Journal of Inflammation Research

Dovepress
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COMMENTARY

Long COVID-19 and Peripheral Serotonin: A Commentary and Reconsideration

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Abstract: We believe there are serious problems with a recently published and highly publicized paper entitled "Serotonin reduction in post-acute sequelae of viral infection." The blood centrifugation procedure reportedly used by Wong et al would produce plasma that is substantially (over 95%) depleted of platelets. Given this, their published mean plasma serotonin values of 1.2 uM and 2.4 uM for the control/contrast groups appear to be at least 30 to 60 times too high and should be disregarded. The plasma serotonin values reported for the long COVID and viremia patients also should be disregarded, as should any comparisons to the control/contrast groups. We also note that the plasma serotonin means for the two control/contrast groups are not in good agreement. In the "Discussion" section, Wong et al state that their results tend to support the use of selective serotonin reuptake inhibitors (SSRIs) for the treatment of COVID-19, and they encourage further clinical trials of SSRIs. While they state that, "Our animal models demonstrate that serotonin levels can be restored and memory impairment reversed by precursor supplementation or SSRI treatment", it should be noted that no data are presented showing an increase or restoration in circulating serotonin with SSRI administration. In fact, one would expect a marked decline in platelet serotonin due to SSRIs' effective inhibition of the platelet serotonin transporter. Wong et al hypothesize that problems of long COVID arise from too little peripheral serotonin. However, given the frequent presence of a hyperaggregation state in long COVID, and the known augmenting effects of platelet serotonin on platelet aggregation, it is plausible to suggest that reductions in platelet serotonin might be associated with a lessening of the cardiovascular sequelae of COVID-19.

Keywords: serotonin, COVID-19, plasma, platelets, long COVID, viral infection

We believe there are serious problems with a recently published and highly publicized paper entitled, "Serotonin reduction in post-acute sequelae of viral infection."[1] The two-part central hypothesis of the study is that COVID-19 and other viral infections: (1) cause a reduction in circulating serotonin (5-hydroxytryptamine, 5-HT), and (2) a reduction in peripheral 5-HT results in reduced vagal activity and thereby adversely affects neurocognitive function.

The authors provide two bar graphs depicting mean concentrations and distributions of plasma 5-HT concentrations in support of the contention that peripheral 5-HT is lower in chronic viral infections. In Figure 1J, the mean plasma 5-HT value for recovered COVID patients of ~1200 nM (1.2 uM, ~210 ng/mL) is contrasted to a substantially lower mean value in long COVID patients. While in Figure 2B the mean plasma 5-HT value for healthy controls of ~2400 nM (2.4 uM, ~420 ng/mL) is contrasted to the lower values obtained for viremia patients.

Unfortunately, the means and distributions of the plasma 5-HT data presented for the contrast/control groups are clearly in error. According to one of the authors of Wong et al, the plasma samples used for the 5-HT determinations were prepared by centrifuging blood at 1500 xg for 10 minutes. This crucial information regarding the centrifugation conditions was not provided in the paper, but rather obtained through personal communication with a corresponding author (M. Levy, November 12, 2023). This force and time of centrifugation typically leads to plasma platelet counts of

Received: 31 January 2024
Accepted: 2 April 2024
Published: 11 April 2024

<10,000 platelets/uL, about 4% or less of those seen in the typical whole blood specimen (250,000 platelets/uL). In fact, similar spins are recommended and used to prepare plasma largely depleted of platelets for use in coagulation assays.[2–4] Given that (1) a marked depletion (4% or less remaining) in platelets is expected for the plasma samples employed, (2) over 99% of blood 5-HT is found in the platelet,[5,6] and (3) typically reported means for whole blood 5-HT are about 1 uM,[7–9] one would expect to see mean 5-HT values for plasma so prepared to be about 0.04 uM or less.

Thus, the reported mean plasma 5-HT values of 1.2 uM and 2.4 uM for the control/contrast groups appear to be at least 30 to 60 times too high and should be disregarded. The plasma 5-HT values reported for the long COVID and viremia patients also should be disregarded, as should any comparisons to the control/contrast groups. It is further noted that the plasma 5-HT means for the two control/contrast groups are not in good agreement.

Several other aspects of the Wong et al paper also deserve comment. There is the general issue regarding the terminology used when reporting blood and plasma 5-HT concentration. It should be clearly stated whether one is determining 5-HT in whole blood (WB), serum, platelet-rich plasma (PRP) or platelet-poor plasma (PPP). The first three sample types provide a measure of platelet 5-HT, given that nearly all circulating 5-HT is sequestered in the platelet. Of the three (WB, serum, and PRP), analysis of whole blood is preferred, as serum 5-HT values can potentially be affected by incomplete release as well as by instability of 5-HT in serum. On the other hand, PRP values can be affected by differences in the yield and characteristics of the platelets collected. The centrifugation procedure used in Wong et al produced plasma that is substantially depleted of platelets, yet not sufficiently platelet poor to provide an estimate of free plasma 5-HT; it thus yields plasma that is neither fish nor fowl.

When determining blood or plasma 5-HT, it is highly beneficial to obtain contemporaneous platelet counts; these were not reported in Wong et al. In the case of PPP, the presence of unwanted residual platelets can thereby be detected. With WB, serum, and PRP samples, however, it is advantageous to be able to express the 5-HT concentration on a per (billion) platelet basis in addition to the more typical concentration units (ng/mL or uM). This can allow a more nuanced interpretation of group differences. For instance, if WB or PRP ng/mL values are low without lower ng per billion platelet values, it suggests that the 5-HT reduction is simply due to lower platelet counts. However, if ng per billion platelet values are low (with or without lower platelet counts), it indicates that one or more of the following factors are at play: reduced platelet lifespan (as all platelet 5-HT is taken up continuously during the platelet's 8–10 day lifespan), reduced platelet exposure to 5-HT, or reduced platelet uptake of 5-HT.

As previously reviewed,[10–13] a large number of studies have reported substantially reduced platelet counts in COVID-19 and the reduction has often been attributed to platelet hyperactivation/hyperaggregability and a presumed resultant shortened platelet lifespan (attributions also supported by animal studies reported by Wong et al[1]). Lowered platelet count is the most parsimonious explanation for the lower mean serum 5-HT values reported for COVID-19 patients in the studies referenced by Wong et al. However, without platelet counts it is not possible to know if other factors (especially 5-HT exposure and uptake inhibition) are playing a role in those previously reported reductions in serum 5-HT.

Wong et al concluded that their studies tend to support the use of SSRIs for the treatment of COVID-19, and they encourage further clinical trials of SSRIs. It is stated in the "Discussion" section that, "Our animal models demonstrate that serotonin levels can be restored and memory impairment reversed by precursor supplementation or SSRI treatment." It should be noted that no data are presented showing an increase in or restoration of 5-HT with SSRI administration. In fact, one would expect a marked decline in platelet 5-HT due to SSRIs' effective inhibition of the platelet 5-HT transporter. All studies of serotonin reuptake inhibitors (SRIs) in COVID-19 would benefit from measuring platelet 5-HT, as this provides an index of bioeffect at the transporter and might facilitate the investigation of factors affecting treatment outcomes.[14] Any study of 5-HT in COVID-19 should either exclude patients receiving SRIs or account for their presence by identifying and subgrouping such patients.

A final area of concern is the lack of clarity regarding the source of the 5-HT hypothesized to act at vagal afferents. At times, the authors seem to suggest that reduced platelet stores of 5-HT lead to diminished vagal activity. At other times, reduced production of 5-HT by enterochromaffin cells of the intestinal wall is posited as underlying a reduced exposure of the vagal afferents in the gut to 5-HT. The statement in the "Discussion" section that the "vagus nerve is an important mediator of sickness behavior, [and] responds to peripheral serotonin levels" is overstated, in that the reference cited in support of the latter contention examined the effects of SSRIs on vagal activity but did not measure 5-HT or apply 5HT to vagal afferents.[15] The evidence provided by Wong et al in support of peripheral 5-HT effects on vagal activity was

limited to calcium influx studies with cultured nodose ganglion neurons, studies of the behavioral and physiological effects of an orally administered 5-HT_3 agonist, and the effects of dietary supplementation with tryptophan.

Despite the abundance of experiments reported by Wong et al, and multiple prior studies of 5-HT and serotonergic agents in COVID-19,[16,17] the role (or roles) of 5-HT in acute and long COVID remains very much an open question. Wong et al hypothesize that problems arise from too little peripheral 5-HT. However, given the frequent presence of a hyperaggregation state in long COVID, and the known augmenting effects of platelet 5-HT on platelet aggregation, it is also plausible to suggest that reductions in platelet 5-HT could be beneficial.

In summary, we reach the following conclusions: (1) the crucial plasma 5-HT data reported in Figures 1J and 2B of Wong et al should be disregarded as clearly and markedly erroneous; (2) reports of circulating 5-HT levels should clearly define how the sample is prepared; (3) platelet counts should be obtained when measuring circulating 5-HT and will significantly improve interpretation of the measurements; and (4) though the role of peripheral 5-HT in long COVID remains unclear, it can be suggested that reduced platelet 5-HT might actually be associated with a lessening of cardiovascular sequelae of COVID-19.

Disclosure

Dr Jeremy Veenstra-VanderWeele reports contracts for clinical trials with Roche, Janssen, Yamo, Acadia, MapLight, and Simons Foundation Clinical Research Associates, and a stipend for editorial work from Springer and Wiley, outside the submitted work.

All authors declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

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