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Biochemical markers in major depression as interface between neuronal network and Artificial Neural Network (ANN)

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Abstract

In our study we have evaluated the theme of the platelet fatty acids composition in 84 subjects with a clinical diagnosis of Major Depression (MD) according to the clinical diagnosis and the Hamilton Rating Scale of Depression (HRSD), compared with 60 apparently normal people. We have analyzed the groups without taking in account therapies, gender and age. The results obtained show the evidence of three fatty acids, Arachidonic Acid (AA), Linoleic Acid (LA), and Palmitic Acid (PA) in a peculiar position with respect to the biochemical characterization of MD. The ratio among the three fatty acids, in the different conditions studied, allows us to do the hypothesis that MD is linked to a possible un-balance of the membrane function. The depression condition, in fact, is characterized by a progressive increase of the degree of un-saturation of the platelet fatty acids in most of the patients, while a consistent group is characterized by an increase of the degree of saturation. These findings in platelets seem to be more specific indicators with respect to red blood cell fatty acids and plasma phospholipid fatty acids.

Introduction

A huge and qualified scientific literature reports about the relationships among fatty acids, oxidative stress and brain with reference to the phenomena which regulate its development, chemical maturation and functional and cognitive aspects. To this relationship is also linked the aspect of the membrane biochemical balance modification which can lead to some psychiatric and behavior pathologies such as MD, Mood Disorders, Schizophrenia etc. In these pathologies a major role is respectively recognized in the decrease and in the increase of the AA in membrane phospholipids (erythrocytes) and/or in plasma phospholipids (Adams et al., 1996; Mahadik and Scheffer 1996; Sublette et al., 2004; Green et al., 2005). Mainly, this aspect, in MD, is considered according to the ratio between n-6 and n-3 fatty acids and in particular within the ratio Arachidonic Acid (AA)/Eicosapentaenoic Acid (EPA), (Adams et al., 1996). These scientific findings, until now, have been considered as a marker of MD. In our work we have considered the platelet lipid fatty acids profile, of a group of people affected by MD and we have obtained results that, from one side, confirm the involvement of the AA, but from another side reveal new aspects and that can open a new way in the understanding of MD.

Method

Participants, in number of 84 (51 females and 33 males, mean age: 60.21, SD: ± 12.27), were patients of a Psychiatric Hospital (Villa Baruzziana) in Bologna (Italy) and the controls, apparently healthy subjects, in number of 60 (38 Males and 22 Females, mean age: 33.97, SD: ± 12.40). The un-balance of the numeric density as long as we want strengthen the ANN learning of the depressive group to reduce the false negatives. The investigation was authorized by the ethic committee of the local Health Authorities. Patients and controls were enrolled without taking in account age, gender and therapy but considering the HRSD (Hamilton, 1960) for the patients and a negative history of depression for the normal.

Blood sampling

Blood samples (10 ml) for platelets from normal volunteers and patients were drawn from the ante-cubital vein. In all

cases blood was anti-coagulated with EDTA (vacutainer, England). Platelets were prepared essentially as described by Iida et al., (1991).

Quantification of fatty acid patterns of platelet phospholipids (PL)

Quantification of fatty acid patterns of platelet phospholipids was performed by GC-MS on a cross linked-FFAP capillary column (50 m x 0.32 mm x 0.52 mm, Hewlett-Packard), following purification of lipid fractions by TLC according to Passi et al. (2003). Conditions: Column, cross linked-FFAP capillary column (50 m x 0.32 mm x 0.52 mm, Hewlett-Packard, injection: 1 ml; split ratio, 2; oven temperature, 80° for 1 min, to 180 °C at 30 °C/min, then *to 240°C at 20 °C/min and hold for 40 min.; injector temperature, 230 °C; carrier gas:* helium. The fatty acids of the two groups were compared and the statistical analysis (Erwin et al., 1992; Kohonen, 1995a, 1997b; Cottrell et al., 1998; Hakkarainen et al., 2004) was done according to the following method: **Student T-Test**. The ANN used was: *SOM (Kohonen ANN)* Algoritm: AB *Mexican Hat*, Training Set: *144 Subjects*, Software: *SOM V.* The network utilized was the classic Self Organizing Map (SOM) modified by the Authors. The ANN used has an output of 20x20 nodes and an input with a number of nodes equal to the entry variables. This ANN has the capacity to map data over a two dimension plane.

Results

The statistical analysis has highlighted the main differences among the parameters investigated.

According to the Student T- Test there is a statistical significance for the parameters investigated: C20:4, C18:2, C16:0, C17:1, C22:6, C18:0, C16:1, C18:1. The progression of the statistical analysis until the ANN application has recognized three fatty acids [Arachidonic Acid (AA), Linoleic Acid (LA) and Palmitic Acid (PA)] as the most significant with respect to the others. Tab.1.
Successively it has been located the minimum number of the parameters studied, to discriminate the pathological subjects from the normal subjects with an ANN.
Administering to the ANN all the fatty acids in an hidden form (without specify the pathological cases from the non pathological cases and excluding the less significant at each successive simulation), it has been possible to discriminate the controls from the pathological subjects. The others ANNs have confirmed the same results of the SOM according to the capacity to discriminate the pathological subjects from the non pathological subjects, always considering the fatty acids evidenced by the statistical analysis.
After the ANN elaboration the data have been mapped over a plane and, "Normal" and "Depressive" patients have been respectively coloured of a rien red, results are shown in fig: 1, 2, 2a. In particular we have obtained three main areas: 1) non depressive patients area, 2) depressive patients area, 3) a mixture of both. The third area was split into two other areas according to the major density of normal and pathological people. The distribution of the subjects gave the possibility to understand which was the different position of the subjects according to the platelet fatty acids composition. The three fatty acids (AA, LA and PA) of all the other groups were elaborated with the same ANN procedures to see in which position of the map they were distributed (fig.3).

NORMAL	C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:1	C18:2	C18:3	C20:3	C20:4	C22:4	C22:5	C22:6
Mean	0.86	20.68	1.47	0.80	11.22	22.18	1.82	19.40	0.48	2.10	14.06	1.62	1.16	2.08
S.D.	0.59	2.15	0.70	0.54	3.00	2.08	0.64	2.68	0.16	0.76	2.41	0.70	0.61	0.80

Platele fatty Acids distribution in Normal

DEPRESSION	C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:1	C18:2	C18:3	C20:3	C20:4	C22:4	C22:5	C22:6
Mean	1.02	17.92	2.02	0.45	12.70	21.14	1.89	16.71	0.73	2.29	19.03	1.60	0.98	1.49
S.D.	0.70	4.46	1.57	0.26	3.01	4.13	0.87	3.35	1.55	0.77	3.83	0.82	0.56	0.80

Platelet fatty Acids distribution in Depressives

VARIABLE	p
C20:4	< 0.01
C18:2	< 0.01
C16:0	< 0.01
C17:1	< 0.01
C22:6	< 0.01
C18:0	< 0.01
VitE	< 0.01
C16:1	< 0.05

Tab.1 - Significance levels of the ANOVA between normal and depressive subjects

Discussion

Our work has documented that AA, LA and PA are the three main fatty acids involved in the biochemical aspect of MD and that they can be considered strong markers of the depressive state.
According to the findings of Green et al. (2005) about the brain fatty acid composition of a strain of depressive rats and Hakkarainen et al. (2004) about the role of omega 3 fatty acids, also in human platelets is confirmed that:

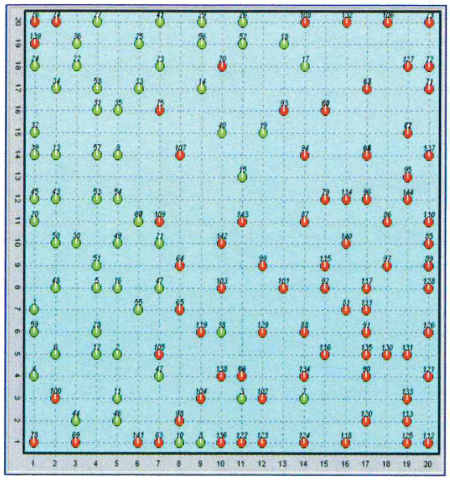


Fig. 1 - The SOM map.

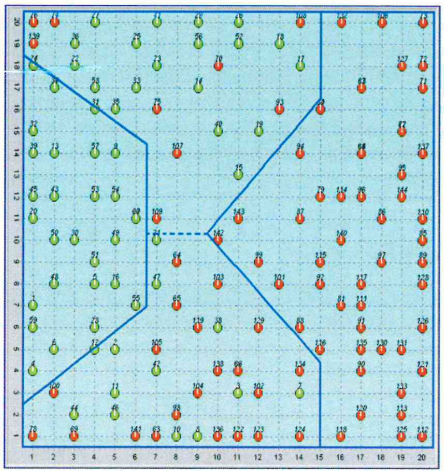


Fig. 2 - Possible subdivision of the map.

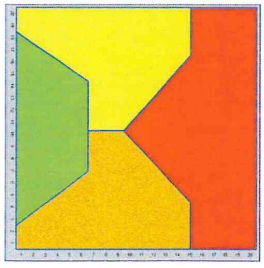
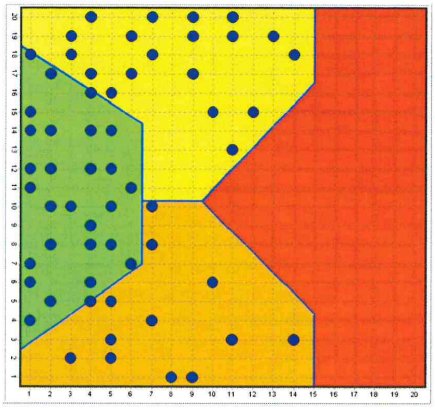


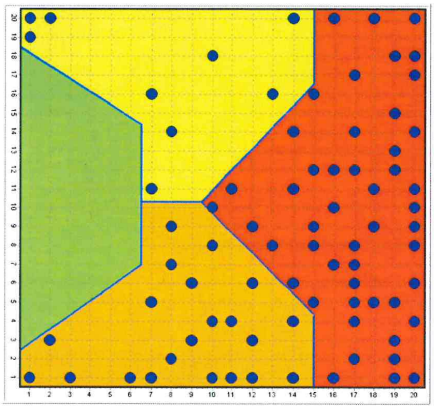
Fig. 2a - Areas of distribution of the cases according to the SOM

1) The AA is the major fatty acid involved in MD.
2) The n-3 fatty acids, as biomarkers in MD are not to be considered of determinant importance.
In any case the AA itself is not enough strong as a marker if it is not considered together with LA and PA. Another observation can be done about the constant sum of the



NORMAL

NO RISK	29	48.3 %
LOW DENSITY	19	31.7 %
HIGH DENSITY	12	20.0 %
DEPRESSION	0	0.0 %
BORDER LINE	0	0.0 %
TOTAL	**60**	**100.0**



DEPRESSION

NO RISK	0	0.0 %
LOW DENSITY	9	10.7 %
HIGH DENSITY	22	26.2 %
DEPRESSION	53	63.1 %
BORDER LINE	0	0.0 %
TOTAL	**84**	**100.0**

Fig. 3 - Distribution of the groups investigated according to the AA, LA, PA.

percentage of AA, LA and PA in all the groups investigated. This allow to consider that the three fatty acids are the major responsible of the membrane balance and that modifications of one of them is responsible of the different state of mobility of the membrane and consequently of the different capacity of the cell communication and, if the hypotheses of the scientific literature are correct, of the modification of the neurotransmission in general and of the serotonin in particular (Sokal, and Rohlf, 1995; Peretti, et al., 2000; Wong et al., 2004). If we calculate the ratio between (AA + LA)/PA=(B Coefficient) of the subjects in fig. 1 and 2 we can realize that from the normal condition the depressive state is reached through an increasing degree of un-saturation of the fatty acids (clockwise). B coefficient increases (fig. 4).

The 4th area which also collects Major Depressive patients is characterized by an increase of the degree of saturation (anti clockwise). B coefficient decreases (fig 5). In both cases it means that the membrane modifies its balance and considering that all the subjects that are in the 2nd, 3rd and 4th area, are clinically diagnosed as MD, there are differences in the biochemical characterization of the pathology. This has not been yet clarified and it will be subject of investigation in the next future. Can we consider different degrees of seriousness of the pathology or can we consider that other aspects of the expression of the pathology must be thought?

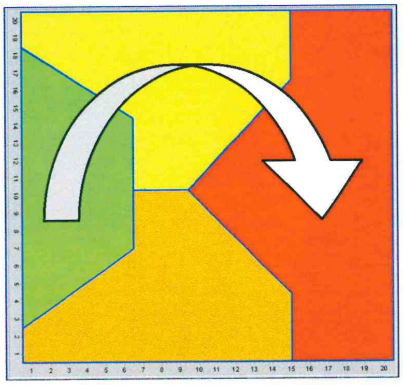


Fig. 4 - (left) increasing degree of un-saturation of the fatty acids (clockwise).

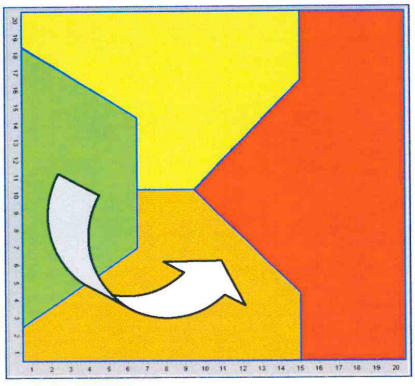


Fig. 5 - (right) increasing degree of saturation of the fatty acids (anti-clockwise)

Conclusion

In conclusion we can assume: 1) AA, LA and PA are the three main lipid biochemical markers in platelets that characterize the MD. 2) The constant sum of the three fatty acids (AA+LA+PA= 53.33 ± 3.43) in all the other conditions studied (Young Sportsmen, Morphea, Scleroderma), gives reason of the role that they play in the membrane balance. In particular the coefficient of correlation between the sum of AA and LA versus PA was highly significant: r= -.66 (p=0.00) 3) The differences of the degrees of un-saturation and of saturation in the platelets of the subjects could be a signal of a different condition within the same pathology. The use of the ANN to analyze the modifications of the fatty acids allows us to confirm, from a biochemical point of view, the clinical diagnosis of MD. 4) It is possible that the blood platelets fatty acids (AA, LA, PA) are strong specific markers in other psychiatric conditions and that they are an easy way to forecast, at least the MD, possibly, other pathologies.

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