Safety and efficacy of live attenuated Japanese encephalitis vaccine SA_{14}-14-2 in human vaccination

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Status of JE vaccination in China

- **Primary hamster kidney cells inactivated vaccine has been used widely since 1970's .**
- **Attenuated SA14-14-2 live vaccine was licensed in 1989 and has been used widely ever since.**

Status of attenuated live JE vaccine production in China

- **Manufacturer increased from one at the beginning of 1989 to 3 in the 1990's.**
- **Products increased from several millions in the early 1990's to 20~30 millions in the late 1990's and over 50 millions in recent years.**

Summary of Virulence of SA14-14-2 Virus

Animals	Inoculation route	SA_{14}-14-2 (Vaccine strain)	SA_{14} (Parent wild virus)
Mice (2.5 week)	I.C.	0	9.5*
	S.C.**	0	>7.0
Hamster	I.C	0	8.0
Rhesus Monkey	I.C.+SP	0	8.5
Nude mice	S.C.	0	>6.0
Mice treated with Cyclophosphamide	S.C.	0	ND

IC, intracerebral; SC, Subcutaneous; IP, Intraperitoneal, ND, No data.
* Log, LD50/ml; ** ic inoculation with diluent

Genotype characteristics

57 nucleotide substitutions
24 amino acid changes

C	**1**
E	**8**
NS1	**5**
NS2b	**2**
NS3	**4**
NS4a	**2**
NS5	**2**

Attenuation Stability

Passages		Virulence (icLD50)	
Animals/cells	No. passages	ic	sc
Mice ic (12-14g)	5	≤2.0	0
Hamster kidney cells(HKC)	17	0	0
HKC+Suckling mice ic	8+1	1.32	0
HKC+Suckling mice ic	17+1	2.68	0

Genetic stability

Passages		E protein gene sequence
Animals/cells	**No. passages**	**No.aa reversion**
HKC	**17**	**0/8**
Suckling mice ic	**1**	**1/8(E107) Phe→Leu**

No.reversion/No.aa substitutions in SA14-14-2 from parent SA14

Phenotypic and Genetic stabilities after mosquitoes(Cx.tritaeniorhynchus) intrathoracical infection

	Virulence				E-gene sequence	
	Suckling mice		2.5wks mice			
	ic	bite	ic	sc	Homology	aa reversion
M_1 1.6×10^4	0/16	0/16				
M_1BHKC_1 1.4×10^7			0/10	0/10	99.9%*	0/8**

* Compared with SA14-14-2 seed virus

** No.reversion/No. aa substitutions in SA14-14-2 from parent SA14

Neuroattenuation and genostability after long storage of vaccines at -20□

No.vaccine	Years of storage	Virus titer logPFU/ml	Neurovirulence (I.C.)	E-gene amino acid reversion
Seed virus	>15	6.66	0/10*	0/8 **
807032	15	5.97	0/10	0/8
880303	14	5.70	0/10	-
891230	13	5.11	0/10	0/8
920103	10	5.20	0/10	0/8
931125	9	5.70	0/10	0/8
941125	8	5.74	0/10	0/8
950213	7	6.35	0/10	-
960309	6	6.24	0/10	0/8
970319	5	5.85	0/10	-
981122	4	5.85	0/10	0/8
990228	3	6.06	0/10	0/8

* No.death/No.tested mice(12-14g)

** No.reversion/No. aa substitutions in SA14-14-2 from parent SA14

Safety studies

in Humans

Safety in JE susceptible children

No. trials	No.Children	ages	Side effects (body-temp□)	Encephalitis Meningitis
1-2	85	8-12	≤37	0
3	47	5-6	≤37.4	0
	979	7-12	-	
4	816	1-6	<38	0
	558512	1-15	-	
5	1964	1-6	<38.5	0
	6000	1-10	>38.6(2)	
			-	

Adverse events in 30 days following vaccination

Event	Vaccinated Group (n=13,266)	Unvaccinated Group (n=12,951)	Risk ratio (95% confidence interval)
Encephalitis	0(0.0)	0(0.0)	undefined
Meningitis	0(0.0)	0(0.0)	undefined
Hospital admission	82(0.6)	114(0.9)	0.70(0.43-1.15)
Severe reaction Consistent with			
Anaphylaxis	0(0.0)	0(0.0)	undefined
Seizure	14(0.1)	15(0.1)	0.91(0.37-2.22)
Fever lasting≥3days	357(2.7)	442(3.4)	0.79(0.56-1.11)
Diarrhea	12(0.1)	11(0.1)	1.06(0.46-2.49)
Upper respiratory Infection	292(2.2)	353(2.7)	0.81(0.55-1.18)
Bronchitis	38(0.3)	44(0.3)	0.84(0.49-1.44)

*accounts for clustering by health center

From J Infect Dis 1997, 176:1366-9 by Dr. Zhengle Liu

Immunogenicity studies

in Humans

Neutralizing antibody response in children

No. test	Places	Virus titers*	Ages	Seroconversion	
				%	GMT
1	Heilongjiang	5.7	8-12	92.0(12/13)**	58
2	Hebei	≥6.0	7-8	100(33/33	≥62.4
3	Jilin	6.5-6.8	13-15	96.3 (26/27)	36
4	Anhui	6.0-6.5	1-6	95.0 (18/19)	50
5	Korea,Souel	≥5.7	1-3	96.0 (65/68)	188
6	Beijing	>-5.7	1-2	91.3(63/69)	20

* PFU/ml ** No. positive /No. tested

Persistence of neutralizing antibody in children living in JE non-endemic area

No. subjects	Seroconversion % (GMT)		
	1 month after 1st dose	1 month after 2nd dose	6 years After2nd dose
27	96.3%(30.5) (26/27)	100%(46.4) (27/27)	88.8%(21.8) (24/27)

Efficacy studies

in Humans

Efficacy studies

studies	district	Year	Vaccinated			unvaccinated		
			Total No.	JE case	Morbidity (1/100000)	Total No.	JE case	Morbidity (1/100000)
1	Guizhou	1988	86146	1	1.16	21135	12	56.7
2	Jiangxi	1989	64027	1	1.56	4546	13	285.9
3	Yunan	1991	29639	2	6.73	29006	46	158.6
4	Anhui GY	1992 ~1996	180700	3	1.67	15636	22	140.7
	Anhui MC		155241	8	5.15	9685	24	247.8

18 dead JE cases were all in the unvaccinated group.

Efficacy observed by case-control

Studies	Years	Place	Ages	Does	Effectiveness (95%CI)
1	1996	Sichuan China	1-6	1	80%(44-93)
				2	97.5%(86-99.6)
2	1999	Nepal	1-16	1	99.3%(94.9-100)
3	2000	Chongqing China	1-6	1	99.3%(92-99)

Data collected from several provinces have shown evident decline of JE morbidity in regions where vaccination campaigns with SA14-14-1-2 live vaccine had been carried out compared with the local historical data and with neighboring counties

Long term efficacy(1989~1999)

- Vaccination schedule

1989 one primary dose for 1~10 years children

No. vaccinated 64027

No. unvaccinated 4546

1990~1999 each year

one primary dose for 1 age children,

one booster dose for 2 ages children

• JE case recorded

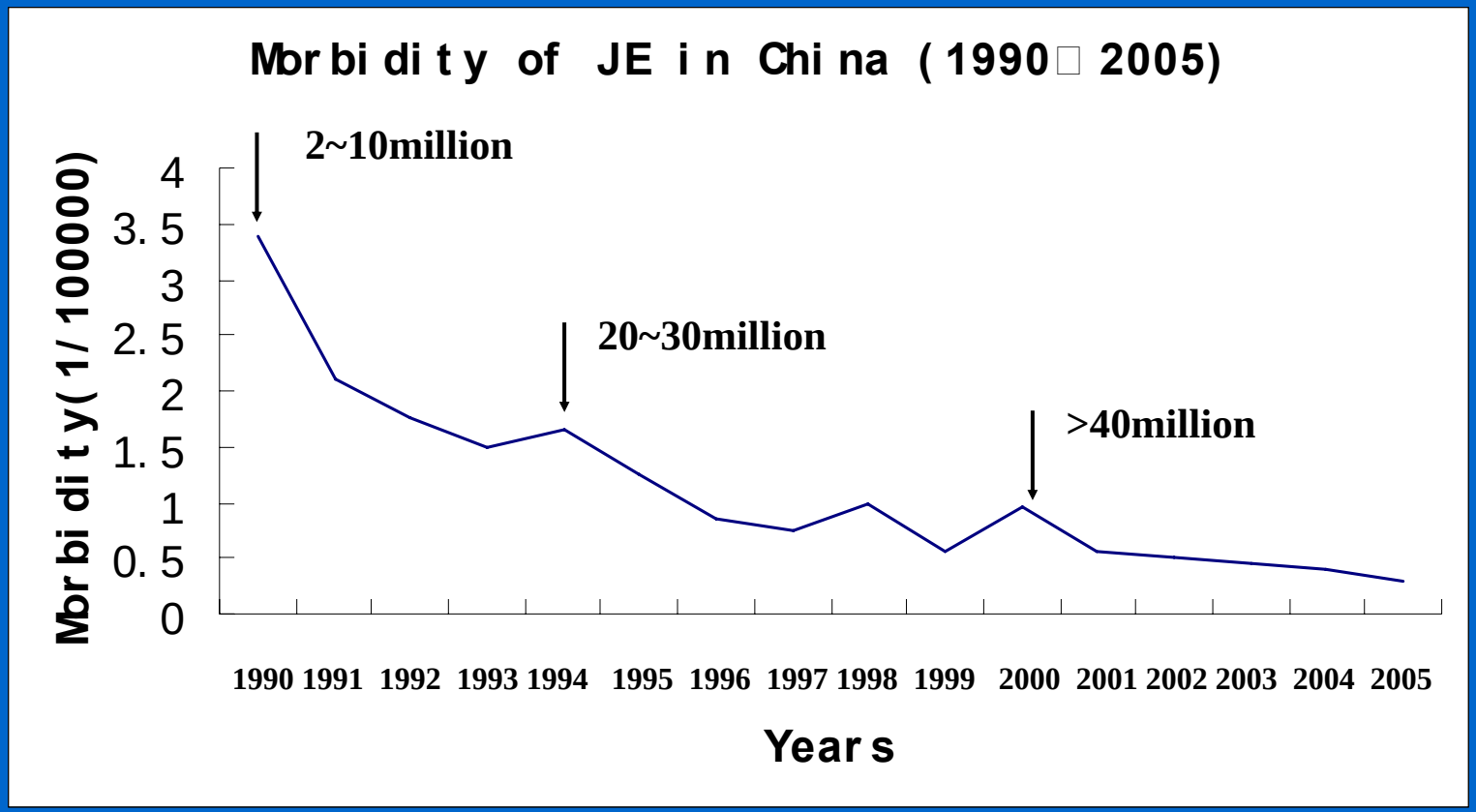
Years	Duration	JE case vaccinated/unvaccinated
1994	5year	1/8
1999	10year	1/12
1989~1999	11year	9/129

Before vaccination 1978~1988 JE total cases 769

Morbidity:Before vac. (1978~1988) 21.89/100000

After vac. (1989~1999) 3.39/100000

Morbidity of JE in China (1990~2005)



The increase of live vaccine production and vaccination resulted in remarkable decline of JE morbidity from2.5/100000 in 1990 to less than 0.5/100000 in 2004 and 2005,the lowest since 1949.

Vaccine production and quality control

Comply with WHO “ Guidelines for the Product and Control of Japanese Encephalitis Vaccine(live) for Human Use”

Vaccine production and quality control

- Establishment of GMP
- SPF Hamster

Vaccine production and quality control

- **Genetic identity(E protein sequence) testing in working seed**
- **IC mice inoculation for attenuation testing**
- **Suckling mice ic inoculation for attenuation stability**

Conclusion

- **The SA14-14-2 virus is highly attenuated with good immunogenicity**
- **The neuroattenuation and genomic characteristics of SA14-14-2 are stable after in vitro and in vivo passages or mosquitoes infections**

Conclusion

- Over the past 15 years ,a total amount of 300 million doses of the vaccine were produced and approximated 200 million children have been vaccinated .

  Decline in JE morbidity was evident and no untoward side reaction related to vaccination was recorded.

Conclusion

- **The results confirm that SA14-14-2 live vaccine is safe and effective for prevention of JE disease in humans**

THANKS