Jpn. J. Infect. Dis., 66, 26-31, 2013

Original Article

Clinical Efficacy of Antivenom and Cepharanthine for the Treatment of Mamushi (*Gloydius blomhoffii*) Bites in Tertiary Care Centers in Japan

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(Received July 31, 2012. Accepted November 2, 2012)

SUMMARY: To our knowledge, no one has conducted a multi-center trial evaluating the efficacy of antivenom and cepharanthine (CEP) for the treatment of mamushi (*Gloydius blomhoffii*) bites. Thus, we conducted a large-scale survey among tertiary care centers in Japan from November 2009 to October 2010 to evaluate the efficacy of antivenom and CEP for the treatment of mamushi bites. We divided the therapeutic interventions received by patients into 4 groups: CEP, antivenom, both CEP and antivenom, and neither CEP nor antivenom. We collected data on age, sex, comorbidities, laboratory measurements, length of hospital stay, and grades of mamushi bites (indication of bite severity ranged from I [mild] to V [severe]). We sent questionnaires to 219 tertiary care centers, of which 114 (52.1%) returned completed questionnaires. Two hundred and thirty-four cases of mamushi bites were reported. Among the severe cases (grades of mamushi bites III, IV, and V), patients administered antivenom had a significantly shorter length of hospital stay than those administered CEP ($P = 0.024$). In contrast, there was no significant difference in the length of hospital stay between mild cases (grades of mamushi bites I and II) ($P = 0.77$). Our results show that antivenom is effective in reducing the length of hospital stay in patients with severe mamushi bites.

INTRODUCTION

The Japanese mamushi, *Gloydius blomhoffii*, is a species of pit viper found throughout Japan, except on the southeast islands. Though the annual number of mamushi bites remains uncertain, some reports estimate 1,000 cases resulting in 10 deaths annually (1). In our previous survey, approximately 400 cases of mamushi bites were treated annually among all tertiary care centers in Japan (2).

The venom of the mamushi snake can cause life-threatening symptoms such as cardiac, pulmonary, and/or renal dysfunction (3–6). Antivenom products neutralize both the hemorrhagic and lethal activities of the venom. An essential and efficient therapy developed for severe cases of mamushi bites involves the rapid intravenous administration of antivenom.

However, antivenom can occasionally produce serious adverse reactions such as primary anaphylaxis and serum sickness. Several authorities have proposed conservative treatments that do not involve the use of antivenom (2). Until 1990, antivenom was administered subcutaneously or intramuscularly in order to avoid adverse reactions. Due to its slow absorption in the human body, clinical doctors believed that antivenom was ineffective for treating mamushi bites (7). Intravenous administration of antivenom began in 1990, leading to the reevaluation of the efficacy of antivenom.

Cepharanthine (CEP), a biscoclaurine (bisbenzylisoquinoline) amphipathic alkaloid isolated from *Stephania cepharantha* Hayata, has been proposed as a possible alternative therapy to antivenom. CEP lessens the inflammation and pain caused by snake bites (2). CEP, or extracts from the plant, are widely used (primarily in Japan) to treat a variety of acute and chronic diseases such as alopecia areata (8), radiotherapy-induced leucopenia (9), malaria (10), and septic shock (11). Despite its use in treating a diverse number of illnesses, CEP does not have the ability to neutralize circulating venom (12,13).

Some single-center cohort reports have evaluated the efficacy of antivenom and CEP without adjusting for severity of mamushi bites (13,14). To our knowledge, multi-center, large population-based studies have not been conducted. Thus, the purpose of our study was to

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evaluate the efficacy of antivenom and CEP alone and in conjunction for the treatment of mamushi bites, after accounting for bite severity, among tertiary care centers in Japan.

MATERIALS AND METHODS

We prepared a questionnaire to examine the clinical characteristics of mamushi bites in tertiary care centers in Japan. We sent the questionnaires to 219 centers in October 2010, and the questionnaires were returned by January 2011. The surveillance period of the questionnaire spanned 1 year, from November 2009 to October 2010. The detailed contents of the questionnaire are illustarated in Fig. 1. The characteristics of each patient are summarized in Table 1.

Therapeutic interventions received by the patients were divided into 4 groups: CEP alone, antivenom alone, both CEP and antivenom, and neither CEP nor antivenom. Information was also collected regarding patient age, sex, comorbidities, grades of mamushi bites, laboratory data, adverse reactions to therapies, in-hospital mortality, and length of hospital stay.

Length of hospital stay was categorized as either ≤ 1 week or >1 week. We analyzed the association between length of hospital stay and different therapeutic interventions after accounting for grades of mamushi bites.

Grade classification for mamushi bites was used to determine the severity of injuries (15); Grade I, redness and swelling around the bitten area; Grade II, redness and swelling including the wrist or foot joint; Grade III, redness and swelling of the elbow or knee joint; Grade IV, redness and swelling of the whole extremity; and Grade V, redness and swelling in parts beyond the extremity or exhibiting systemic symptoms. Grades of mamushi bites are rather complicated, therefore we combined the 5 grades of mamushi bites into 2 groups: mild (grades of mamushi bites I and II) and severe (grades of mamushi bites III, IV, and V).

Statistical analyses: Categorical variables were compared using Fisher's exact test, and continuous variables were compared using ANOVA. Data were considered statistically significant if the *P* values were less than 0.05. All statistical procedures were performed using JMP software (version 7.0 for Windows; SAS, Inc., Cary, N.C., USA).

1. How many cases did you treat from November 2009 to October 2010? ( ) cases.
2. Please fill in details of the cases that you have treated.

Case

	Case1	Case2	Case3	Case4	Case5	Case6	Case7	Case8	Case9	Case10
Age										
Gender										
Comorbidity										
Mamushi Grade (I–V)										
Leukocytes										
Hct										
PLT										
CK										
BUN										
Cre										
LDH										

Treatment

Antivenom										
Side effect of antivenom										
CEP										
Steroid										
Local wound care										

Course

Maximum grade of injured area										
Length of ICU stay										
Length of hospital stay										
Patient outcome										
Causes of death										
Complication										
Others										

Fig. 1. Questions on the clinical treatment of mamushi (*Gloydius blomhoffii*) bites. Hct, hematocrit; PLT, platelet; CK, creatine kinase; BUN, blood urea nitrogen; Cre, creatinine; LDH, lactate dehydrogenase; CEP, cepharanthine.

Table 1. Clinical characteristics of mamushi (*Gloydius blomhoffii*) bites

Variable	No. of cases (%) (n = 234)
Age (yr)	57 ± 24
Sex, male	148 (63)
Comorbidity	112 (48)
Hypertension	30 (13)
Diabetes mellitus	13 (6)
Grades of mamushi bites	
I	66 (28)
II	74 (32)
III	52 (22)
IV	33 (14)
V	5 (2)
Unknown	4 (2)
Laboratory value	
Leukocyte (/mm^3)	7400 ± 3100
PLT (×10^4/mm^3)	20.9 ± 7.4
Hct (mg/dL)	40.4 ± 5.4
CK (IU/L)	1297 ± 6028
BUN (mg/dL)	16.5 ± 9.1
Cre (mg/dL)	0.74 ± 0.59
LDH (IU/L)	263 ± 209
Treatment	
CEP	28 (12)
Antivenom	85 (36)
Both CEP and antivenom	26 (11)
Neither CEP nor antivenom	71 (30)
Unknown	24 (10)
Side effect of antivenom	
Anaphylaxis	2 (1.8)
Mild reactions	8 (7.2)
Outcome	
In hospital mortality	0 (0)
Length of hospital stay (days)	5.3 ± 5.1

Values are expressed as mean ± standard deviation or no. of patients (%).
Abbreviations are in Fig. 1.

RESULTS

Completed questionnaires were received from 114 (52.1%) tertiary care centers. Two hundred and thirty-four cases of mamushi bites (148 men, 86 women) were reported (Table 1). The mean (SD) age of the cases was 57 (24) years. One hundred and twelve (48%) patients had an underlying condition, the most common of which were hypertension and diabetes mellitus.

Regarding grades of mamushi bites, the bites of 66 of 234 patients were grade I (28%); 74 patients, grade II (32%); 52 patients, grade III (22%); 33 patients, grade IV (14%); and 5 patients, grade V (2%). In addition, the creatine kinase (CK) levels were variable among the patients and were generally elevated with increasing grade severity (Fig. 2).

CEP alone was administered to 28 patients, and 85 patients were treated with antivenom alone. Both CEP and antivenom were administered to 26 patients, and

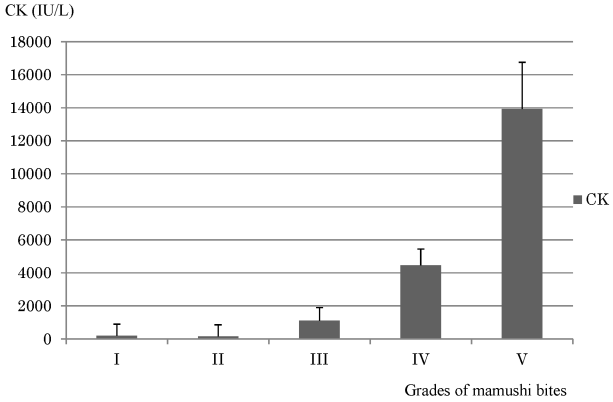


Fig. 2. Association between grades of mamushi bites and creatine kinase (CK) level on admission. Bars represent the mean ± SEM.

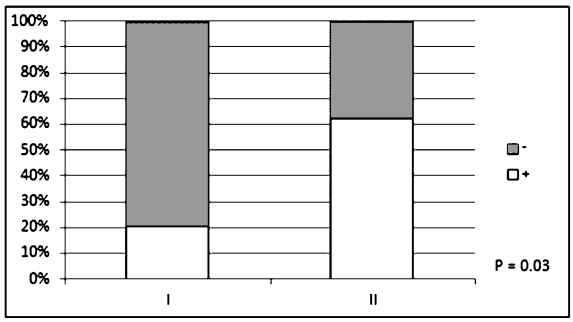
Table 2. Comparison among the 4 groups administered cepharanthine (CEP) alone, antivenom alone, both CEP and antivenom, and neither CEP nor antivenom

Variable	CEP (n = 28)	Antivenom (n = 85)	CEP + antivenom (n = 26)	Neither CEP or antivenom (n = 71)	P
Age (yr)	60.1 ± 4.3	59.7 ± 2.5	55.0 ± 4.4	57.3 ± 2.7	0.74
Men	20 (71)	47 (55)	16 (62)	45 (63)	0.45
Comorbidity	11 (55)	34 (40)	11 (42)	42 (59)	0.40
Grades of mamushi bites					
I	11	19	2	34	
II	9	32	7	16	
III	6	19	11	9	
IV	2	14	6	8	
V	0	1	0	4	
Leukocyte (/mm^3)	7064 ± 594	6930 ± 343	7918 ± 617	7643 ± 379	0.37
PLT (×10^4/mm^3)	18.2 ± 1.4	21.1 ± 0.8	20.9 ± 1.4	21.4 ± 0.8	0.22
Hct (mg/dL)	40.3 ± 1.1	40.4 ± 0.6	41.0 ± 1.1	40.1 ± 0.7	0.91
CK (IU/L)	1589 ± 1251	976 ± 704	1397 ± 1251	1898 ± 810	0.86
BUN (mg/dL)	15.8 ± 1.9	17.6 ± 1.0	15.7 ± 1.9	16.3 ± 1.2	0.71
Cre (mg/dL)	0.77 ± 0.12	0.71 ± 0.07	0.70 ± 0.46	0.82 ± 0.08	0.69
LDH (IU/L)	295 ± 43	246 ± 24	246 ± 44	294 ± 27	0.50
Outcome					
Length of hospital stay (days)	5.6 ± 1.0	5.3 ± 0.6	4.7 ± 1.0	5.6 ± 0.6	0.89

Values are expressed as mean ± standard deviation or number of patients (percentage).
Abbreviations are in Fig. 1.

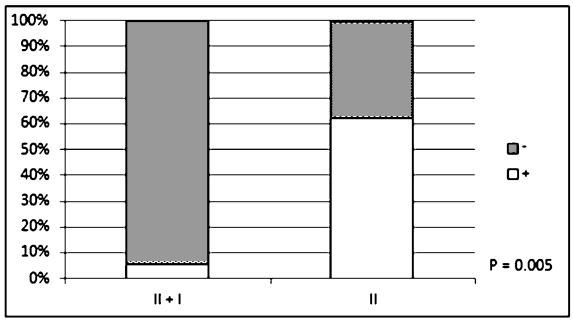
A

	+	-	Total
I	7	27	34
II	5	3	8
Total	12	30	42



B

	+	-	Total
II + I	1	16	17
II	5	3	8
Total	6	19	25



C

	+	-	Total
I	7	27	34
I + II	1	16	17
Total	8	43	51

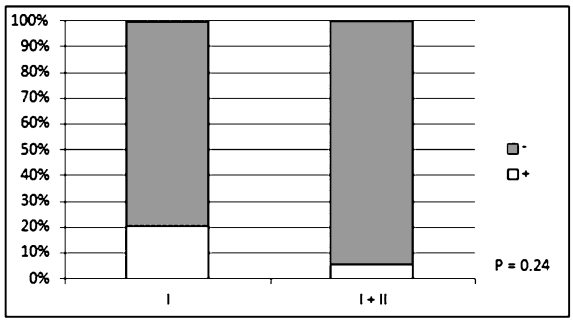
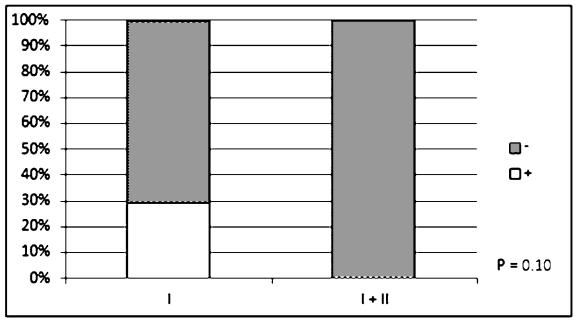


Fig. 3. Association between lengths of hospital stay and treatment in the severe group (grades of mamushi bites III, IV, and V). A: Comparison between antivenom alone (I) and cepharanthine (CEP) alone (II). B: Comparison between CEP alone (II) and both antivenom (I) and CEP (II). C: Comparison between antivenom alone (I) and both antivenom (I) and CEP (II). +, length of hospital stay >1 week; —, length of hospital stay ≤ 1 week.

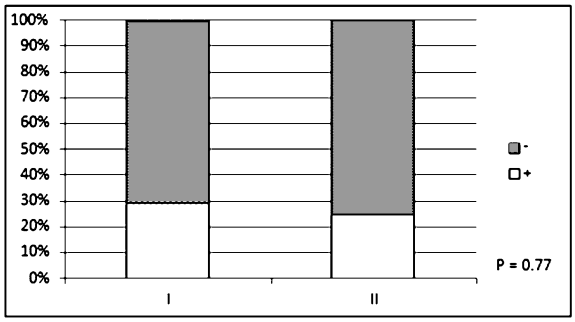
A

	+	-	Total
I	15	36	51
I + II	0	9	9
Total	15	45	60



B

	+	-	Total
I	15	36	51
II	5	15	20
Total	20	51	71



C

	+	-	Total
II + I	0	9	9
II	45	15	20
Total	45	24	29

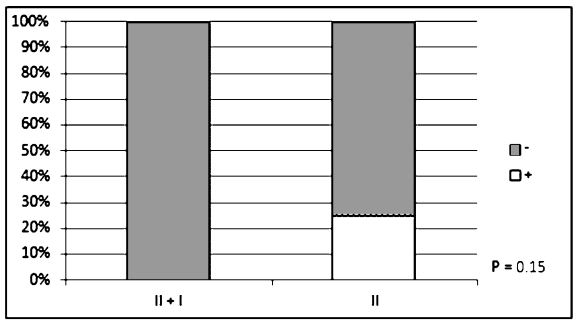


Fig. 4. Association between length of hospital stay and treatment in the mild group (grades of mamushi bites I and II). A: Comparison between antivenom alone (I) and both antivenom (I) and CEP alone (II). B: Comparison between antivenom alone (I) and CEP alone (II). C: Comparison between CEP alone (II) and both antivenom (I) and CEP (II). +, length of hospital stay >1 week; —, length of hospital stay ≤ 1 week.

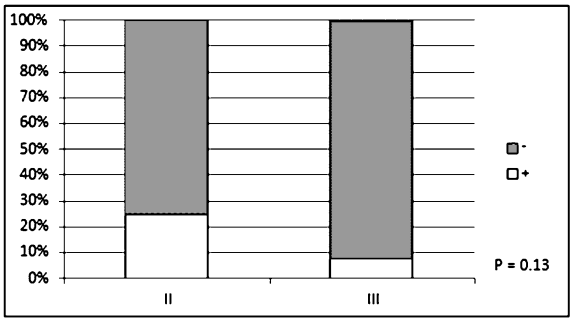
neither CEP nor antivenom was administered to 71 patients. Severe adverse reactions to antivenom occurred only 2 patients (1.8%) with anaphylaxis (Table 1). We observed no significant differences in age, sex, laboratory data, or length of hospital stay among the 4 treatment groups (Table 2).

After adjusting for grades of mamushi bites in the severe-grade group, patients receiving antivenom alone had a significantly shorter length of hospital stay than those given CEP alone ($P = 0.03$). Moreover, patients administered both CEP and antivenom had a significantly shorter length of hospital stay than those administered CEP alone ($P = 0.005$). However, no significant difference was observed in length of hospital stay between patients administered antivenom alone and those administered both antivenom and CEP ($P = 0.24$) (Figs. 3A, 3B, 3C). Interestingly, no significant differences were observed in the length of hospital stay in the mild-grade group (Figs. 4A, 4B, 4C).

As for CEP, no significant differences were observed

A

	+	-	Total
II	5	15	20
III	4	46	50
Total	9	61	70



B

	+	-	Total
II	5	3	8
III	7	14	21
Total	12	17	29

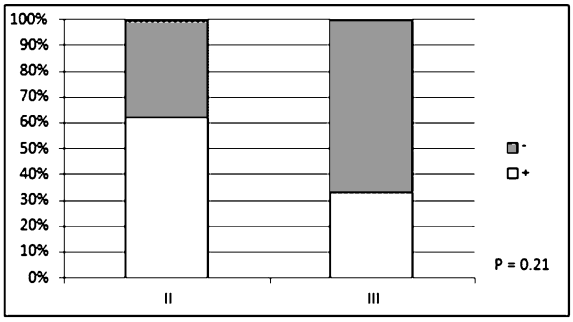


Fig. 5. Association between length of hospital stay and CEP administration. A: Comparison between CEP alone (II) and neither CEP nor antivenom (III) in mild group. B: Comparison between CEP alone (II) and neither CEP nor antivenom (III) in severe group. +, length of hospital stay >1 week; —, length of hospital stay ≤ 1 week.

in length of hospital stay between patients administered only CEP and those administered neither CEP nor antivenom in both mild- and severe-grade groups (Figs. 5A, 5B).

DISCUSSION

In this multi-center, large population-based cross-sectional analysis, we determined that without accounting for the grades of mamushi bites, there was no significant difference in the length of hospital stay or survival of patients receiving CEP alone, antivenom alone, both CEP and antivenom, or neither CEP nor antivenom. Since the severity of mamushi bites differed among these groups, stratification by severity was required in order to gauge the efficacy of each treatment accurately.

In severe cases (grades of mamushi bites III, IV, and V), our data determined that antivenom is the major contributing factor for reducing the length of hospital stay. In contrast, no significant differences were observed in the length of hospital stay in the mild group (grades of mamushi bites I and II).

Some reports have evaluated the efficacy of antivenom and CEP in a single-center cohort study (13,14). Makino et al. evaluated 114 cases and reported that patients administered antivenom alone had a significantly shorter hospital stay than those administered CEP alone ($P < 0.01$). However, the percentage of patients administered antivenom was relatively higher than that of patients administered CEP in severe cases (grades of mamushi bites IV and V) (50% versus 33%, $P = 0.06$) (13). On the other hand, Kochi et al. evaluated 50 cases and reported that patients administered antivenom alone had a significantly longer hospital stay than those administered CEP alone because of the higher severity in antivenom group (14).

Given the data from our survey, we conclude that the majority of mamushi bites should be initially categorized by the grades of mamushi bites. In severe cases (grades of mamushi bites III, IV, and V), antivenom should be administered in order to neutralize the venom as well as reduce the length of hospital stay.

Regarding adverse reactions to antivenom, we reconfirmed that the incidence of such occurrences are low. In a recent national survey, it was reported that the incidence of adverse reactions to antivenom was only 2.4%, including mild cases (2). In the present survey, 9.0% of patients had adverse reactions, which is identical to that reported in clinical trial data (4).

Although adopting a more conservative approach to mamushi bite treatment—one that does not involve the use of antivenom—could reduce the risk of overtreatment and exposure to unnecessary complications, it is not necessary. Adverse reactions can be treated with careful monitoring and appropriate treatment. Before adopting such an approach, clinicians must accept the possibility that even patients with mild injuries may require more aggressive treatment with antivenom if their clinical status begins to deteriorate while in the hospital. Here, we emphasize that CEP by itself does not have the ability to neutralize circulating venom, even though it is used clinically to treat various other medical conditions.

One limitation of this study is the fact that we did not receive data from all tertiary care centers in Japan. In addition, retrospective, questionnaire-based studies do not always yield reliable clinical data. Finally, this study did not obtain the time from receiving injuries to administration of treatment.

In conclusion, antivenom was effective for reducing the length of hospital stay in severe cases of mamushi bites. Furthermore, future large-scale prospective studies evaluating the clinical efficacy of antivenom are required.

Acknowledgments We acknowledge the staff at all 219 tertiary care centers in Japan.

This work was supported by a Grant for Research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest None to declare.

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