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Original Article

Methisoprinol for children with early phase dengue infection: a pilot study

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Abstract

Background Dengue fever is associated with many health complications and medical costs. Furthermore, there is currently no approved dengue antiviral medication or vaccine. Empiric evidence has suggested that patients who received supplemental methisoprinol therapy had faster recovery times and fewer complications.

Objective To determine the effects of oral methisoprinol on the clinical course and laboratory findings of children with early phase dengue infection.

Methods We conducted a randomized, double-blind study from June to September 2012 on 22 children aged 2.7-16.8 years with laboratory-confirmed early dengue infection. Subjects had not previously received antithrombotic agents, nor did they have bleeding disorders or immunodeficiency. We randomized the subjects to receive either oral methisoprinol (100 mg/kg BW/day, divided into four doses) or placebo for 72 hours, with 11 subjects per group. The primary endpoint was fever clearance time (FCT), and secondary endpoints were platelet nadir, white blood cell (WBC) nadir, maximum hemoconcentration, length of hospital stay, and development of complications.

Results The mean decrease in WBC count was less with methisoprinol than with placebo [1.14 (SD 0.84) vs 2.60 (SD 3.12) x 10^9/L; P=0.004]. In addition, the mean decrease in platelet count was less in patients on methisoprinol [38.36 (SD 58.3) vs. 50.46 (SD 73.42) x 10^9/L; P=0.046]. No significant differences between the two groups were found for FCT (P=0.158), length of hospital stay (P=0.511), hemoconcentration, or dengue complications.

Conclusion Methisoprinol initiated at an early phase in dengue infection reduced the anticipated leukopenia by 56% and thrombocytopenia by 24%. Hence it can be used along with standard approved fluid and antipyretic therapy.

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Keywords: *Dengue, leukopenia, methisoprinol, thrombocytopenia*

Dengue fever remains a serious public health concern in tropical regions as it continues to claim many lives year after year. The Philippines is known to be endemic for the four antigenic subtypes of dengue viruses, with no locality or social stratum spared from it. Dengue mortality in the country numbered 872 out of the 178,644 reported cases (case fatality rate/CFR 0.5%).[1] The majority (40%) belong to the 1-10 years age group.[2] The World Health Organization (WHO) estimates there to be 50–100 million dengue infections worldwide every year and over 2.5 billion people, 40% of the world's population, are now at risk from dengue.[3] Presently though, there is still no approved antiviral treatment for dengue. While there are protocols for effective standard supportive treatment with fluids and transfusions, the 'race for the cure' is still in progress, including supplemental therapeutic modalities used by several health practitioners and even physicians who claim their effectiveness based on practice-based evidence. These include homeopathic remedies and trial-stage antiviral products, among others. Off-label use of methisoprinol

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for supplementary dengue treatment in the Philippines has only been testimonial in nature.[4]

Methisoprinol is a synthetic complex of inosine and dimethylaminoisopropanol (as p-acetamidobenzoate).[5] It belongs to the class of other antivirals, used as a direct-acting antiviral for the systemic treatment of viral infections and as an immunostimulant/immunomodulator.[6] Methisoprinol has already been proven effective in human and animal models either singly or in combination for many viral diseases.[7-11] It is marketed in the Philippines for the following indications: subacute sclerosing panencephalitis (SSPE), varicella, mumps, rhinovirus, influenza A, measles, encephalitis, herpes zoster, herpes simplex labialis, hepatitis A, acute viral B hepatitis, herpes genitalis, as well as immunorestoration in conditions such as pre-acquired immune deficiency syndrome (AIDS)/persistent generalized lymphadenopathy and AIDS-related complex, neoplastic disease, anergy and hypoergy prior to major surgery, and severe burns.[5]

Methisoprinol was first postulated as an adjuvant for dengue fever in Thailand,[12] but no other studies have confirmed nor refuted its claims. We aimed to determine the effects of oral methisoprinol treatment on the clinical course of early phase dengue infection in pediatric patients and specifically, to establish the effect of methisoprinol on dengue patients' fever clearance time (FCT), threshold of leukopenia (nadir white blood cell/WBC counts), threshold of thrombocytopenia (nadir platelet counts), maximum hemoconcentration, duration of confinement, and prevention of dengue complications.

Methods

We performed a randomized (allocation ratio 1:1), double-blind, pilot group study of 22 children hospitalized in Saint Louis Hospital, University of the Sacred Heart (SLU-HSH), Baguio City, Philippines for early dengue fever. Patients were eligible if they were aged 2 to 18 years 11 months, with clinical symptoms suggestive of dengue fever, as defined by the WHO Dengue Guidelines 2009,[13] and laboratory confirmation of very early or early acute infection, via *Dengue Duo Pack®* (*SD Bioline: Standard Diagnostics Inc, Korea*) at the time of admission. Patients must be NS1 positive and in the febrile phase to qualify for the study. The additional serology results (immunoglobulin (Ig) G and IgM) were used to distinguish primary from secondary infection. Patients were excluded if they were NS1-negative, assessed to have severe dengue infection, critical or in the recovery phase of dengue fever, previously diagnosed with bleeding diathesis, platelet disorder or immunodeficiency disorder, previously ingested aspirin, ibuprofen or other antithrombotic medication within the previous 48 hours, pregnant or lactating, unable to swallow oral medications (non per orem order), malnourished (<2 SD weight-for-height or BMI for age), or if consent was unobtainable. Permission from patients' attending physicians was also secured verbally or in writing. Patients and their parents/guardians were informed of the nature of the planned intervention, that placebo would be used and that improvement would be monitored through several laboratory tests. The medical risks associated with taking the medication were explained. A consent form was filled in duplicate by the patient and/or parents/guardians. The consent form was available in English, Filipino, and *Iluko*. It was explained to them in the vernacular on the occasion that a patient could not understand these. The consent forms and research proposal were submitted and approved by the Bioethics Committee of the Saint Louis University Hospital of the Sacred Heart.

Patients were randomly assigned to either the methisoprinol group (syrup provided at a therapeutic dose of 100mg/kg/day), or the placebo group. Both the actual medication and placebo preparations had identical presentation without distinctive marks except for a letter coding (the identity of the products was only revealed after data collection was complete). Assignment of treatment was done randomly with *Random Allocation Software® ver 1.0*, in blocks of 10 patients. The intervention was started immediately after laboratory confirmation of early dengue. Twelve doses (72 hours of medication) were completed, which also corresponded to the maximal period of viremia. Research assistants were responsible to administer, or supervise the administration of the correct intervention. A checklist for scheduling of dosages was also placed at the bedside for the patient/guardian to fill. Clinical care, including other treatment such as intravenous fluids were at the discretion of the attending physician, following WHO/Department

of Health (DOH)/ Philippine Pediatric Society (PPS) Dengue Guidelines.[14] Study subjects were not allowed to take any other adjuvants (probiotics, vitamin supplements, or herbal remedies) during the observation.

Patient profiles, diagnoses, classification, date and time of admission and discharge, vital signs and laboratory results were recorded in case record forms. Clinical history and significant examination findings were also recorded. Daily vital signs including temperature, obtained by aural digital thermometer (after ear canal patency was ensured) and laboratory results were logged on a daily basis. Laboratory determinations for serial hemoglobin, hematocrit, and platelet counts were done as clinically indicated, according to the attending physician's discretion. Inpatient laboratory determinations were done in the SLU-HSH Laboratory. Extent of hemoconcentration and thrombocytopenia variation was compared to normal values for age and sex. Fever clearance time, and duration of confinement, progression to dengue shock syndrome (DSS), need for intensive care unit (ICU) care, need for blood transfusion, and mortality were noted accordingly.

The primary endpoint of the study was FCT, defined as the time from fever onset to the start of the first 48-hour period during which the temperature remained below 37.8^{o}C. Secondary endpoints were: 1) leukopenia threshold, defined as the lowest (nadir) WBC count, 2) thrombocytopenia threshold, defined as the lowest (nadir) platelet count, 3) maximum hemoconcentration calculated as [(maximum hematocrit recorded during inpatient period) – (mean normal hematocrit of age- and sex-matched population value)]/(mean normal hematocrit of age- and sex-matched population value) x 100], 4) duration of confinement, and 5) number of patients who developed DSS, were admitted to the ICU, transfused with blood products, and other complications such as bradyarrythmia or carditis, effusions, hepatitis or encephalitis.

Based on the existing hospital census for 2011, there were 46 patients with dengue infection admitted between the June to August period, the months which are considered to be high-risk by the Department of Health. Ninety-six percent of cases treated were classified as uncomplicated dengue infection (all cases of dengue except DSS, stages 3 and 4). Computing for 90% confidence interval at 5% margin of error, the estimated candidate population was 41 patients. Supposing that half of these were in the early phase of dengue infection, then the ideal sample would be 20 patients.

Data remained blinded until the database was finalized and ready for analysis. Statistical analysis was performed with *SPSS Version 2.0*. A two-sided P value $\leq$0.05 was considered to be significant for all parameters. The null hypothesis was that isoprinosine had no effect on fever clearance, maximum hemoconcentration, leukopenia, thrombocytopenia, confinement duration, and development of dengue complications. Fisher's exact test (Chi-square when applicable) and T-test were used to compare values between groups. There were some patients who had existing comorbidities while they were treated in the hospital, such as pneumonia, typhoid, and acute tonsillopharyngitis. Likewise, pre-admission anemia, baseline laboratory determination variability, and obesity may have also been present. These covariates thought to influence the time to resolution of fever, recovery, and laboratory results, were accounted for in the final model.

Results

During the study period, there were a total of 131 children with dengue infection admitted to SLU Hospital. Having met the clinical inclusion criteria and consenting to participate, 42 patients were identified as potential subjects, of which 15 had negative NS1 and were subsequently excluded. Three patients refused admission, one patient withdrew consent, one patient was rejected due to incomplete monitoring, and two patients did not meet the age criteria. Hence, 22 children were randomly assigned to either the methisoprinol group or the placebo group (11 subjects per group) (**Figure 1**).

Almost all subjects had primary infections (IgG negative). Only one subject had a secondary infection and belonged to the placebo group. Prior to admission, the most common presenting symptoms of the subjects were myalgia, body aches and pains (15/22), anorexia/ nausea (11/22), and abdominal pain (9 /22). While there were 2 obese patients in the placebo group, there was no observed significant difference to the methisoprinol group with normal BMIs. Fourteen of

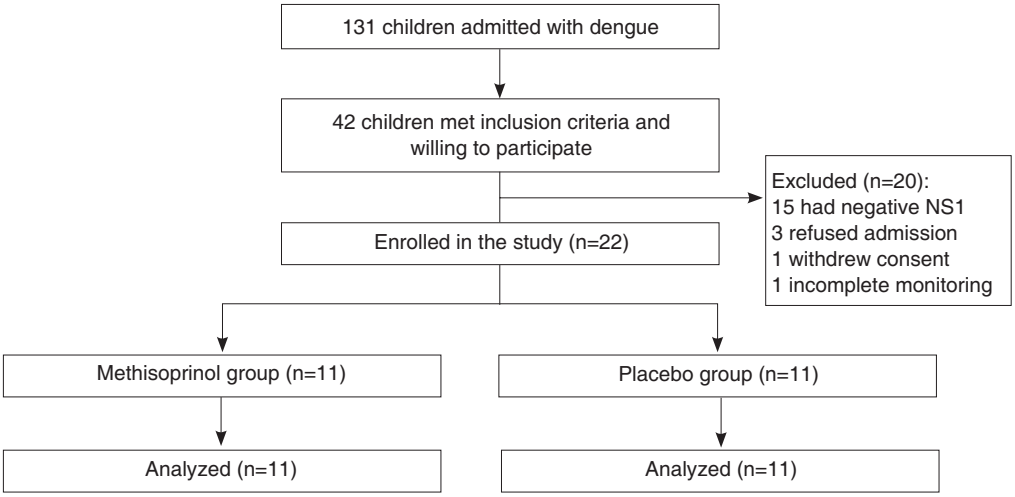

Figure 1. Study flow chart

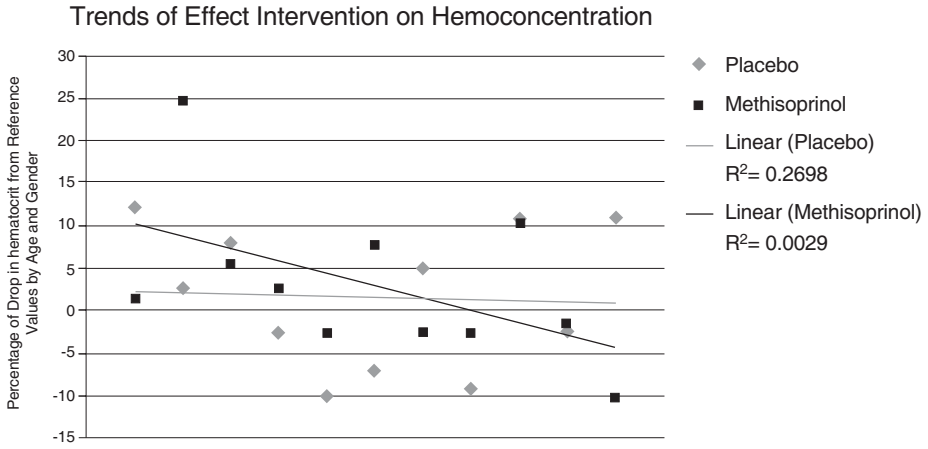

Figure 2. Scatterplot and linear regression of the effects of intervention on hemoconcentration in %. Each plot in the graph shows the percentage change in hematocrit level from reference values for age and gender of each of the patients with serial hematocrit determinations during confinement with methisoprinol (■) and placebo (◆) treatment.

22 subjects manifested two or fewer key complaints, suggesting that reliance on clinical symptoms coupled with timely laboratory determination are important to catch patients at the early stage of the disease. Three patients had pre-existing or concurrently treated comorbidities. Two patients had acute upper respiratory tract infections and one patient had pediatric community-acquired pneumonia (class B – minimal risk). These conditions did not significantly alter the results.

We also found that 18 subjects were leukopenic at admission. None of the subjects showed the classic ≥20% increase in hematocrit concentration (hemoconcentration) based on reference values for age and gender, nor at admission baseline levels. Baseline platelet status was normal in the majority (19/22). Baseline characteristics of the two groups are summarized in **Table 1**.

We found that 15 subjects were febrile at the time of admission, 5 subjects were afebrile at admission but

Table 1. Baseline characteristics of subjects

Characteristics	Methisoprinol group (n=11)	Placebo group (n=11)
Mean age, years (SD)	10.72 (4.71)	10.3 (4.52)
Gender, n		
Male	4	3
Female	7	8
BMI, n		
Normal	11	9
Overweight	0	2
Serologic subclassification, n		
Early to late acute (NS1+,IgM+)	2	4
Very early acute (NS1+, IgM-)	9	7
Clinical classification		
Dengue without warning signs	7	6
Dengue with warning signs	4	5
Mean onset of fever prior to admission, hours (SD)	68.82 (27.21)	58.55 (38.13)
Key symptoms prior to admission		
Anorexia/nausea	4	7
Rash	4	3
Myalgia, body aches and pains	6	9
Abdominal pain	4	5
Tourniquet test positive	4	1
Fluid accumulation	0	2
Lethargy	1	1
Mucosal bleeding	1	1
Number of key symptoms		
2 or fewer	8	6
More than 2	3	5
Mean WBC count on admission, x 10^9/L (SD)	3.68 (1.25)	4.59 (3.45)
Normal for age	2	2
Low for age	9	9
Mean platelet count on admission, x 10^9/L (SD)	216.27 (30.19)	190.91 (91.52)
Normal	11	8
Low	0	3
Mean hematocrit at admission, % (SD)	40.76 (3.46)	39.52 (3.20)

*WBC: white blood cell

developed fever afterwards during confinement, and 2 subjects did not develop more fever at all during confinement after a history of reported fever at home shortly before admission. Fevers seemed to clear faster in the placebo group than in the methisoprinol group, but this result was not statistically significant.

The mean lowest (nadir) WBC counts obtained during confinement while medicating was recorded and compared to baseline values at the time of admission. The methisoprinol group had significantly lesser magnitude of WBC drop compared to the placebo group. Hence, methisoprinol reduced the anticipated leukopenia by 1.5 x 10^9/L or 56%.

The mean lowest (nadir) platelet count obtained during confinement was also recorded and compared to baseline values at admission. A smaller drop in platelet count was observed in the methisoprinol group than in the placebo group. Hence, methisoprinol reduced the anticipated thrombocytopenia by 12 x 10^9/L or 24%.

Patients in the placebo group were hospitalized longer than the methisoprinol group, but this difference was not statistically significant.

The mean highest hematocrit level recorded during confinement (excluding admission) in the methisoprinol group was higher than that of the placebo group. With regards to mean maximum hemoconcentration based on age-matched reference values, and baseline admission hematocrit level, they were not statistically different (P=0.724 and P=0.913). Similarly, the "discharge hematocrit," or the last hematocrit taken after the patient was considered to be stable was not different between the two groups (P=0.182). Scatterplot and trending analysis in **Figure 2** supports this lack of hematocrit difference between groups. Since R^2 values in both arms of treatment were <1, there was very little consistent, predictable and significant relationship between medicating with either methisoprinol and hemoconcentration.

All admitted patients were on IV fluid therapy and their treatment was according to protocols for dengue infection management. In addition, we also compared complications in the two groups. The outcomes are shown in **Table 2**.

Table 2. Treatment outcomes between the methisoprinol and placebo groups

Parameters	Methisoprinol group (n=11)	Placebo group (n=11)	P value
Mean fever clearance time (SD), hours	109.07 (22.87)	89.47 (34.00)	0.158
Mean lowest WBC count during study (SD), x 10^9/L	2.33 (0.58)	2.37 (0.93)	0.906
Mean decrease in WBC count (SD), x 10^9/L	1.14 (0.84)	2.60 (3.12)	0.004
Mean lowest platelet count during study (SD), x 10^9/L	177.91 (21.20)	140.45 (18.21)	0.195
Mean decrease in platelet count (SD), x 10^9/L	38.36 (58.3)	50.46 (73.42)	0.046
Mean length of stay (SD), hours	84.33 (23.86)	90.68 (20.53)	0.511
Mean highest hematocrit level during study (SD), %	41.88 (4.92)	40.34 (3.21)	0.393
Mortality, n	-	-	
Complications, n			
Required fresh frozen plasma	2	-	*
Experienced bradycardia/carditis	1	3	0.586
Pleural effusion (radiologic confirmed)	-	1	*
Transferred to ICU	-	-	
Encephalitis	-	-	
Hepatitis	-	-	
Epistaxis	2	1	0.476
Allergic reaction	-	-	

*P value could not be computed because of a zero value in one of the groups.

Discussion

Leukopenia usually precedes the onset of the critical phase of dengue and has been associated with severe disease.[13] In our study, the WBC decrease in the methisoprinol group was 56% less than the anticipated WBC count decrease in the placebo group. Hence, if the theorized effect of methisoprinol were true, its immunostimulant role may have produced an increase in the overall WBC count, and effectively arrested or lessened the leukopenia and severity of dengue infection. Methisoprinol's immunomodulatory effect can probably be explained based on its ability to normalize deficient or dysfunctional cell-mediated immunity, by evoking a Th1-type response which in turn, initiates T-lymphocyte maturation and differentiation as well as potentiation of induced lymphoproliferative responses in mitogen- or antigen-activated cells. Similarly, the drug has been shown to modulate T-lymphocyte and natural killer cell cytotoxicity, T8 suppressor and T4 helper cell functions, and also to increase the number of IgG and complement surface markers. It increases cytokine IL-1 production and enhances IL-2 production, upregulating the expression of the IL-2 receptor in vitro.[14] It significantly increases endogenous IFN-gamma secretion and decreases IL-4 production in vivo.[15] It has been shown to potentiate neutrophil, monocyte and macrophage chemotaxis and phagocytosis. As an antiviral, in vivo, methisoprinol enhances potentiation of depressed lymphocytic mRNA protein synthesis and translational ability, while inhibiting viral ribonucleic acid (RNA) synthesis achieved by yet to be clarified degrees of incorporation of inosine-mediated orotic acid into polyribosomes. It also inhibits of polyadenylic acid attachment to viral messenger RNA and engages in molecular reorganization of lymphocyte intramembrane plasma particles that result in a nearly threefold increase in density.[16-22] The effect of the test drug on platelet counts, on the other hand, was unexpected and the exact mechanism of methisoprinol action on the platelets cannot be explained by the existing literature.

The paucity of severe dengue complications is consistent with the anticipated absence of antibody-dependent enhancement in primary infections.[23] Since the population under study had mostly primary dengue infections, this scenario was anticipated. It is

important to note, however, that our study is limited to the effects of methisoprinol in early dengue infection alone, and the full spectrum of its effect on later stages and more severe dengue cases was not assessed.

Methisoprinol was hypothesized to decrease fever duration, but this was not observed in our study. There were also no significant decreases in the recovery period or admission duration in our results. This may be explained by the fact that the number of hours of confinement is only, at best, an approximate reflection of the time of improvement or recovery of a dengue patient. Patients are not necessarily discharged at the time-point of clinical recovery. Patients may also be discharged earlier than others, based on the anticipated level of parental care and supervision at home or the amenability to follow-up. In our study, patients were not monitored after confinement, and no attempt was made to determine if or when laboratory parameters resumed to normal.

It is recommended that further studies be made in a hospital setting with more patient admissions, i.e., public hospitals in known dengue hotspots in the country and other Asian locales. We should allow for recruitment of outpatient subjects, but with assigned, dedicated research assistants to help monitor home-bound patients. Trials using different strengths (dosaging), frequency and durations for the test medication are also recommended, as well as using in vitro studies to investigate the antiviral/immunomodulatory and antithrombocytopenic effects of the medication on the dengue virus in a local setting.

In conclusion, oral methisoprinol at 100 mg/kg/day, divided into four doses, for 72 hours, do not reduce fever clearance time or length of hospital stay, nor do it diminish or prevent hemoconcentration or the development of complications in children with uncomplicated acute primary dengue infection. However, methisoprinol is observed to reduce leukopenia by 1.5 x 10^9/L (56%) and thrombocytopenia by 12 x 10^9/L (24%) compared to controls. Hence, methisoprinol could be used along with the standard, approved fluid and antipyretic therapy for dengue treatment.

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