Neutralizing antibody activity of human intravenous immunoglobulin (IVIG) against enteroviruses causing hand, foot and mouth disease

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Intravenous immunoglobulin (IVIG) is the blood product containing IgG antibodies extracted from the plasma of over one thousand healthy donors. It is mainly used for prophylaxis and treatment of several infectious and non-infectious diseases. In Thailand, IVIG is routinely prescribed as a therapeutic agent for pediatric patients who developed neurological signs and symptom from enteroviruses associated hand, foot and mouth disease.

Objective

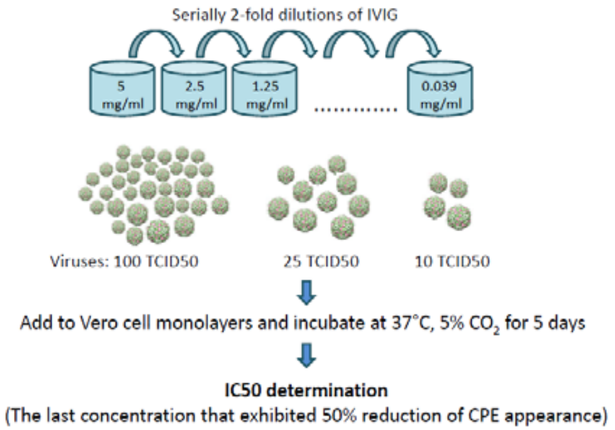
To conduct cytopathic effect (CPE) based microneutralization (microNT) and plaque reduction neutralization (PRNT) assays for determining the neutralizing antibody activity of IVIG against enterovirus 71 (EV71) and Coxsackievirus A16 (CA16) strains in Thailand

Materials

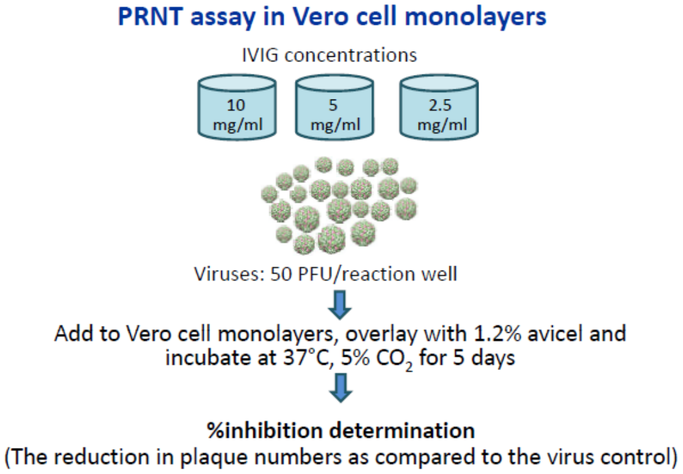
- **IVIG**: 4 lots of IVIG produced from 2 manufacturers in Asian countries
- **Viruses**: 4 strains of EV71 genotypes B5 and C4 (B5/2011, B5/2013, C4a/2014, and C4b/2006) and 2 strains of CA16 genotype B (B/2011 and B/2012)

Methods

CPE-based microNT assay in Vero cell monolayers



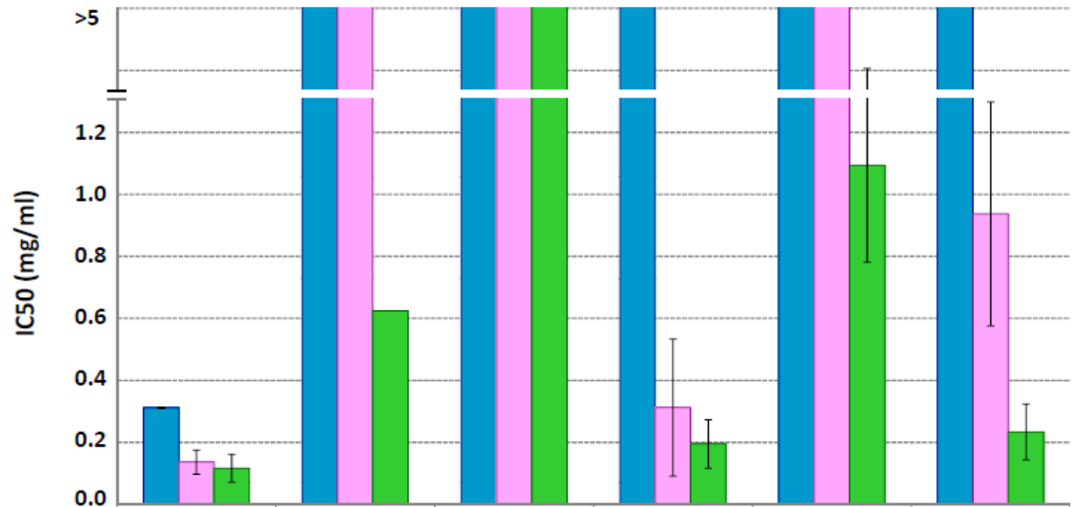
PRNT assay in Vero cell monolayers



Results

- All 4 lots of IVIG yielded similar 50% inhibitory concentration (IC50) against all virus strains assayed. The variation in IC50 concentration fell within 2 folded differences at all check points (Figure 1).
- At the virus concentration of 100 TCID50, only B5/2011 virus was inhibited with IC50 of 0.312 mg/ml; while the other 3 EV71 and 2 CA16 strains was not.
- At the virus concentration of 10 TCID50, 3 EV71 strains (B5/2011, B5/2013, and C4b/2006) yielded the IC50 values varying from 0.078 to 0.625 mg/ml, while C4a/2014 was the only strain not inhibited with all IVIG concentrations assayed. Both CA16 strains yielded the IC50 values varying from 0.156 to 1.25 mg/ml.

PRNT was conducted using 3 EV strains (EV71-B5/2011, EV71-C4a/2014 and CA16/2012) against 3 IVIG concentrations. The result showed that IVIG at concentration of 10 mg/ml was toxic to Vero cells, and thus, the result could not be obtained. At concentration of 2.5 and 5 mg/ml, IVIG poorly inhibited EV71-C4a/2014 (<50% inhibition); while they effectively inhibited both CA16/2012 and EV71-B5/2011 (Table 1 and Figure 2). The result of PRNT was correlated to those obtained by TCID50 based assay.



	EV71 B5/2011	EV71 B5/2013	EV71 C4a/2014	EV71 C4b/2006	CA16/2011	CA16/2012
100 TCID50	0.312	>5	>5	>5	>5	>5
25 TCID50	0.1365	>5	>5	0.31225	>5	0.9375
10 TCID50	0.117	0.625	>5	0.195	1.09375	0.234

Figure 1. The average of IC50 values from 4 lots of IVIG against EV71 and CA16 isolates by TCID50-based microNT assay

Table 1. %inhibition of IVIG against EV71 and CA16 viruses by PRNT assay

Test viruses	%inhibition using PRNT assay		
	2.5 mg/ml	5 mg/ml	10 mg/ml
EV71 B5/2011	88%	97%	cell toxic
EV71 C4a/2014	32%	31%	cell toxic
CA16/2012	100%	100%	cell toxic

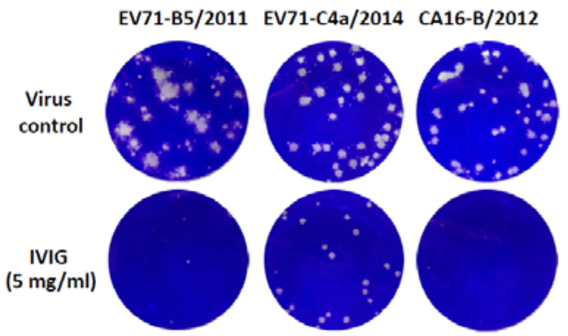


Figure 2. PRNT assay

Conclusion

We showed that IVIG prepared from blood samples of Asian people contained neutralizing antibodies activity against various EV71 and CA16 isolates. Nevertheless, percentages of neutralization varied according to virus strain such that inhibitory activity of IVIG against EV71-C4a/2014 was very poor. This suggests the necessity to regularly explore for cross reactivity between NT antibody in IVIG and the circulating EV strains.

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