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BWP Report on viral safety of plasma-derived and urine-derived medicinal products with respect to Zika virus

Introduction

Zika virus (ZIKV) is an emerging arthropod-borne 50 nm enveloped RNA virus belonging to the *Flaviviridae* family, *Flavivirus* genus. It was first identified in a rhesus monkey in the Zika Forest of Uganda in 1947 and isolated from a human in 1968 in Nigeria[1].

Sporadic cases have been reported since the 1960s in Africa and Asia but in 2007 a major epidemic occurred in Yap Island (Federated States of Micronesia). In 2013 and 2015, additional outbreaks occurred on islands and archipelagos of the Pacific region including a large outbreak in French Polynesia. An outbreak was also reported in Cape Verde. More recently, the virus has caused widespread outbreaks across Central and South America, Mexico, and the Caribbean. The Zika epidemic in the Americas continues to evolve and expand geographically. Autochthonous (locally acquired) infections have been observed in USA since July 2016. It is also spreading recently to Asia (recent autochthonous infections reported in Singapore during the summer of 2016).

The incubation period is thought to range from 3 to 12 days. Most ZIKV infections in humans are asymptomatic (up to 80%). Symptomatic infections are characterised by a self-limiting febrile illness of 2 to 7 days duration accompanied by transient arthritis/arthralgia with possible joint swelling mainly in the smaller joints of the hands and feet, maculo-papular rash often spreading from the face to the body, conjunctival hyperaemia or bilateral non-purulent conjunctivitis, and general non-specific symptoms such as myalgia, asthenia, and headaches.

Neurological manifestations and congenital anomalies have been temporally and spatially associated with ZIKV disease outbreaks. Association of ZIKV infection with Guillain-Barré syndrome (GBS) cases has been reported during outbreaks in Polynesia and in Brazil. In Brazil, there has also been a marked increase in the incidence of microcephaly in regions most affected by the ZIKV epidemic. The potential association of ZIKV with microcephaly has been established[2]. Recently,

[1] Hayes EB. Zika virus outside Africa. Emerg Infect Dis. 2009; 15(9):1347-50.
[2] http://www.cdc.gov/media/releases/2016/s0413-zika-microcephaly.html

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considerable amounts of ZIKV have been detected in the brain from a foetus with microcephaly and severe brain injury[3].

ZIKV is transmitted to humans primarily through the bite of an infected *Aedes* species mosquito (*A. aegypti* and *A. albopictus*). These are the same mosquitoes that spread dengue and chikungunya viruses. In addition, intrauterine, perinatal and sexual transmissions have been reported[4, 5, 6]. Possible transfusion-transmission has been reported in Brazil. In French Polynesia, 3% of samples from asymptomatic blood donors contained detectable ZIKV RNA during the outbreak in French Polynesia in 2013-14, indicating the likelihood of transmission by blood transfusion. Nearly 1% of blood donations from Puerto Rico were found positive for ZIKV RNA[7] and probable transfusion-transmitted Infections[8, 9] have been reported from Brazil.

Zika has a similar epidemiology, clinical presentation and transmission cycle in urban environments as dengue and chikungunya, although it generally causes milder illness.

Discussion

Plasma-Derived Medicinal Products

The virus safety of plasma-derived medicinal products is based on a combination of several safety measures, i.e. donor selection, testing and efficient virus inactivation/removal steps applied at manufacture, see EMA guideline on plasma-derived medicinal products[10]. These safety measures are addressed as follows:

Donor selection

Plasma used for manufacture of plasma-derived medicinal products in Europe is currently sourced in EU-Member states and the USA. There is some risk that asymptomatic donors returning from affected areas might donate viraemic plasma[11,12,13]. Considering the effective virus inactivation/removal steps (see discussion below) used for manufacture of plasma-derived medicinal products, exclusion measures for donors for plasma for fractionation is not considered to be necessary.

Feasibility of testing plasma donors for Zika Virus RNA

The appropriate testing measures for ZIKV RNA would be NAT testing. Antibody tests are not considered suitable for exclusion of viraemic donors.

Several NAT tests are now available (WHO 2016)[14] and some have already obtained a CE marking. Recently, a candidate WHO international standard for Zika virus for nucleic acid amplification

[3] Mlakar J, Korva M Tul N et al. 2016. Zika Virus Associated with Microcephaly. N Engl J Med. 2016 Feb 10. [Epub ahead of print] PubMed PMID: 26862926. DOI: 10.1056/NEJMoa1600651
[4] Foy BD, Kobylinski KC, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011 May; 17(5):880-2.
[5] Musso D, et al. Potential Sexual Transmission of Zika Virus. Emerg Infect Dis. 2015 February; 21: 359-361.
[6] Besnard M, Lastère S, Teissier A, Cao-Lormeau VM, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill. 2014; 19(13):pii=20751.
[7] Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Galel SA, Williamson PC, Busch MP, Alsina JO, Climent-Peris C, Marks PW, Epstein JS, Nakhasi HL, Hobson JP, Leiby DA, Akolkar PN, Petersen LR, Rivera-Garcia B. Screening of Blood Donations for Zika Virus Infection - Puerto Rico, April 3-June 11, 2016. MMWR Morb Mortal Wkly Rep. 2016 Jun 24; 65(24):627-8. doi: 10.15585/mmwr.mm6524e2
[8] Motta IJF, Spencer BR, Cordeiro da Silva SG, et al. Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med. DOI: 10.1056/NEJMc160726
[9] Barjas-Castro, M. L., Angerami, R. N., Cunha, M. S., Suzuki, A., Nogueira, J. S., Rocco, I. M., Maeda, A. Y., Vasami, F. G.S., Katz, G., Boin, I. F.S.F., Stucchi, R. S.B., Resende, M. R., Esposito, D. L.A., de Souza, R. P., da Fonseca, B. A. and Addas-Carvalho, M. (2016), Probable transfusion-transmitted Zika virus in Brazil. Transfusion, 56: 1684–1688. doi:10.1111/trf.13681
[10] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf
[11] Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 2014;19(14)
[12] http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM486360.pdf
[13] http://ecdc.europa.eu/en/publications/Publications/01-08-2016-RRA-eighth-update-Zika%20virus-Americas,%20Caribbean,%20Oceania.pdf
[14] Document " Zika Product Pipeline" available at http://www.who.int/csr/research-and-development/zika/en/

technique (NAT) was made available[15] from Paul-Ehrlich-Institut (PEI). Specific validation for testing plasma pools for fractionation has not yet been performed.

FDA has recommended to test blood donations for transfusion for ZIKV-RNA (ID-NAT) or to implement approved pathogen reduction technologies (FDA, 2016)[16]. However, plasma donor testing for ZIKV is not considered necessary as a safety measure for plasma-derived medicinal products (source plasma) in the USA.

Value of testing plasma donors for Zika Virus RNA

A limited number of viraemic blood donations have been investigated in French Polynesia and the viraemic concentrations from asymptomatic blood donors ranged from less than ca. 4 $\log_{10}$ to more than 6 $\log_{10}$ genome equivalents per ml. Such concentrations would be diluted in plasma pools for fractionation consisting of more than 1000-10,000 donations. Therefore, testing of pools for fractionation would only detect plasma pools with very high virus loads. While exclusion of highly contaminated plasma pools would be considered a valuable safety measure for certain viruses where the inactivation/removal capacity of the manufacturing process is limited, this is not the case for ZIKV where efficient virus inactivation/removal is expected (see discussion below).

In conclusion, testing donors giving plasma for fractionation or pools for fractionation is not considered necessary taking into account the effective virus inactivation/removal steps. This is in line with the current safety strategy for plasma for fractionation with respect to West Nile Virus (WNV), dengue virus (DENV) and chikungunya virus (CHKV) where donor exclusion and testing measures were not implemented[17].

Virus inactivation for plasma derivatives/ Risk assessment for plasma-derived medicinal products

Effective methods with different mechanisms for inactivation/removal of enveloped viruses are required and have been implemented in the manufacture of plasma-derived medicinal products[18]. In general these include manufacturing steps such as SD-treatment (solvent-detergent), liquid heat inactivation (pasteurisation) and virus filtration. These steps have an excellent record for inactivation of the wide range of different species of enveloped viruses tested to date.

Product specific validation of virus inactivation is performed using a panel of model viruses such as HIV, a herpes virus, and a flavi/pestivirus (in most cases Bovine Viral Diarrhoea Virus (BVDV). It could be shown that emerging flaviviruses such as West Nile Virus (WNV) or Chikungunya virus (CHKV) were equally inactivated[19, 20]. Given the experience with inactivation of different flaviviruses and pestiviruses, there is no scientific rationale against the assumption that these steps are effective for inactivation/removal of ZIKV. A recent study from the Paul-Ehrlich-Institut (PEI) verified the high susceptibility of ZIKV to liquid heat treatments (pasteurisation) and solvent-detergent treatment. In addition, infectious virus particles were removed by virus filtration[21].

In conclusion, the virus reduction capacity of the established steps for inactivation/removal of enveloped viruses is considered sufficient for the ZIKV safety of plasma-derived medicinal products.

[15] http://www.pei.de/EN/information/license-applicants/standard-and-referencematerials/who/zika-virus-rna/zika-node.html;jsessionid=54B3B9F8BC2FF3F5772CF1E5F7BA992E.1_cid344
[16] http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM518213.pdf
[17] http://www.ema.europa.eu/docs/en_GB/document_library/Position_statement/2009/09/WC500003789.pdf
[18] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf
[19] Kreil TR, Berting A, Kistner O, Kindermann J. West Nile virus and the safety of plasma derivatives: verification of high safety margins, and the validity of predictions based on model virus data. Transfusion. 2003 Aug; 43(8):1023-8.
[20] Leydold et al. Chikungunya virus and the safety of plasma products. Transfusion 52:2122-30, 2012.
[21] Blümel J, Musso D, Teitz S, Miyabayashi T, Boller K, Schnierle B, Baylis S. Inactivation and removal of Zika virus during manufacture of plasma derived medicinal products. Transfusion, *in press*

Urine-Derived Medicinal Products

ZIKV RNA has been detected in the urine from infected persons, with the possibility that the RNA load may be higher than found in blood and the virus may be detectable in urine for a longer period than in blood[22]. Medicinal products such as urokinase and hormones are produced from large pools of urine. It was noted that some manufacturers (i.e. for hormone preparations) collect their urine starting material from South America (Argentina). There is no regular health check at each donation of urine and given the high number of asymptomatic infections or persons with non-specific symptoms due to ZIKV-infection, the possibility that ZIKV may enter the urine pools cannot be excluded.

There is no published scientific information about the stability of infectious ZIKV in urine. Urine has been known as a route for excretion of viruses for a long time and manufacturers of urine-derived medicinal products have been requested to investigate their manufacturing process for virus inactivation/removal[23]. When drafting the Guideline on the adventitious agent safety of urine-derived medicinal products (EMA/CHMP/BWP/126802/2012), the available data from 2011 provided assurance that there is efficient clearance of viruses, which may contaminate the urine pool, by defined steps in the manufacturing processes. More specifically, for urokinase, dedicated viral clearance steps often consist of pasteurisation and nanofiltration steps. With regard to the urine-derived hormones, virus clearance is attributed to a combination of process steps, which are specific to the individual manufacturing processes, such as alkali treatment, precipitation or chromatographic steps. According to the Guideline on the adventitious agent safety of urine-derived medicinal products, manufacturers have been encouraged to incorporate nanofiltration to further improve clearance of highly resistant, small non-enveloped viruses and several manufacturing processes include such a virus filtration step. There is little doubt that virus filtration (nanofiltration) steps which have been validated for efficient removal of model viruses such as BVDV as well at the usual pasteurisation (60°C for 10h) steps will be effective for inactivation/removal of ZIKV. However, for the ZIKV-inactivation/removal capacity of other steps such as precipitations and chromatography a product-specific assessment is warranted.

Manufacturers have been requested to perform a product specific risk assessment based on the following criteria:

- countries where the urine collection takes place;
- measures taken to exclude urine donations from Zika virus infected donors as far as possible;
- risk evaluation regarding potential contamination of urine pools based on quantitative data as far as feasible (prevalence, assumed virus burden);
- virus inactivation/removal steps in the manufacturing process with particular focus on model viruses chosen and mechanism for reduction of viral infectivity by these steps;
- relevance of the results obtained with the model viruses used in validation studies with regard to inactivation/removal capacity for Zika virus;
- additional measures taken to reduce the risk (such as medical questionnaire and donor education to ensure that donors with symptoms suggestive of Zika virus infection do not donate urine).

[22] Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis. 2015 Jan; 21(1):84-6.
[23] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/05/WC500187391.pdf

Based on a product-specific risk assessment it was concluded that there is no risk with regard to transmission of Zika virus by urine-derived medicinal products as currently authorised in the EU. The manufacturing processes for these products contain complementary steps with inactivation/removal capacity for enveloped viruses, which are considered sufficient for Zika virus safety of urine-derived medicinal products. Manufacturers have stopped sourcing urine from Brazil.

Conclusions

Plasma-Derived Medicinal Products

It is expected that the manufacturing process of plasma-derived medicinal products (including solvent-detergent treated plasma) efficiently inactivates or removes ZIKV. Therefore, no additional safety measures such as exclusion of plasma donors or NAT testing are considered necessary.

Urine-Derived Medicinal Products

ZIKV can be excreted in urine. Based on a product-specific risk assessment it was concluded that the manufacturing processes of urine-derived medicinal products have the potential to inactivate/remove ZIKV. Additional safety measures such as screening of urine donors or urine donations or the deferral of donors returning from affected areas are not considered necessary for these products as currently authorised in the EU. However, product-specific risk assessment should be updated on a regular basis since ZIKV is currently spreading to other areas.