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OPEN PEER REVIEW

Combination of Propranolol and Heated 4T1 Cells Elicits Beneficial Response Against Mouse Model of Breast Cancer

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

0 comments

No public comments in this round.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Respected Editor-in-Chief

With greetings and devotion

The responses to the respected reviewers are sent as an attachment.

Thank you

Respected first reviewer

With greetings and thanks for your fair review, I respectfully submit the following:

1- The work presented at the 12th International Congress of Immunology and Allergy of Iran was part of our initial pilot work, which was carried out to determine the appropriate dose of propranolol. The results of the continuation of the work indicated the greatest efficacy for the 6 mg/kg dose. Unfortunately, however, due to some limitations in completing the tests, reporting the final results took a little longer. Considering the word-count limitations of the journal Zahedan J Res Med Sci and to avoid repeating the reported results, the results related to the most effective dose, i.e., 6 mg/kg, were reported.
2- The use of the 6 mg/kg dose of propranolol as an adjuvant in mice is entirely precedented in previous articles; for example, the following articles can be mentioned:

a) Propranolol efficacy as a novel adjuvant for immunization against *Toxoplasma gondii* tachyzoites. Exp Parasitol. 2018 Nov;194:60-66. doi: 10.1016/j.exppara.2018.09.014. Epub 2018 Sep 22.

b) Evaluation of the adjuvant activity of propranolol, a Beta-adrenergic receptor antagonist, on efficacy of a malaria vaccine model in BALB/c mice: Iran J Allergy Asthma Immunol. 2014 Oct;13(5):307-16.

3- However, regarding the article that you mentioned (https://doi.org/10.1016/j.biopha.2018.05.002) as having similarity to our work, I respectfully submit a few points: At the outset, perhaps our delay in publishing the full text, given the reporting of part of the work at the 12th International Congress of Immunology and Allergy of Iran, may have played a role. However, on careful reading of the article, points of difference are also seen that justify the difference in the design and aim of the work. First, in our study the aim was to design an effective therapeutic vaccine (theraccine) against cancer. Naturally, for the vast majority of cancers, other than viral cancers, a conventional (preventive) vaccine is basically not relevant, and immunotherapy begins when the symptoms of the disease have actually appeared. For this reason, we started immunotherapy on day 11, when all the studied mice showed a palpable and real tumor (The vaccination was initiated on day 11 after 4T1 injection when all mice brought up a palpable tumor.). In other words, we have reconstructed the real conditions of a cancer. Whereas in the study

you referred to, immunotherapy began on the third day, i.e., when the symptoms had not started. For this reason, we obtained a response with the 6 mg/kg dose of propranolol, and the study you referred to with the 3 mg/kg dose of propranolol.

Second, there is basically a completely substantive difference between the two articles. We induced the tumor (injection of tumor cells), whereas in the study you have in mind, the tumor was transplanted into the mice. Naturally, tumor transplantation is not the normal way of developing a tumor. Finally, we also have the natural killer cell cytotoxicity test in our study, which the study you have in mind does not have.

Thank you.

Respected second reviewer

With greetings and thanks for your fair review, I respectfully submit the following:

1- As requested, the statistical results of Figure 1 were added to the text more completely.

2- Regarding Figure 2, the results of each specific day were compared with each other. For example, on day 27, the tumor size data of the three existing groups were compared with each other.

3- With the utmost respect, I submit that for comparing several data sets the Kruskal-Wallis test was used; after it was determined that there was a statistical difference among all the data, the Wilcoxon test with Bonferroni adjustment was used for pairwise comparison of the groups (group T with V1, T with V2, and V1 with V2). This is exactly as, in the parametric approach, the On-way Anova test is used for comparing several data sets and then a post-hoc test such as the Tukey test is used for pairwise comparison of the data. When the data are analysed in software such as SPSS, the software itself naturally applies the Bonferroni adjustment while performing the Tukey test. Now, the respected reviewer's question may be why the Kruskal-Wallis method and not ANOVA was used. In answer to this question, two points are necessary: First, the Kaplan-Meier test, which was used in the analysis of Graph 1 to assess the survival rate, is inherently a nonparametric test. On the recommendation of the statistics specialist, for consistency of procedure, the other tests also used the nonparametric Kruskal-Wallis method and not the parametric ANOVA method. The second reason is that in nonparametric tests, unlike parametric methods, the assumption of normal distribution of the data does not apply. Unfortunately, based on an incorrect practice, many researchers perform ANOVA without a test confirming the normality of the data. Basically, in a nonparametric test there is no need to perform a normality test, because no such assumption applies. Of course, in nonparametric tests the probability of

the data becoming significant is greatly reduced. Well, it is natural that when a sensitive test, such as the evaluation of a cancer immunotherapy method, is underway, one should act conservatively and use stricter statistical methods. In that case, the significance of the data will be more robust.

Thank you.

2 Revision 1

0 comments

No public comments in this round.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Respected first reviewer

With greetings, courtesy and many thanks for your meticulous review, I respectfully submit the following:

1- Although the statistical tests had been carried out under the supervision of a statistics specialist, as requested, another biostatistics specialist was also consulted.

2- The software used for the statistical work in this research was not SPSS; rather, MedCalc software was used for this research. In the first part of the statistical work in the Materials and Methods section, the type of software and its version were stated. The reason for choosing this software was my access to the original licence of this software. Conscientiously and religiously, I have no interest whatsoever in using cracked software. In this software, unlike the higher versions of SPSS, Bonferroni correction is still necessary.

3- With your permission, due to lack of access to an original GraphPad licence, Excel 2016 software was used to draw the graphs.

4- As you requested, the tumor size results were analysed by the Repeated measures analysis of variances (ANOVA) method, in the MedCalc software environment. This was stated in the statistics part of the Materials and Methods section. However, no particular change was observed in the results of Figure 2. In any case, the test output is presented on the next page:

Repeated measures ANOVA

Number of subjects	15

Within-subjects factors

Factor
DAY_12
DAy_17
DAY_22
DAY_27
DAy_32
DAY37

Between-subjects factors (subject groups)

group	n
0	5
1	5
2	5
Total	15

Sphericity

Method	Epsilon
Greenhouse-Geisser	0.272
Huynh-Feldt	0.295

Test of Between-Subjects Effects

Source of variation	Sum of Squares	DF	Mean Square	F	P
Groups (group)	560821.330	2	280410.665	32.08	<0.001
Residual	104890.283	12	8740.857		

Test of Within-Subjects Effects

Source of variation		Sum of Squares	DF	Mean Square	F	P
Factor	Sphericity assumed	4265399.067	5	853079.813	394.13	<0.001
	Greenhouse-Geisser	4265399.067	1.361	3133766.971	394.13	<0.001
	Huynh-Feldt	4265399.067	1.475	2891417.198	394.13	<0.001
Group x Factor interaction	Sphericity assumed	559989.750	10	55998.975	25.87	<0.001
	Greenhouse-Geisser	559989.750	2.722	205710.809	25.87	<0.001
	Huynh-Feldt	559989.750	2.950	189802.169	25.87	<0.001
Residual	Sphericity assumed	129868.576	60	2164.476		
	Greenhouse-Geisser	129868.576	16.333	7951.148		
	Huynh-Feldt	129868.576	17.702	7336.247		

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I am most grateful for your attention and the time you devoted.

3 Revision 2

2 comments · 15 Feb 2020 – 15 Feb 2020

Associate Editor

ACCEPT 15 Feb 2020

Accept

Editor-in-Chief

ACCEPT 15 Feb 2020

Accept