To Zika and destroy: an antimalarial drug protects fetuses from Zika infection

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Zika virus & human pregnancy

Zika virus (ZIKV) is a member of the Flaviviridae family of RNA viruses and is closely related to other flaviviruses such as dengue virus, West Nile virus and yellow fever virus [1]. ZIKV was first isolated from a Rhesus macaque in the Zika forest in Uganda in 1947 [2] but, until recently, it was not considered a significant pathogen because it caused a self-limiting illness characterized by mild fever, headache, arthralgia, conjunctivitis and rash. However, during recent epidemics, ZIKV has been associated with devastating neurological sequelae in adults, such as Guillain-Barré syndrome. Furthermore, ZIKV infection in pregnant women can induce fetal complications, such as microcephaly, intrauterine growth restriction and fetal demise [3]. The rapid spread of ZIKV across the Americas and the virus' serious threat to fetal development propelled WHO to declare the ZIKV epidemic an international public health emergency in early 2016. Given the lack of antiviral therapies that are effective against ZIKV, we urgently need to develop therapies to prevent ZIKV-induced fetal defects in infected pregnant women.

Animal models of ZIKV infection in pregnancy

In preclinical work aimed at developing such therapies, researchers have developed several mouse models of ZIKV infection, many of which require overcoming the natural murine immune resistance to ZIKV [4]. In the model our research team has used pregnant mice lacking an interferon receptor subcutaneously injected with ZIKV. In this model, ZIKV entered the placenta, replicated within fetoplacental trophoblasts and fetal endothelial cells and caused extensive placental damage including reduced placental size and increased numbers of apoptotic trophoblasts and nucleated fetal erythrocytes [5]. Moreover, ZIKV was able to cross the placenta, which normally serves as a formidable barrier to pathogens, to replicate within the brain and impair fetal brain development [5–7].

Autophagy & the placental barrier

In seeking a therapeutic that could prevent ZIKV placental infection, we drew on previous studies showing that autophagy, a cellular pathway that targets intracellular components for lysosomal degradation, is a critical component of the placental barrier against pathogens [8]. Additionally, ZIKV infection induces autophagic flux in human skin cells, suggesting that autophagy plays a role in ZIKV pathogenesis [9]. We first confirmed that ZIKV also induces autophagy in human trophoblasts *in vitro* and in mouse placentas *in vivo* [10]. Next, we showed that upon ZIKV infection, pregnant mice deficient for a key autophagy gene, *Atg16L1* (*Atg16L1^{HM}*), had significantly lower ZIKV titers in both the placentas and fetal brains than the wild-type mice. Moreover, pups from ZIKV-infected

Atg16l1^{HM} mice were larger than those from ZIKV-infected wild-type mice, and placentas from *Atg16l1^{HM}* mice were significantly less damaged by ZIKV infection than placentas from wild-type mice. However, *Atg16l1^{HM}* mice had similar maternal serum and brain ZIKV titers as wild-type mice. Further experiments confirmed that deletion of *Atg16l1* exclusively in trophoblasts was sufficient to limit ZIKV maternal–fetal transmission. However, deletion of *Atg16l1* exclusively in trophoblasts did not reduce ZIKV titers in fetal heads, supporting a previous report that autophagy plays a role in ZIKV infection of the fetal neurons [11]. Together, these findings suggest that autophagy promotes ZIKV infection in the placenta.

A new use for an old drug: hydroxychloroquine limits ZIKV infections in pregnancy by inhibiting autophagy

Given that loss of a key autophagy gene reduced placental ZIKV transmission, we tested the autophagy inhibitors bafilomycin A1, 3-methyladenine and chloroquine and found that all three were able to reduce ZIKV titers in human trophoblasts *in vitro* [10]. Hydroxychloroquine (HCQ), the hydroxyl derivative of chloroquine, was an attractive candidate drug for *in vivo* tests in mice for several reasons. First, like chloroquine, HCQ inhibits autophagy by blocking autophagosome–lysosome fusion and lysosomal degradation [12]. Second, HCQ is an antimalarial drug that has also been used for years to treat chronic autoimmune disorders, particularly systemic lupus erythematosus, in pregnant women [13]. Finally, HCQ has antiviral effects against flaviviruses, HIV and SARSV [14,15].

We administered HCQ to ZIKV-infected mice and found that it greatly reduced ZIKV titers in placentas and fetal heads. Moreover, ZIKV-induced placental damage was prevented by HCQ treatment, and pups from HCQ-treated ZIKV-infected mice were larger than those from untreated ZIKV-infected mice [10]. Finally, our data suggested that the anti-ZIKV effects of HCQ depend on HCQ's ability to inhibit autophagy, as placental and fetal viral titers were equivalent between *Atg16l1^{HM}* mice treated with and without HCQ [10].

Our work provides a new perspective in understanding the mechanism of HCQ's anti-ZIKV effects during pregnancy. Recent studies have shown that ZIKV nonstructural proteins block the Akt–mTOR pathway in human fetal neural stem cells, which may contribute to autophagy activation as mTOR negatively regulates autophagy [11]. Future work untangling the ZIKV–autophagy interaction will help elucidate the molecular mechanism underlying why autophagy inhibition blocks ZIKV infection and maternal–fetal transmission. Although the mechanisms by which loss or inhibition of autophagy reduces ZIKV infection are currently unknown, our human *in vitro* and mouse *in vivo* data indicate that HCQ is a promising therapeutic candidate to prevent adverse pregnancy outcomes in ZIKV-infected women.

Taking HCQ treatment from the bench to the bedside to treat pregnant ZIKV-infected women

To be used clinically to combat ZIKV infections in pregnancy, HCQ must be demonstrated to be safe for use during pregnancy. Several studies suggest that it is. For example, in a retrospective study of pregnant patients with chronic rheumatologic conditions, 133 women taking HCQ were compared with a control group of 53 pregnant women not taking HCQ. No significant differences in outcomes, such as live birth rate and infant vision, hearing, growth or development, were seen between the two groups [16]. In a prospective follow-up study of women taking HCQ during pregnancy to treat rheumatologic disease, no significant congenital malformations or vision or neurodevelopmental defects were observed [17]. Finally, a systematic review and meta-analysis examined the effects of HCQ on pregnant women with autoimmune diseases and concluded that HCQ did not appear to increase the risk of adverse pregnancy outcomes [13]. Given these studies, HCQ continues to be recommended by the American Congress of Obstetricians and Gynecologists for treatment of lupus and other rheumatological disorders throughout pregnancy. Given that HCQ is relatively inexpensive and readily available globally, including in low- and middle-income countries, HCQ may prove to be an ideal therapeutic to treat pregnant women who test positive for ZIKV.

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