



RESEARCH NOTE

In silico analysis suggests repurposing of ibuprofen for prevention and treatment of EBOLA virus disease [version 1; peer review: 2 approved]

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Abstract

The large 2014/2015 Ebola virus outbreak in West Africa points out the urgent need to develop new preventive and therapeutic approaches that are effective against Ebola viruses and can be rapidly utilized. Recently, a simple theoretical criterion for the virtual screening of molecular libraries for candidate inhibitors of Ebola virus infection was proposed. Using this method the 'drug space' was screened and 267 approved and 382 experimental drugs as candidates for treatment of the Ebola virus disease (EVD) have been selected. Detailed analysis of these drugs revealed the non-steroidal anti-inflammatory drug ibuprofen as an inexpensive, widely accessible and minimally toxic candidate for prevention and treatment of EVD. Furthermore, the molecular mechanism underlying this possible protective effect of ibuprofen against EVD is suggested in this article.

Keywords



drug space , NSAID , molecular libraries



This article is included in the **Emerging Diseases and Outbreaks** gateway.

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Approval Status

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1. Mattia Mori , Istituto Italiano di Tecnologia, Rome, Italy		
2. Ayub Darji , Autonomous University of Barcelona, Barcelona, Spain		
Any reports and responses or comments on the article can be found at the end of the article.		



This article is included in the **Ebola Virus** collection.

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Introduction

The recent Ebola virus outbreak in West Africa caused (as of April 8, 2015) a total of 25,556 confirmed cases, including 10,587 deaths (World Health Organization, Ebola data and statistics). Although reports of new, confirmed cases of Ebola seemed to decrease in April of 2015 to approximately 30 cases per week, medical and aid organizations are warning that the current crisis is not over. Furthermore, there remains a risk for future epidemics. Therefore, there is an urgent need to develop new preventive and therapeutic approaches that can be rapidly utilized. New drugs for the treatment of EVD should be safe, efficacious, easy to manufacture and inexpensive in order to be successfully deployed in African countries. In contrast to the significant progress recently achieved in development of an effective Ebola virus vaccine¹, therapeutic options are still limited². The main obstacle represents identification of an appropriate therapeutic target, which has been largely hampered by the time and money-consuming development of new drugs, especially for neglected diseases. Furthermore, the registration of newly identified drugs, like favipiravir, for potential usage in EVD patients takes a relatively long period of time, even though it has been shown to be effective in non-human primate models. In order to avoid these obstacles, several approaches for repurposing of approved drugs for the treatment of EVD patients have been proposed in literature^{3–7}.

Recently, we proposed a relatively simple theoretical criterion for the fast virtual screening of molecular libraries for candidate inhibitors of Ebola virus infection⁸. Using this criterion, which is based on calculation of the average quasi-valence number (AQVN) and the electron-ion interaction potential (EIIP) - parameters determining long-range interaction between biological molecules^{9–13} - we selected 267 approved and 382 experimental drugs as candidates for treatment of EVD. Further detailed analysis of these drugs, including molecular docking, revealed ibuprofen as an inexpensive, widely accessible and minimally toxic candidate for potential prevention and treatment of EVD. The molecular mechanism underlying the possible protective effect of ibuprofen against EVD is suggested.

Material and methods

AQVN and EIIP molecular descriptors

Recently, we proposed a theoretical criterion for selection of candidate inhibitors of Ebola virus infection⁸. This criterion is based on the calculation of EIIP and AQVN which are determined by following equations:

$$Z^* = \frac{1}{N} \sum_{i=1}^m n_i Z_i \quad (1)$$

where:

i - Type of the chemical element

Z_i - Valence of the i -th chemical element

n_i - Number of the i -th chemical element atoms in the compound

m - Number of types of chemical elements in the compound

N - Total number of atoms

$$EIIP = 0.25 \frac{Z^* \sin(1.04 \pi Z^*)}{2\pi} \quad (2)$$

The EIIP values calculated according to the equation (2) are in Rydbergs (Ry = 13.6 eV).

The AQVN and EIIP molecular descriptors determine the long-distance ($>5\text{\AA}$) intermolecular interactions in biological systems¹⁴. This approach showed that molecules which potentially block Ebola virus infection are placed within AQVN range (2.3–2.7) and EIIP range (0.829–0.954 Ry), respectively. Using this theoretical criterion the drug library encompassing 267 approved drugs selected as candidate inhibitors of the Ebola virus infection (Candidate Ebola Drugs Database, CEDD) was established¹⁵. This drug library was used for selection of an approved drug, which represents the optimal candidate for prevention and treatment of EVD.

GP1 receptor modelling

The modelling of glycoprotein GP1 from Ebola virus was previously described in 16.

Ligand optimization

Ligands were built in VEGA ZZ¹⁷, protonated according to physiological conditions and optimized on semi empirical PM6 level of theory using MOPAC 2009.

Molecular docking

Ligand and receptor were prepared in VEGA ZZ¹⁷. The docking was carried out with Autodock Vina (version 1.1.2.)¹⁸. In both cases the whole receptor conformational space was searched, using grid boxes with dimensions $60 \times 60 \times 60$ and $30 \times 30 \times 30 \text{\AA}^3$. The docking was carried out with weighting of hydrophilic interactions, and the corresponding parameter value in the docking configuration was set to -1.20 (compared to default weight: hydrogen = -0.587439). The exhaustiveness was set to 250. The conformations with lowest binding energy values were chosen. The calculations were carried out on the PARADOX Cluster computer.

Results and discussion

We screened the CEDD library¹⁵ for optimal candidates for prevention and treatment of EVD. To achieve this end, we used the following criteria: a) high efficacy of binding of drug to the Ebola virus glycoprotein GP1; b) low toxicity and accessibility (nonprescription drugs); and c) low cost. By mining of CEDD using these criteria, we selected ibuprofen as the best candidate. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). Its mode of action, as for other NSAIDs, is not completely understood, but may be related to prostaglandin synthetase inhibition. It achieves this effect on prostaglandin synthesis by inhibiting cyclooxygenase (COX I and II isotypes), an enzyme that is present in at least one isoform most of the tissues of the body and which is responsible for production of not only prostaglandins but also prostacyclins and thromboxane.

Generally, ibuprofen is minimally toxic drug. A meta-analysis of eight placebo-controlled studies showed that application of non-prescription doses of ibuprofen (800–1200 mg/day) over 10 days caused no significant adverse events¹⁹. Similar results were obtained

in a prospective study of ibuprofen use in healthy volunteers taking the maximum permitted, non-prescription dose of ibuprofen (1200 mg/day) for 10 days²⁰. It was demonstrated that administration of ibuprofen in children is safe. A randomized, community-based study on the safety of ibuprofen in febrile children aged 2 years old (5 or 10 mg/kg) showed that the risk of hospitalization for gastrointestinal bleeding associated with this drug was 17 per 100,000²¹. It was also shown that fecal blood loss associated with application of prescription doses of ibuprofen (2400 mg/day) did not exceed the normal range²². Even in the case of post-surgical usage for pain control in children undergoing tonsillectomy, ibuprofen administration is considered to be safe and does not increase risk of hemorrhages²³. Accordingly, we believe that it would be crucial to better understand the potential risk and benefits of ibuprofen usage in experimental models of EVD. Taken together, these data indicate that ibuprofen could be safely used in nonprescription doses in EVD patients, with potential antiviral effects as well as to alleviate the symptoms.

To assess specificity of ibuprofen as a potential inhibitor of the Ebola virus infection, distribution of all approved NSAIDs in AQVN/EIIP space was analyzed. Results presented in (Table 1 and Figure 1) show that only ibuprofen and its isomer dexibuprofen are located within the domain of the AQVN/EIIP space. Interestingly, most of the experimentally verified inhibitors of the Ebola virus infection are located in the same space (e.g. chloroquine, amodiaquine, brincidofovir, etc)⁸. This suggests that ibuprofen and dexibuprofen are the only drugs from the NSAID group that potentially have anti-Ebolavirus effects, which should be tested both *in vitro* and *in vivo*.

A previous *in silico* study suggested that Elastin Microfibril Interface Located Proteins (EMILINs) are involved in interaction between GP1 and endothelial extracellular matrix (ECM)¹⁶. Docking of ibuprofen to the GP1 model gave conformations which bound to EMILINs binding domain on GP1¹⁶. The binding site of ibuprofen on GP1 spans the edge of this region and consists of Thr 338, Ser 340, Gln 344 and Ala 415¹⁶. The intermolecular interactions between ibuprofen and binding site amino-acids include hydrogen bonds of carboxyl group with Thr 338, Ser 340 and Gln 334. Additional stabilization is provided through aromatic and hydrophobic interaction with Ala 415. This binding site is placed between two loops, which provide the possibility of stabilizing a particular conformation, and therefore possibly blocking receptors. The appropriately high binding energy of -9.0 kcal/mol favors this assumption. The binding conformation is presented in Figure 2. At this stage it can be hypothesized that ibuprofen prevents interaction between Ebola virus and ECM by blocking the interaction between GP1 and EMILIN. There are some literature data that support our current hypothesis. EMILIN-1 is a glycoprotein expressed in the vascular tree that binds to the TGF- β 1 precursor and prevents its processing by cellular protease furin²⁴. It was shown that Emilin-1 knockout mice display increased TGF- β 1 signaling in the walls of their blood vessels, leading to peripheral vasoconstriction and arterial hypertension²⁵. These matrix-dependent changes in the vascular hemodynamics caused by TGF- β 1 and EMILIN-1 are important because they ultimately affect the cardiovascular morbidity and mortality rates. Recently, it was shown that activation of the TGF- β 1 signaling pathway by Ebola virus plays an important role in pathogenesis of EVD²⁶.

These findings suggest the possibility that binding of GP1 to EMILIN-1 prevents its interaction with TGF- β 1, which results in activation of TGF- β 1 signaling pathway. Binding of ibuprofen to GP1 could prevent GP1/EMILIN-1 interaction allowing EMILIN-1 to keep control of TGF- β 1 signaling pathway.

Table 1. AQVN and EIIP molecular descriptors of nonsteroidal anti-inflammatory drugs.

NSAID	Formula	AQVN	EIIP [Ry]
Aspirin	C9H8O4	3.238	0.1179
Diflunisal	C13H8F2O3	3.077	0.0720
Salicylic acid	C7H6O3	3.250	0.1202
Salsalate	C14H10O5	3.310	0.1296
Ibuprofen	C13H18O2	2.485	0.0954
Dexibuprofen	C13H18O2	2.485	0.0954
Naproxen	C14H14O3	2.839	0.0169
Fenoprofen	C15H14O3	2.875	0.0036
Ketoprofen	C16H14O3	2.909	0.0092
Dexketoprofen	C16H14O3	2.909	0.0925
Flurbiprofen	C15H13FO2	2.774	0.0390
Oxaprozin	C18H15NO3	2.973	0.0337
Loxoprofen	C15H18O3	2.667	0.0693
Indomethacin	C19H16ClNO4	2.976	0.0347
Tolmetin	C15H15NO3	2.882	0.0008
Sulindac	C20H17FO3S	2.905	0.0076
Etodolac	C17H21NO3	2.667	0.0693
Ketorolac	C15H13NO3	3.000	0.0439
Diclofenac	C14H11Cl2NO2	2.867	0.0067
Aceclofenac	C16H13Cl2NO4	3.000	0.0439
Nabumetone	C15H16O2	2.667	0.0693
Piroxicam	C15H13N3O4S	3.277	0.1251
Meloxicam	C14H13N3O4S2	3.333	0.1319
Tenoxicam	C13H11N3O4S2	3.454	0.1317
Droxicam	C16H11N3O5S	3.500	0.1260
Lornoxicam	C13H10ClN3O4S2	3.454	0.1317
Isoxicam	C14H13N3O5S	3.333	0.1319
Mefenamic acid	C15H15NO2	2.788	0.0345
Meclofenamic acid	C14H11Cl2NO2	2.867	0.0067
Flufenamic acid	C14H10F3NO2	2.867	0.0067
Tolfenamic acid	C14H12ClNO2	2.867	0.0067
Celecoxib	C17H14F3N3O2S	2.950	0.0249
Rofecoxib	C17H14O4S	3.111	0.0835
Valdecoxib	C16H14N2O3S	3.111	0.0835
Parecoxib	C19H18N2O4S	3.046	0.0608
Lumiracoxib	C15H13ClFNO2	2.788	0.0345
Etoricoxib	C18H15ClN2O2S	2.974	0.0342
Firocoxib	C17H20O5S	2.884	0.0003
Nimesulide	C13H12N2O5S	3.333	0.1319
Clonixin	C13H11ClN2O2	2.966	0.0308
Licofelone	C23H22ClNO2	2.694	0.0626

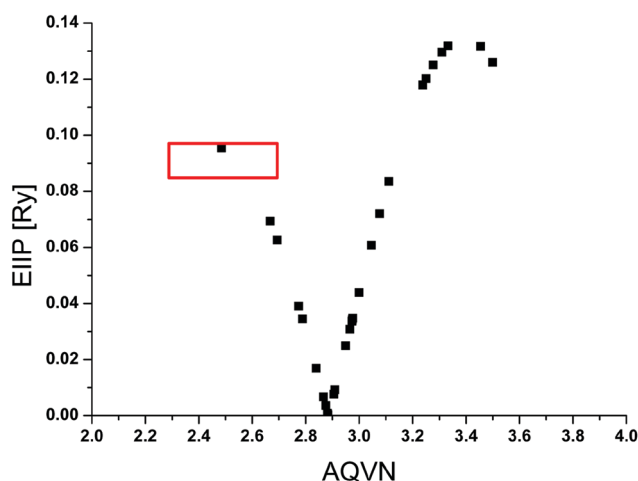


Figure 1. Distribution of nonsteroidal anti-inflammatory drugs in the AQVN/EIIP space. Domain of inhibitors of Ebola virus infection⁸ is marked in red.

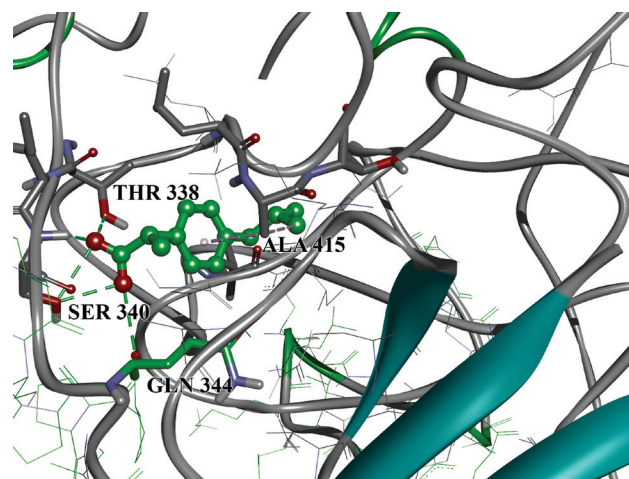


Figure 2. Ibuprofen docked to GP1 with marked amino-acid residues. Green dotted lines: hydrogen bonds; grey: hydrophobic interactions.

In conclusion, presented results should encourage further investigation of ibuprofen and ibuprofen-inspired drugs as inexpensive, low-toxic and wide-accessible candidates for prevention and its usage in the treatment of EVD.

Data availability

F1000Research: Dataset 2. Approved and experimental drugs selected as candidate for treatment of EVD, [10.5256/f1000research.6110.d42877](https://doi.org/10.5256/f1000research.6110.d42877)¹⁵

Author contributions

VV, SP and MG conceived and designed the study. VP developed the analysis tools. VV, SG, NV, MS and DB analyzed the data. VV,

SP and MG wrote the paper. All authors agreed to the final content of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

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I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Agnandji ST, Huttner A, Zinser ME, *et al.*: Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe - Preliminary Report. *N Engl J Med*. 2015. [PubMed Abstract](#) | [Publisher Full Text](#)
2. Olszanecki R, Gawlik G: Pharmacotherapy of Ebola hemorrhagic fever: a brief review of current status and future perspectives. *Folia Med Cracov*. 2014; 54(3): 67–77. [PubMed Abstract](#)
3. Madrid PB, Chopra S, Manger ID, *et al.*: A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. *PLoS One*. 2013; 8(4): e60579. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Kouznetsova J, Sun W, Martínez-Romero C, *et al.*: Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerg Microb Infect*. 2014; 3: e84. [Publisher Full Text](#)
5. Ekins S, Freundlich JS, Coffee M: A common feature pharmacophore for FDA-approved drugs inhibiting the Ebola virus [v2; ref status: indexed, <http://f1000r.es/4wt>]. *F1000Res*. 2014; 3: 277. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Litterman N, Lipinski C, Ekins S: Small molecules with antiviral activity against the Ebola virus [v1; ref status: indexed, <http://f1000r.es/523>]. *F1000Res*. 2015. 4: 38. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Ekins S, Coffee M: FDA approved drugs as potential Ebola treatments [v2; ref status: indexed, <http://f1000r.es/554>]. *F1000Res*. 2015; 4: 48. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Veljkovic V, Loiseau PM, Figadere B, *et al.*: Virtual screen for repurposing approved and experimental drugs for candidate inhibitors of EBOLA virus infection [v1; ref status: indexed, <http://f1000r.es/51s>]. *F1000Res*. 2015; 4: 34. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Veljkovic V, Veljkovic N, Este J, *et al.*: Application of the EIIP/ISM bioinformatics concept in development of new drugs. *Curr Medic Chem*. 2007; 14(4): 441–53. [PubMed Abstract](#) | [Publisher Full Text](#)
10. Veljkovic V, Mouscadet JF, Veljkovic N, *et al.*: Simple criterion for selection of flavonoid compounds with anti-HIV activity. *Bioorg Medic Chem Lett*. 2007; 17(5): 1226–32. [PubMed Abstract](#) | [Publisher Full Text](#)
11. Veljkovic N, Glisic S, Perovic V, *et al.*: The role of long-range intermolecular interactions in discovery of new drugs. *Exp Opin Drug Disc*. 2011; 6(12): 1263–70. [PubMed Abstract](#) | [Publisher Full Text](#)
12. Maga G, Veljkovic N, Crespan E, *et al.*: New *in silico* and conventional *in vitro*

- approaches to advance HIV drug discovery and design. *Exp Opin Drug Discov*. 2013; **8**(1): 83–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Veljkovic N, Glisic S, Prljic J, *et al.*: Simple and general criterion for “in silico” screening of candidate HIV drugs. *Curr Pharm Biotechnol*. 2013; **14**(5): 561–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 14. Veljkovic V: A theoretical approach to preselection of carcinogens and chemical carcinogenesis. Gordon & Breach New York. 1980.
[Reference Source](#)
 15. Veljkovic V, Loiseau PM, Figadère B, *et al.*: Dataset 2 in: Virtual screen for repurposing approved and experimental drugs for candidate inhibitors of EBOLA virus infection. *F1000Res*. 2015.
[Data Source](#)
 16. Veljkovic V, Glisic S, Muller CP, *et al.*: In silico analysis suggests interaction between Ebola virus and the extracellular matrix. *Front Microbiol*. 2015; **6**: 135.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 17. Pedretti A, Villa L, Vistoli G: VEGA—an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. *J Comput Aided Mol Des*. 2004; **18**(3): 167–173.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Trott O, Olson AJ: AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010; **31**(2): 455–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Kellstein DE, Waksman JA, Furey SA, *et al.*: The safety profile of nonprescription ibuprofen in multiple-dose use: a meta-analysis. *J Clin Pharmacol*. 1999; **39**(5): 520–32.
[PubMed Abstract](#)
 20. Doyle G, Furey S, Berlin R, *et al.*: Gastrointestinal safety and tolerance of ibuprofen at maximum over-the-counter dose. *Aliment Pharmacol Ther*. 1999; **13**(7): 897–906.
[PubMed Abstract](#) | [Publisher Full Text](#)
 21. Lesko SM, Mitchell AA: The safety of acetaminophen and ibuprofen among children younger than two years old. *Pediatrics*. 1999; **104**(4): e39.
[PubMed Abstract](#)
 22. Porro GB, Corvi G, Fuccella LM, *et al.*: Gastro-intestinal blood loss during administration of indoprofen, aspirin and ibuprofen. *J Int Med Res*. 1977; **5**(3): 155–60.
[PubMed Abstract](#)
 23. Yaman H, Belada A, Yilmaz S: The effect of ibuprofen on postoperative hemorrhage following tonsillectomy in children. *Eur Arch Otorhinolaryngol*. 2011; **268**(4): 615–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
 24. Raman M, Cobb MH: TGF-beta regulation by Emilin1: new links in the etiology of hypertension. *Cell*. 2006; **124**(5): 893–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Zacchigna L, Vecchione C, Notte A, *et al.*: Emilin1 links TGF-β maturation to blood pressure homeostasis. *Cell*. 2006; **124**(5): 929–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Kindrachuk J, Wahl-Jensen V, Safronetz D, *et al.*: Ebola virus modulates transforming growth factor β signaling and cellular markers of mesenchyme-like transition in hepatocytes. *J Virol*. 2014; **88**(17): 9877–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Ayub Darji

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Current manuscript by Veljkovic *et al.*, deals with the identification of inhibitors against Ebola virus infection. Authors took advantage of existing approved and experimental drugs and, based on *in silico* analysis, proposed ibuprofen as a potential candidate for prevention and treatment of Ebola virus infection. EIIP and AQVN calculation, previously described by authors, was employed to define ibuprofen as the best candidate against Ebola virus infection.

The manuscript is adequately presented and can be considered as acceptable for indexation.

Minor points:

Ebola virus infection is initiated by interaction between the virus glycoprotein GP1 and its cognate receptor(s). Putative receptor binding domain of GP1, key residues involved in GP1 protein folding or structure and a putative receptor binding pocket has been proposed and mapped to the N-terminal 150 amino acids of GP1 (33-185 residues).

Authors should include in the discussion as how interaction of ibuprofen with GP1 (residues, Thr 338, Ser 340, Gln 344 and Ala 415) will influence the receptor binding domain.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 24 Aug 2015

Veljko Veljkovic, University of Belgrade, Mihajla Petrovica 12-14, 11001 Belgrade, Serbia

It was previously suggested that Elastin Microfibril Interface Located Proteins (EMILINs) are involved in interaction between GP and endothelial extracellular matrix (ECM) (Veljkovic *et al* . 2015) The binding site of ibuprofen on GP overlaps with the proposed domain of GP involved in GP/EMILINs interaction. This suggests that ibuprofen could modulate the virus/ECM interaction.

Competing Interests: No competing interests were disclosed.

Reviewer Report 05 June 2015

<https://doi.org/10.5256/f1000research.6904.r8913>

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Mattia Mori

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In the work of Veljkovic *et al.*, *in silico* methods were used with the aim of identifying inhibitors of Ebola virus infection. Particularly, authors focused their investigation on approved and experimental drugs and proposed ibuprofen as a candidate for prevention and treatment of Ebola virus. It is well known that Ebola virus represents an international health emergency and that there is an urgent need for therapeutics and prophylactic drugs.

In the present manuscript, authors applied a previously described and well-established criterion, based on EIIP and AQVN calculation, to select ibuprofen as the best candidate for Ebola virus disease treatment. Moreover, a mechanism of action consisting on GP1 inhibition has been proposed.

It is the opinion of the present referee that some minor issues should be fixed before the manuscript may be considered as acceptable for indexation:

1. Since molecular docking of ibuprofen was performed against the putative target GP1, it would be important to report the predicted binding energy (in kcal/mol) or score, and to compare this value with estimated or experimental affinity of reference GP1 ligands. Moreover, in a repurposing perspective, it would be interesting if authors could comment on the approximated dosage of ibuprofen they expect for providing anti-Ebola activity.
2. Ibuprofen has a serum half-life of 1.8 to 2.0 hours. Did authors consider this aspect when claiming that ibuprofen could be also administered to patients to prevent Ebola virus disease.
3. Liver and gastrointestinal tissues are among the most injured by Ebola virus infection. Moreover, it is well known that ibuprofen can cause serious gastrointestinal toxicity and should be used with caution in person with coagulation defects. Did authors considered

ibuprofen side effects in the context of Ebola infection treatment? Following the question 1., a comment on the expected dosage would help to clarify most of these issues.

4. It is not clear how ibuprofen can alleviate the symptoms of Ebola virus disease, also considering the proposed mechanism of action.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reader Comment 23 Jun 2015

Thomas Hoenen, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

With respect to reviewer comment #3 it is worth pointing out that Medicines Sans Frontieres, the WHO, the CDC, and other health organizations specifically advise AGAINST the use of Ibuprofen and other NSAIDs due to the risk of adverse effects. This is an important point that should, in my opinion, be addressed by the authors.

See also:

<http://www.ammi.ca/media/69846/Ebola%20Clinical%20Care%20Guidelines%202%20Sep%202014.pdf>

<http://www.slamviweb.org/es/ebola/FHFfinal.pdf>

www.cdc.gov/vhf/ebola/ppt/ebola-101-cdc-slides-for-us-healthcare-workers.pptx

The short version:

<http://www.who.int/csr/disease/ebola/photos/ebolablock-poster15.jpg>

Competing Interests: No competing interests were disclosed.

Author Response 24 Aug 2015

Veljko Veljkovic, University of Belgrade, Mihajla Petrovica 12-14, 11001 Belgrade, Serbia

Response to Mattia Mori

1. It was demonstrated that several host proteins interact with GP1. This indicates (i) that more than one receptor/co-receptor is involved in the Ebola virus infection and (ii) that entry inhibitors have different mode of actions because they could bind different targets. All experimentally proven entry inhibitors of Ebola virus are selected *in vitro* and their exact binding site on GP or receptor/co-receptor is not known. For these reasons, there are no "reference GP ligands" whose binding properties could be compared with ibuprofen.

Without initial experimental data any suggestion of dosage of ibuprofen in treatment

of EVD would be highly speculative because the low toxicity of this drug allows its application in a very broad dosing range (100 – 2400 mg per day).

2. Although the plasma half-life of ibuprofen is short (1.9 to 2.2 hours), without experimental data it is not possible to predict antiviral effect of the residual concentration of this drug. Fourteen to twenty-four hours after administration of single dose of 800 mg of ibuprofen, the plasma level of this drug and its metabolites is < 250 ng/mL. For comparison, after administration of the therapeutic dose of 300 mg of Maraviroc, the plasma concentration of this HIV entry inhibitor after 10 hours is <100 ng/mL (Abel *et al.*, 2009).
3. Application of ibuprofen in the early stage of disease should be safe because hemorrhagic effect of EVD appears in the late stage of the illness. This is confirmed by the safe use of ibuprofen in the treatment of Ebola patients in Sierra Leone during the 2014/2015 outbreak without side effects (Ansumana *et al.*, 2015).
4. We proposed that ibuprofen acts as an entry inhibitor preventing spread of virus in the body of Ebola patients (like HIV entry inhibitor Maraviroc which prevents spread of virus in the body of HIV patients).

Response to reader's comment (Thomas Hoenen)

Despite the recommendation of WHO, CDC and some other organizations against ibuprofen in therapy of EVD, this drug was safely used in treatment of EVD patients in Sierra Leone during 2014/2015 Ebola outbreak (Ansumana *et al.* 2015).

Competing Interests: No competing interests were disclosed.

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