Reviewer's report

Title: The Effects of Stress on Myocardial Apoptosis in myocardial infarction

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Reviewer: Lucia Carboni

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The manuscript entitled “The Effects of Stress on Myocardial Apoptosis in myocardial infarction” by Wang et al. reports experiments aimed at investigating the molecular mechanisms of the observed association between myocardial infarction and major depressive disorder. Markers of apoptosis in the heart were compared in groups subjected to chronic unpredictable stress or myocardial infarction (MI) versus sham-operated animals. The main finding was that in MI rats the amount of one marker of apoptosis was greater in the subgroup that displayed depressive-like behaviours as compared with animals that showed lower scores in these tests.

The subject is interesting, although the approach used is not especially innovative (e.g.: Kubera et al., Prog Neuropsychopharmacol Biol Psychiatry, 35: 744–759, 2011). In particular, the association between apoptosis and chronic mild stress was evaluated in a previous study from the same authors (Wang et al., Hum. Psychopharmacol. 2011, 26:95-101), while an association between MI and apoptosis is well recognized.

In addition, there are several issues in the experimental design, in the choice of the methods and in the statistical analysis of the results.

Major compulsory revisions

1. Experimental design

About the experimental design, from the section entitled “subjects” of the methods it appears that the group labelled as MI-depressed was selected within the MI group because of the response in the open field and sucrose preference tests. The animals were labelled as MI-depression by splitting the MI group based on results obtained in the tests aimed at evaluating depressive-like behaviours. Therefore, the results in these tests cannot be displayed as results of the experimental groups, since they were adopted to define the groups themselves. If the belonging to a group was defined based on results in sucrose intake and open field tests, performing statistical analyses after the selection is meaningless. Thus, the results section is reduced to the comparison of bax/bcl-2 and caspase levels in the four groups. Moreover, the selection criteria to include an animal in the MI-depressed group should be clearly defined beforehand and described in the methods section.

Also, the title is misleading since no stress plus MI infarction group was examined.

The sham, MI, and MI-depressed groups experienced surgery, whereas the
chronic unpredictable stress-depressed group did not. I wonder if this difference may introduce bias. Reasons for this choice should be given in the methods.

2. Choice and description of the methods

Using RT PCR as a quantitative method for measuring the level of gene expression is not adequate, since quantitative real time RTR PCR methods are now available. End-point RT PCR as adopted in this study is not sufficiently quantitative to allow accurate comparisons of expression levels.

The method for measuring the expression levels of Bax and Bcl-2 is not sufficiently detailed. The indicated provider apparently does not show its products or protocols in a web site. The American provider does not list the indicated kit among its products. Additional information should be provided to ensure reproducibility of the data. In particular, the method for quantitative measure of immunostaining should be reported in detail.

The method for measuring caspase activity was not described, unless if expression level was meant. If this is the case, the concerns expressed for bax and blc-2 hold for caspase as well. In addition, if levels instead of activity were investigated, in the results the comparisons should be described as “difference between caspase expression levels”, not as “difference between caspase activity”, as it is in the current version.

3. Statistical analysis

The concerns about statistical analysis of behavioural data were already discussed in the experimental design section.

The statistical analysis of expression levels should be carried out with one-way ANOVA tests, followed by post hoc tests which include correction for multiple comparisons if the cut-off level for significance was reached. Repeated Student’s t tests are not adequate to compare four independent groups, since higher rates of false positive results are obtained.

Minor essential revisions

In the abstract, information should be provided about the animal model used to reproduce major depression symptoms. Thus, chronic mild stress should be mentioned in the abstract as well.

Abstract, 6th line in first paragraph: “makers” should be “markers”.

Chronic mild stress: how was the isolation carried out if all rats were singly housed as stated in the section “subjects”?

There are several language mistakes in figure legends.

There are format inconsistencies in the References, e.g. in 7, 21, 24, 26, 30, 31, 35, 37 and 43: journal title is not abbreviated; 27: abbreviation is wrong.

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I declare that I have no competing interest