Model

MARIA BOGDAN[1], ISABELA SILOSI[2], PETRA SURLIN[3], A.A. TICA[4], OANA SORINA TICA[5], T.A. BALSEANU[6], ANNE-MARIE RAUTEN[7], D. CIOLOCA[8], A. CAMEN[9]

[1]*Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania*
[2]*Department of Immunology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[3]*Department of Periodontology, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[4]*Department of Pharmacology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[5]*Department of Obstetrics-Gynecology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[6]*Department of Physiology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[7]*Department of Orthodontics, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[8]*Department of Surgery, University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania*
[9]*Department of Dentoalveolar Surgery, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania*

ABSTRACT: *Purpose*. The purpose of the present study was to investigate whether the co-administration of aripiprazole and fluoxetine could produce impaired liver function in Wistar rats by means of liver fatty acid binding protein (L-FABP) and hemojuvelin (HJV) serum levels. Furthermore, the experiment intended to assess the salivary levels of L-FABP and HJV and to determine whether they correlate with the serum levels of the two markers. *Materials and Methods*. Adult male Wistar rats were randomly assigned to four groups: control (saline 10ml/kg), aripiprazole (4.05 mg/kg), fluoxetine (10 mg/kg) and aripiprazole + fluoxetine (4.05 mg/kg + 10 mg/kg). The drugs were administered by gavage, daily at the same hour, along a 6 week period. L-FABP and HJV levels were determined in serum, from intraventricular blood, and in saliva. Also from intraventricular blood, serum levels for aspartate aminotransferase (ASAT) and alanine amino transferase (ALAT) were assessed. *Results.* Positive and statistically significant correlations between serum and salivary levels of L-FABP and HJV were found. Aripiprazole + fluoxetine group experienced increased serum L-FABP levels than aripiprazole and fluoxetine groups, and salivary L-FABP as compared to aripiprazole group; but it registered decreased levels for serum and salivary HJV, for ASAT and ALAT than aripiprazole and fluoxetine groups, and for salivary L-FABP compared to fluoxetine group. *Conclusions.* The data indicate that: aripiprazole coprescribed with fluoxetine do not cause additional alterations in liver function; L-FABP and HJV levels can be helpful as biomarkers for impaired function of hepatocytes; and that their salivary determination can replace serum determination.

KEYWORDS*: aripiprazole, fluoxetine, liver function, L-FABP/FABP1, HJV/RGM-C*

Introduction

Drugs are an important risk factor for initiating liver injury or hepatotoxicity. More than 1000 drugs and toxins have been implicated in drug-induced liver injury (DILI), which represents up to 10% of all adverse drug reactions [1]

Fluoxetine is an antidepressant drug within the serotonin selective reuptake inhibitors (SSRI) category, which have potential for both pharmacokinetic and pharmacodynmic interactions [2]. It inhibits metabolism of other drugs, so it presents a high risk of drug interactions [2,3]. Fluoxetine can cause mild increases in liver enzymes, and there were reported cases in which fluoxetine appeared to have precipitated hepatitis, which remitted when treatment was withdrawn [4].

Aripiprazole, a second-generation antipsychotic, has been shown to be effective as an adjunctive therapy in major depressive disorder and treatment-resistant depression [5,6]. It is a partial agonist of D_2/D_3 and $5\text{-}HT_{1A}$ receptors with rather weak antagonistic effects at $5\text{-}HT_{2A}$, $5\text{-}HT_7$, and H_1 receptors [7,8].

Liver Fatty Acid Binding Protein (L-FABP/FABP1) belongs to a family of molecules that coordinate lipid responses in cells, and is expressed in very high levels in liver, intestine and kidneys [9,10]. It was named after the tissue in which it was first discovered and it was found to have hepatoprotective properties [11].

Hemojuvelin (HJV) is a glycoprotein, member of the three-gene Repulsive Guidance Molecule family in mammals. HJV (or RGM-C) is mainly expressed in hepatocytes but also in cardiac and skeletal muscle, and plays a key role in iron homeostasis [12, 13].

The purpose of the present study was to investigate whether the co-administration of aripiprazole and fluoxetine could produce impaired liver function in Wistar rats by means of L-FABP/FABP1 and HJV/RGM-C serum levels. Furthermore, the experiment intended to assess the salivary levels of L-FABP/FABP1 and HJV/RGM-C and to determine whether they correlate with the serum levels of the two markers.

Materials and Methods

The study was approved by The Ethic Commitee from The University of Medicine and Pharmacy of Craiova.

Twenty adult male Wistar rats (255-270g) were housed in pairs in polycarbonate cages, in a temperature-controlled room (22 ± 1°C) and under 55-60% humidity with a 12 h dark-light cycle. The animals were fed standard laboratory chow and water ad libitum, and the wood shaving was changed every two days. For each animal an individual file was opened, registering the weight values measured in the morning between 9 and 10 o'clock, the used drug and the doses administered according to weight. The drugs were administered orally by gavage, daily at the same hour, along a 6 week period.

The animals were divided into four groups of five, and consulting the literature [14,15] we selected the drugs' doses: group I - control (C) (saline 10 ml/kg); group II – aripiprazole (A) (4.05 mg/kg); group III – fluoxetine (F) (10 mg/kg) and group IV – aripiprazole + fluoxetine (AF) (4.05 mg/kg + 10 mg/kg). The blood volume collected from rats, which allows their survival in optimal conditions for the study, is very small and the baseline values of the subjects included in the study could not be obtained. Therefore, the control group was introduced in the experimental protocol, which lived in the same conditions as the rest of the animals, to provide normal values for each determination.

24 hours after the last drug administration, the rats were anesthetized, using Sevoflurane 5% diluted in a mixture of 70% nitrous oxide and 30% oxygen, given in a special cage for the induction of anesthesia, and 4 ml of intraventricular blood were collected in 2 gel vacutainers. After centrifugation, the supernatant was collected and frozen at -80°C until processing samples.

L-FABP/FABP1 and HJV/RGM-C levels were measured, both in serum and in saliva, by ELISA technique using Quantikine kits from R&D Systems, USA with ASYS Expert Plus (ASYS HITECH GMBH, Austria) according to the manufacturer's instructions.

Saliva was collected on filter paper strips (PerioPaper, Oraflow Inc., Smithtown, NY, USA) introduced in the animal's oral cavity for 10 sec, according to the previously described technique, applied in humans, for collecting the gingival crevicular fluid and saliva [16].

Samplings were done prior to beginning of drug administration, as well as after the last administration, before animals' sacrifice. The volume was measured with a precalibrated device (Periotron 8000, Oraflow Inc., Smithtown, NY, USA) set for saliva, designed to measure volumes of 10^{-6}l (µl). The absorbed liquid was diluted in 100µl phosphate-buffered saline (PBS) in polypropylene tubes, the obtained samples being frozen at –20°C until their utilization.

Additionally, serum levels for aspartate aminotransferase (ASAT) and alanine amino transferase (ALAT) were assessed. by absorbance photometry using commercial kits obtained from Roche Diagnostics, USA, with an automated analyzer (Cobas Integra 400 Plus, Roche, Switzerland).

The collected data were statistically analyzed with a dedicated software (SPSS 16.0, Chicago, IL, USA). Differences between groups were calculated using the Mann Whitney *U* test and for correlations among groups the Pearson test was used. Results are presented as mean ± SD (standard deviation) $P < 0.05$ being considered statistically significant.

Results

Table 1 presents serum levels of L-FABP and HJV. Serum levels of L-FABP were significantly increased ($P < 0.05$) in group 4 (AF group) compared to group 1 (group C), and were also significantly different between groups 2, 3 (A and F groups) and group 4 ($P < 0.01$ for F group; $P < 0.001$ for A group).

Regarding HJV, all three drug treated groups displayed a significantly increase in these values when compared to C group ($P < 0.001$ for F group; $P < 0.01$ for A and AF groups). Also, there were registered significantly differences between A, F groups and AF group ($P < 0.001$ for F group; $P < 0.01$ for A group).

 DOI: 10.12865/CHSJ.41.02.05

Table 1. Serum levels of L-FABP and HJV in the four groups of rats

Variables	Groups			
	C	A	F	AF
L-FABP (ng/ml)	0.8708± 0.3664	0.5856± 0.3246*	0,8302± 0,0681*	1,8452± 0,3767†
HJV (pg/ml)	857.3840± 333.0377	2430.6262± 344.2762†*	2992,0272± 276,4045†*	1542,7406± 78,7431†

Values are mean ± SD. C: control group; A: aripiprazole group; F: fluoxetine group; AF: aripiprazole + fluoxetine group. † $P < 0.05$ compared to corresponding data in C group; * p<0.05 versus AF.

Table 2 presents salivary levels of L-FABP and HJV. Compared to the initial evaluation in the same group, a significant increase of final L-FABP salivary levels was observed in F group ($P < 0.01$) and in AF group ($P < 0.05$); also A group and F group showed significantly different values ($P < 0.05$) versus AF group's values. Final salivary HJV levels were significantly higher in all study groups ($P < 0.05$) compared to the initial evaluation in the same group, but no significantly different results were found between groups.

Table 2. Salivary levels of L-FABP and HJV in the three drug treated groups of rats

Variables	Groups					
	A		F		AF	
	Ai	Af	Fi	Ff	AFi	AFf
L-FABP (ng/ml)	0.0930± 0.0060	0.0858± 0.0312*	0,0788± 0,0050	0,1402± 0,0269#*	0,0890± 0,0107	0,1166± 0,0132#
HJV (pg/ml)	18.0026± 0.2554	23.3854± 4.6623#	18,1034± 0,5515	21,8768± 3,6675#	17,2778± 1,0679	21,0232± 2,8527#

Values are mean ± SD. A: aripiprazole group (Ai: initial value; Af: final value); F: fluoxetine group (Fi: initial value; Ff: final value); AF: aripiprazole + fluoxetine group (AFi: initial value; AFf: final value).
# $P < 0.05$ versus initial evaluation in the same group; * p<0.05 versus AF.

Positive and statistically significant ($P < 0.05$) correlations between serum and salivary levels of L-FABP and HJV were found, as shown in Table 3 (a, b). There was a very strong positive correlation for L-FABP in all three drug treated groups, and for HJV in aripiprazole group.

Table 3. Correlations between serum and salivary levels of L-FABP (a) and HJV (b) in the three drug treated groups of rats

Serum levels of L-FABP	Salivary levels of L-FABP		
	A	F	AF
A	0.8562	-	-
F	-	0.9199	-
AF	-	-	0.8272

(a)

Serum levels of HJV	Salivary levels of HJV		
	A	F	AF
A	0.8775	-	-
F	-	0.6685	-
AF	-	-	0.6183

(b)

A: aripiprazole group; F: fluoxetine group; AF: aripiprazole + fluoxetine group.

Additionally, ASAT and ALAT values were registered in the four groups of rats (Table 4).

ASAT levels were significantly different in all three drug treated groups ($P < 0.05$ for A and F groups; $P < 0.01$ for AF group) compared to C group, and between F and AF group ($P < 0.05$). However all three drug treated groups experienced reduced values as against the ones from C group.

No significantly different results were found in any group for ALAT compared to C group; again A and AF groups showed decreased levels than C group (only F group exhibited higher values). Significantly different results were found for F group as compared to AF group.

Table 4. Biochemical markers in the four groups of rats

Variables	Groups			
	C	A	F	AF
ASAT (UI/L)	148.764± 16.8484	106.064± 11.3571†	125,992± 16,9997$^{\dagger *}$	99,25± 8,7457†
ALAT (UI/L)	56.654± 12.0522	49.91± 6.0493	59,654± 5,0576*	47,134± 4,7930

Values are mean ± SD. C: control group; A: aripiprazole group; F: fluoxetine group; AF: aripiprazole + fluoxetine group. $^{\dagger}P < 0.05$ compared to corresponding data in C group; * $p<0.05$ versus AF

Discussion

The clinical presentation of drug-induced liver injury can range from asymptomatic, often self-limiting, and transient increase in liver function tests to jaundice and severe life threatening acute liver failure and seldom to chronic liver disease [17]. Hepatotoxicity and cardiac toxicity are the main reasons of withdrawal from the market for a drug, or termination in drug development in phase I-III [18].

The abundance of studies in the last years regarding the pharmacologic interventions in major depressive disorder and treatment-resistant depression shows the interest for these adults' mental illnesses. Safe and effective use of the new therapeutical strategies requires a thorough understanding of drug interactions; unfortunately, there aren't enough informations upon the side effects of drug combinations.

Drug-drug interactions may aappear when an SSRI, like fluoxetine, is coadministered with another drug metabolized through the cytochrome P450system. 2D6 and 3A4, two of the isoenzymes of the cytochrome P450 system, are responsible for the metabolism of more than 80% of currently marketed drugs [2,19]; fluoxetine presents low inhibition on 2C and 3A4 isoenzymes and high inhibition on 2D6 isoenzyme [2,19]

Regarding hepatotoxicity, beside the associated serum biochemical markers, such as ASAT and ALAT, potential biomarkers have been studied in the recent years. Even though none of the members of the FABP family are tissue-specific, they have drawn interest as early and sensitive serum markers of tissue damage [20]. Researches demonstrated that elevation of serum L-FABP level after hepatocellular injury was larger and faster than that of ALAT, and that serum L-FABP is a very sensitive marker of liver injury [20, 21].

In the present experiment, statistically higher values were found for serum L-FABP in AF group as compared to C group; also statistically differences were registered between both serum and salivary levels in A, F groups and AF group. In A group, no significant changes were recorded either between initial and final salivary levels of L-FABP, or between its serum levels and those in C group.

Data from similar experiments are lacking, and to our knowledge, no studies are available which have analyzed the effects of aripiprazole and fluoxetine on L-FABP in humans or rats.

In this study, for ASAT concentrations in A, F and AF groups significant changes were found, but their results were significantly lower when compared to C group. As for ALAT, no significantly different results were found in any group in comparison to those in C group. Both for ASAT and for ALAT, F group registered the highest concentrations, statistically significant as compared to AF group.

Endogenous soluble HJV protein was observed in human and rodent serum, yet its source, quantity, and physiologic role *in vivo* are still not fully clarified [22, 23]. Researches indicate that hepatic expression of HJV has the greatest physiologic role in systemic iron homeostasis regulation *in vivo* [22].

 DOI: 10.12865/CHSJ.41.02.05

The current experiment showed that all three drug treated groups exhibited a significantly increase in serum values of HJV when compared to C group. Also, there were registered significantly differences between A, F groups and AF group. Regarding final salivary HJV levels, no significantly different results were found between groups.

Analyzing the values obtained for the group treated with the combination of drugs, there were found increased levels for serum L-FABP than in aripiprazole anf fluoxetine groups, and for salivary L-FABP as compared to aripiprazole group. But there were registered decreased levels for serum and salivary HJV, for ASAT and ALAT than in aripiprazole anf fluoxetine groups, and for salivary L-FABP compared to fluoxetine group.

To the best of our knowledge, this is the first experiment to determine L-FABP/FABP1 and HJV/RGM-C serum levels in rats treated with aripiprazole and/or fluoxetine; moreover, this study is the first attempt to assess the salivary levels for L-FABP/FABP1 and HJV/RGM-C after administration of aripiprazole and/or fluoxetine, and to determine possible correlations between serum and salivary values of these markers.

Recently, the significant advances in techniques for detection of biomarkers in the oral cavity have made possible to use saliva as a diagnostic fluid for different conditions such as caries and periodontal disease, autoimmune and infectious diseases, psychological and genetic disorders, malignancies and legal issues [24].

The significantly increased final values of salivary L-FABP and HJV compared to initial values, observed in the three treated groups, demonstrate that both drugs determine salivary detectable changes in these biomarkers' concentration. Positive correlations, either strong or very strong, between serum and salivary levels of L-FABP and HJV indicate that the analyze of these biomarkers can be achieved using saliva instead of blood determination.

Conclusions

In conclusion, the results suggest that the combination of the two drugs do not cause additional alterations in liver function. The findings indicate that L-FABP/FABP1 and HJV/RGM-C levels can be helpful as biomarkers for impaired function of hepatocytes, and that their salivary determination can replace serum determination.

Further experimental and clinical studies should evaluate the changes in the serum levels of these markers and the correlation with their salivary levels, in various drug treatments.

Acknowledgment

This paper was published under the frame of European Social Found,Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893.

References

1. Hebels DG, Jetten MJ, Aerts HJ, et al. Evaluation of database-derived pathway development for enabling biomarker discovery for hepatotoxicity, Biomark Med. 2014;8(2):185-200.
2. Teter CJ, Kando JC, Wells BG. Major Depressive Disorder. In Wells BG. (Ed): Pharmacotherapy: A Pathophysiologic Approach, 8th edition, 2011, McGraw Hill Medical, USA, 1173-1190.
3. Rang HP, Dale MM, Ritter JM, et al. Antidepressant drugs. In Hyde M. (Ed): Rang and Dale's Pharmacology, Seventh Edition, 2012, Elsevier, UK, 564-583.
4. Aronson Jk. Meyler's Side Effects of Psychiatric Drugs. 2009, Elsevier, 57-63.
5. Rogoz Z. Combined treatment with atypical antipsychotics and antidepressants in treatment-resistant depression: preclinical and clinical efficacy. Pharmacol Rep 2013; 65:1535-1544.
6. Nelson JC, Rahman Z, Laubmeier KK, et al. Efficacy of adjunctive aripiprazole in patients with major depressive disorder whose symptoms worsened with antidepressant monotherapy. CNS Spectr 2014; 19:528-534.
7. Rang HP, Dale MM, Ritter JM, et al. Antipsychotic drugs. In Hyde M. (Ed): Rang and Dale's Pharmacology, Seventh Edition, 2012, Elsevier, UK, 553-563.
8. Crismon ML, Argo TR, Buckley PF. Schizophrenia. In Wells BG. (Ed): Pharmacotherapy:. A Pathophysiologic Approach, 8th edition, 2011, McGraw Hill Medical, USA, 1147-1172.
9. Inoue M, Takahashi Y, Fujii T, et al. Significance of downregulation of liver fatty acid-binding protein in hepatocellular carcinoma, World J Gastroenterol. 2014;20(46):17541-17551.
10. Smathers RL, Petersen DR. The human fatty acid-binding protein family: evolutionary divergences and functions, Hum Genomics. 2011;5(3):170-191.
11. Gong Y, Wang G, Gong Y, et al. Hepatoprotective role of liver fatty acid binding protein in acetaminophen induced toxicity. BMC Gastroenterology 2014; 14:44.
12. Severyn CJ, Rotwein P. Conserved Proximal Promoter Elements Control Repulsive Guidance Molecule C/Hemojuvelin Gene Transcription in Skeletal Muscle. Genomics 2010; 96:342–351.
13. Nili M, David L, Elferich J, et al. Proteomic Analysis and Molecular Modeling Characterize the Iron-Regulatory Protein, Hemojuvelin/Repulsive Guidance Molecule C. Biochem J 2013;452: 87–95.

14. Shastry CS, Shafeeque AA, Ashwathnarayana BJ. Effect of combination of aripiprazole with carbamazepine and fluvoxamine on liver functions in experimental animals. Indian J Pharmacol 2013; 45:121-125.
15. Erdemir F, Atilgan D, Firat F, et al. The effect of Sertraline, Paroxetine, Fluoxetine and Escitalopram on testicular tissue and oxidative stress parameters in rats, Int Braz J Urol. 2014;40(1):100-108.
16. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodontal Res 1986; 21:101-112.
17. Navarro VJ, Senior JR. Drug-related hepatotoxicity, N Engl J Med 2006;354:731–739.
18. Watkins P. Drug safety sciences and the bottleneck in drug development, Clin Pharmacol Ther. 2011;89:788–790.
19. Wells BG, DiPiro JT, Schwinghammer TL, et al. Major Depressive Disorder. In Wells BG. (Ed): Pharmacotherapy Handbook, 9th edition, 2015, McGraw Hill Education, USA, 712-730.
20. Ishimura S, Furuhashi M, Watanabe Y, et al. Circulating Levels of Fatty Acid-Binding Protein Family and Metabolic Phenotype in the General Population, PLoS ONE 2013; 8: e81318.
21. Pelsers MM, Morovat A, Alexander GJ, et al. Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients, Clin Chem 2002; 48: 2055–2057.
22. Core AB, Canali S, Babitt JL. Hemojuvelin and bonemorpho- geneticprotein (BMP) signaling in iron homeostasis. Front.Pharmacol 2014; 5: 104.
23. Chen W, Sun CC, Chen S, et al. A novel validated enzyme-linked immunosorbent assay to quantify soluble hemojuvelin in mouse serum. Haematologica 2013; 98: 296-304.
24. Barbosa da Silva AC, da Silva DR, de Macedo Ferreira SA, et al. Salivary Diagnostics, Current Reality and Future Prospects, In Mandeep Singh Virdi (Ed): Emerging Trends in Oral Health Sciences and Dentistry, 2015, InTech, 673-689.

Corresponding Author: Maria Bogdan, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş St., 200349 Craiova, Romania; e-mail: bogdanfmaria81@yahoo.com

 DOI: 10.12865/CHSJ.41.02.05