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Efficacy and safety of natural acetylcholinesterase inhibitor huperzine A in the treatment of Alzheimer's disease: an updated meta-analysis

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Abstract The objective of this study was to provide an updated meta-analysis of the efficacy and safety of huperzine A (HupA) in Alzheimer's disease (AD). We searched for randomized trials comparing HupA with placebo in the treatment of AD. The primary outcome measures were mini-mental state examination (MMSE) and activities of daily living scale (ADL). Data were extracted from four randomized clinical trials and analyzed using standard meta-analysis and meta-regression methods. Oral administration of HupA for 8–24 weeks (300–500 μg daily) led to significant improvements in MMSE and ADL. The results of meta-regression showed that the estimated effect size of MMSE and ADL was increased over the treatment time. Most adverse events were cholinergic in nature and no serious adverse events occurred. Huperzine A is a well-tolerated drug that could significantly improve cognitive performance and ADL in patients with AD.

Keywords Alzheimer's disease · Huperzine A · Acetylcholinesterase inhibitor · Clinical trials · Meta-analysis · Meta-regression

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Introduction

Alzheimer's disease (AD) is the common cause of dementia in late life, affecting approximately 10% of people aged at least 65 years worldwide. Health care of people with AD has become an increasing burden for the family, society and economy. AD is a progressive neurodegenerative disorder associated with a global impairment of higher mental function, and presenting with impaired memory as the main symptom. A significant correlation has been found between a decrease in cortical cholinergic activity and the deterioration of mental test scores in patients with AD. Based on the cholinergic hypothesis of AD, cholinergic enhancement strategies have been at the forefront of efforts to pharmacologically palliate the cognitive impairments. Among the various therapeutic approaches, cholinesterase inhibitors (ChEIs) are the first group of compounds that have produced modest improvements in cognitive function of AD patients. Four ChEIs, tacrine, donepezil, rivastigmine and galantamine, have been approved by the US FDA for the treatment of mild or moderate AD.

Huperzine A (HupA), a novel alkaloid isolated from the Chinese herb *Huperzia serrata*, is a potent, highly selective, reversible acetylcholinesterase (AChE) inhibitor (Tang et al. 1989; Wang et al. 2006; Zhang et al. 2008). Pharmacokinetic studies have shown that HupA is absorbed rapidly, distributed widely in the body and eliminated at a moderate rate with the property of slow and prolonged release after oral administration. When compared with

tacrine, donepezil and rivastigmine, HupA has better penetration of the blood–brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action (Bai et al. 2000). HupA is more potent than tacrine, rivastigmine and galantamine in terms of AChE inhibition activity with the least anti-butyrylcholinesterase activity among tested inhibitors, suggesting a better selectivity and tolerability profile (Giacobini 2004). Beyond the potent AChE inhibition, HupA exerted multifunctional effects on several molecular targets, including modulation of β-amyloid peptide processing, reduction of oxidative stress, neuroprotection against apoptosis, and regulation of the expression and secretion of nerve growth factor and its signaling, holding a promise as a disease-modifying agent in the treatment of AD (Ved et al. 1997; Zhang and Tang 2006; Zhang et al. 2008).

Huperzine A was approved for the treatment of AD in China in 1994 and has been widely used to improve the memory deficits in elderly people and patients with benign senescent forgetfulness, AD, and vascular dementia (Kelley and Knopman 2008; Little et al. 2008; Yan and Tang 2006). HupA has generated considerable interest in recent years in the US and other Western counties (Mazurek 2000). The HupA derivatives ZT-1, bis-HupA, bis-HupA-tacrine, as well as different formulation of HupA are also being developed as new anti-AD drugs in several countries (Wong et al. 2003; Ma et al. 2007). Although many clinical trials have claimed that HupA could improve cognitive performance and activities of daily living (ADL) on AD patients, only a few are randomized controlled clinical trials with different treatment durations and assessment time points. No published controlled clinical trials of HupA have been reported outside China. A multicenter, double-blind, placebo-controlled therapeutic Phase II study has been carried out in more than 30 sites in the US but its results in detail have not been published. A recent systematic review from Cochrane database has presented beneficial effects of HupA on the improvement of general cognitive function, global clinical assessment, behavioral disturbance and functional performance on AD patients (Li et al. 2008). However, the results were limited by the low quality of individual studies (Dong et al. 2002; Zhou et al. 2004) and improper inclusion of one center report (Liu et al. 1995).

In our present study, we performed an updated meta-analysis of placebo-controlled randomized clinical trials of HupA in patients with AD. Moreover, a bivariate repeated measures meta-regression analysis with the duration of treatment as covariate was performed to take full advantage of available data, evaluate the efficacy of HupA in AD more precisely and investigate the possible causes of the heterogeneity.

Methods

Identification of trials

We systematically searched the literature to identify randomized trials of HupA in AD. The probing strategy included searching the English-language literature using the Cochrane Library and Medline, and the Chinese-language literature using the Chinese Biomedical Literature Analysis and Retrieval System for Compact Disc (CBM-DISC) from January 1980 to May 2008. The key search terms were: huperzine A (or its trademark names in China such as Ha Bo Yin, Shuang Yi Ping) and Alzheimer's disease, and the limits were randomized controlled trials and human. Recent review articles and published reports of clinical trials were manually cross-referenced, as were all references and bibliographies from retrieved articles.

Two raters reviewed the Methods section of all articles identified. Articles meeting the inclusion criteria were then rated on quality by two raters using the Jadad scale, which is simple to use and has been validated. Disagreements regarding inclusion and quality were settled by consensus discussion.

Inclusion criteria:

1. A randomized placebo-controlled design.
2. Participants with AD and without current diagnosis of any other psychiatric or neurological disorder, aged older than 50 years.
3. Outcome measures of cognitive performance and ADLs in AD patients.

Exclusion criteria:

1. Trials with fewer than 20 participants in each arm.
2. Trials evaluating dementia caused by diseases other than AD.

Outcome variables:

The studies used a range of scales and measures to record changes in participants and only cognitive outcome measures and ADLs scale were discussed in detail here.

Cognitive outcome measures

Mini-mental state examination was selected as the primary variable to evaluate the effects of HupA on cognitive function. It includes 11 questions on orientation, memory, concentration, language and praxis, and uses a scale of

0–30 with a higher score indicating less impairment (Folstein et al. 1975). This scale is an objective measure of cognitive function.

Activities of daily living scale

All the included studies used the activities of daily living scale (ADL) of Lawton and Brody to assess ADLs (score range 14–56) (Lawton and Brody 1969). We selected it as the second variable in our meta-analysis. A negative value with this scale indicates improvement in patient's abilities.

The trial duration and the time for evaluation were not the same in the different studies. The outcome data at endpoint in each study were chosen for meta-analysis, because the endpoint selected according to clinical protocol is more reliable and the endpoints of individual trials cannot be changed in a meta-analysis.

Safety was assessed by the monitoring of treatment-emergent adverse events, clinical laboratory evaluations and the recording of vital signs.

Data extraction

Data were extracted from the published reports by two independent reviewers. Disagreements were resolved by discussion with another reviewer. For each trial, the following data were documented: publication year, patient population, diagnosis criteria, primary variable, sample size, and treatment regimen. The efficacy of HupA was evaluated based on patient cognitive performance and ADLs assessment. All the efficacy data were collected if trials reported results at more than one follow-up time. Wherever possible, outcomes from the intention-to-treat (ITT) population were used, and if this was not possible per protocol outcomes were extracted.

Statistical methods

1. The endpoint outcome data in each study were chosen for the meta-analysis. Mean difference in the changes of mean score from baseline between HupA group and placebo group was used to measure HupA treatment effects. A positive value indicated a beneficial effect for MMSE and a negative value indicated a beneficial effect for ADL scores.
2. Test of heterogeneity: If H_0 in the test of heterogeneity could not be rejected at the α level, a fixed-effect model was used to estimate a common parameter for all studies; otherwise, a random-effect model was chosen. In this study, the α level was set at 0.2.
3. Standard statistical model: Fixed-effect model and random-effect model (Normand 1999; Whitehead 2002).
4. A bivariate repeated measures meta-regression was used with duration of treatment as the covariate (Van Houwelingen et al. 2002). The estimation method was restricted maximum likelihood (REML). The overall estimates of the difference in mean change from baseline between HupA and placebo groups at specific time points were chosen to assess treatment difference.
5. Statistical analysis was performed using the statistical software SAS9.13.

Results

Trials and patients

Eleven articles were selected, which covered an open-label study (Mazurek 2000), two center reports (Liu et al. 1995; Yang and Jiang 1996), two positive controlled clinical trials (Xu et al. 1999; Chen et al. 2000), and six non-positive treatment controlled trials (Dong et al. 2002; Zhou et al. 2004; Xu et al. 1995; Zhang et al. 2002; Yang et al. 2003; Zhang et al. 2006). Four trials, with more than 20 participants in each arm, were included in the meta-analysis based on the inclusion and exclusion criteria (Xu et al. 1995; Zhang et al. 2002; Yang et al. 2003; Zhang et al. 2006). All trials had been performed in China. A total of 474 patients were included in the study, with 235 in the HupA group and 239 in the control group. The number of patients in the individual studies ranged from 65 to 197. Trial durations ranged from 8 to 24 weeks.

Quality assessment

All trials mentioned the type of randomization in "Methods" section. Two trials (Yang et al. 2003; Zhang et al. 2006) were single-blind, and the other two trials (Xu et al. 1995; Zhang et al. 2002) were double-blind. There was a description of withdrawals and dropouts in two trials (Zhang et al. 2002, 2006). Only one trial (Zhang et al. 2002) used the full analysis set based on the intent-to-treat principle; the other three trials used the per protocol set.

Patient population

All four studies enrolled male and female patients according to the diagnosis of AD, for example, Hachinski ischemic score $\leq$4, and CT or MRI findings consistent with the diagnosis or clinical symptoms. Table 1 included a

Table 1 Study characteristics of all included clinical trials

Study ID	Study	Publication year	Patient population	Diagnosis criteria	Primacy variable	Sample size	Treatment regimen
1	Si-sun Xu et al.	1995	Age: >50, MMSE (range) 13–23	DSM3-R	MMSE, HDs, ADL, MQ	103	400 μg/day, 8 weeks
2	Zhen-xin Zhang et al.	2002	Age (range) 50–80, MMSE (range): 10–26	DSM4	ADAS-cog, MMSE, ADL, ADAS-non-Cog, CIBIC plus	197	400 μg/day, 12 weeks
3	Chu-yu Yang et al.	2003	Age (range) 65–90, MMSE: <=26	DSM4	MMSE, CDR, ADL	65	300 μg/day, 16 weeks
4	Ming-lian Zhang et al.	2006	Age (range) 52–78, MMSE (mean ± SD): 15±4	DSM3-R	MMSE, ADL	109	500 μg/day, 24 weeks

description of the study characteristics and demographics of the participants in the studies.

Treatment regimen

The active medication contained 50 μg HupA in each tablet. Patients in the HupA group received HupA tablets orally for 8–24 consecutive weeks. The mean dose ranged from 300 to 500 μg per day. Patients in the control group received blank tablets, except those in study 4 received *Salvia miltiorrhiza* tablets. In one study, vitamin E (100 mg/day) was given to all participants in treatment and control groups as routine treatment (Zhang et al. 2002).

Meta-analysis

Table 2 showed the MMSE summary statistics for each study at each time point. The effect size of each study was shown in Fig. 1a. Random-effect model was used to estimate the pooled effect size. The pooled effect size of HupA versus placebo was 3.52 (95% CI, 2.23–4.80, $\tau^2 = 1.12$), indicating a beneficial effect of HupA.

Table 3 showed the ADL score summary statistics for each study at each time point. The effect size of each study was presented in Fig. 1b. Random-effect model was used to estimate the pooled effect size. The pooled effect size of HupA versus placebo was −4.50 (95% CI, −7.05 to −1.96, $\tau^2 = 4.03$). A negative value indicated an improvement in condition.

Table 2 HupA studies: summary statistics for MMSE across time

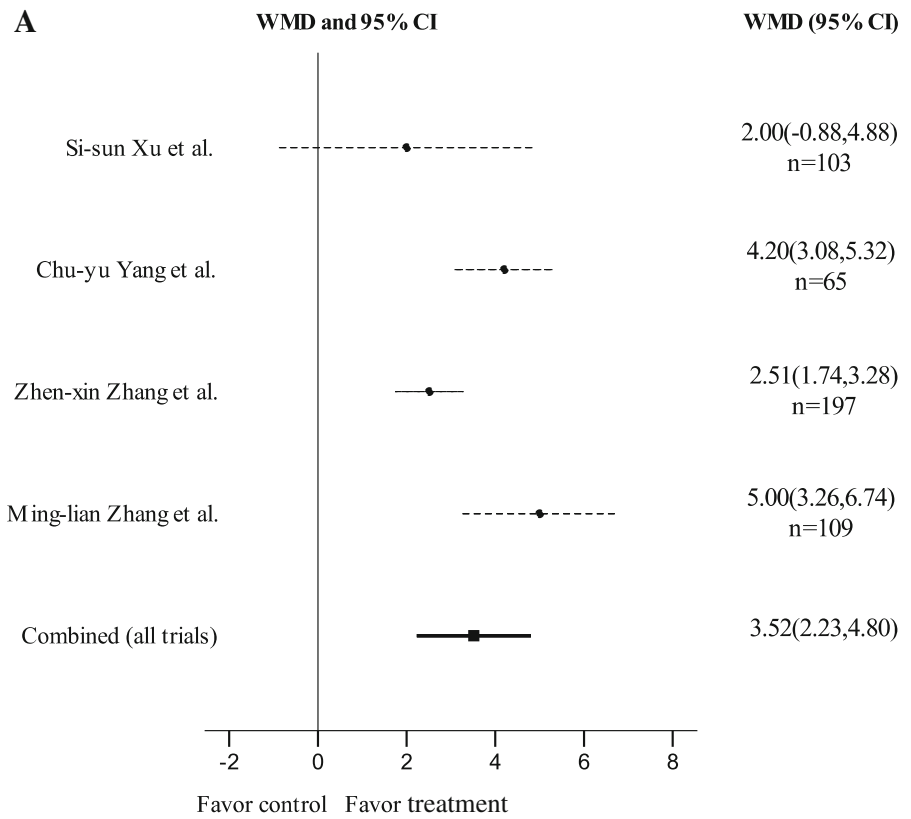
Week	Study	HupA			Placebo		
		Number	Mean	Standard deviation	Number	Mean	Standard deviation
0	1	50	16.00	5.0	53	14.00	5.0
0	2	98	19.00	5.0	99	19.00	5.0
0	3	35	18.80	2.3	30	18.00	2.3
0	4	52	14.00	4.0	57	15.00	4.0
6	2	98	20.87	5.0	99	18.96	5.0
6	4	52	15.00	4.0	57	15.00	4.0
8	1	50	19.00	5.0	53	15.00	5.0
12	2	98	21.70	5.0	99	19.19	5.0
12	4	52	15.00	4.0	57	15.00	4.0
16	3	35	23.10	2.3	30	18.10	2.3
18	4	52	17.00	4.0	57	15.00	5.0
24	4	52	19.00	5.0	57	15.00	5.0

Meta-regression

Table 4 showed the overall estimated difference of mean change in MMSE and ADL between HupA and placebo at 8, 16 and 24 weeks post-treatment.

Figure 2 showed the relationship between duration of treatment and the mean changes from baseline in MMSE and ADL for the placebo group and the HupA group.

1. MMSE: For placebo group, the regression line was mean change from baseline = 0.3388 + 0.0034 × (duration of treatment) (standard errors of intercept and slope were 0.3362 and 0.0193, respectively). For the HupA group, the regression line was mean change from baseline = 0.3778 + 0.2178 × (duration of treatment) (standard errors of intercept and slope were 0.5027 and 0.0214, respectively). There was a significant time effect on the mean change in MMSE in the HupA group. The estimated regression line of the treatment difference measure on treatment duration was difference of mean change = 0.0390 + 0.2144 × (duration of treatment), with standard errors of 0.4075 and 0.0274 for intercept and slope, respectively.
2. ADL: For the placebo group, the regression line was mean change from baseline = −0.0405 + 0.0059 × (duration of treatment) (standard errors of intercept



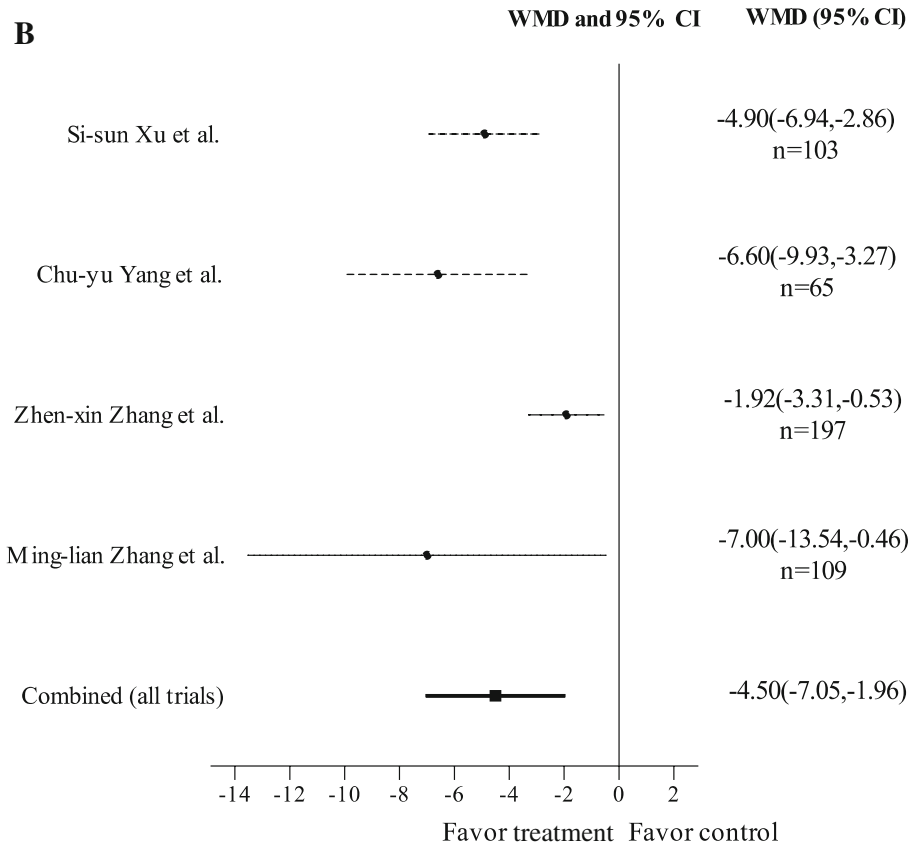


Fig. 1 Forest plot with the weighted mean difference (WMD) on **a** MMSE and **b** ADL of HupA relative to placebo in AD with 95% CI of the trials included in the meta-analysis. The *dashed horizontal lines* indicate the standard Wald confidence intervals of individual trials. The *solid horizontal lines* indicate the random effect estimate of all trials. Test for heterogeneity on MMSE: $Q = 11.1172$; $df = 3$; $P = 0.0111$. Test for heterogeneity on ADL: $Q = 11.3976$; $df = 3$; $P = 0.0098$

Table 3 HupA studies: summary statistics for ADL across time

Week	Study	HupA			Placebo		
		Number	Mean	Standard deviation	Number	Mean	Standard deviation
0	1	50	33.00	10.0	53	31.00	9.0
0	2	98	38.00	10.0	99	37.00	11.0
0	3	35	46.10	6.9	30	47.50	6.0
0	4	52	37.00	15.0	57	39.00	17.0
6	2	98	35.51	10.0	99	36.87	10.0
6	4	52	36.00	17.0	57	40.00	18.0
8	1	50	29.00	9.0	53	31.90	7.0
12	2	98	35.61	10.0	99	36.53	10.0
12	4	52	37.00	18.0	57	41.00	17.0
16	3	35	39.50	7.5	30	47.50	6.1
18	4	52	35.00	12.0	57	40.00	18.0
24	4	52	32.00	13.0	57	41.00	20.0

and slope were 0.6997 and 0.0725, respectively). For the HupA group, the regression line was mean change from baseline=$-2.6666-0.0827\times$ (duration of treatment) (standard errors of intercept and slope were 1.4116 and 0.0922, respectively). The estimated regression line of the treatment difference measure on duration of treatment was difference in mean change=$-2.7071-0.0886\times$ (duration of treatment), with standard errors of 1.5755 and 0.1172 for intercept and slope, respectively.

Safety and tolerability

A total of 474 patients with AD received at least one dose of study medication in the four clinical trials included in the meta-analysis. No adverse effects on vital signs, blood test results or electrocardiogram results were seen (Table 5). Most adverse effects were mild, only occasionally moderate in intensity and generally diminished with time despite continuation of treatment. Of those adverse effects, some mild peripheral cholinergic side effects such as nausea or vomiting and diarrhea were more likely occur in the HupA group than in the placebo group; however, differences were not significant. Most other non-cholinergic-induced adverse effects were considered unrelated to the study drug. There were no clinically significant differences in abnormal laboratory test parameters, vital signs or cardiovascular parameters between the study groups.

Discussion

This meta-analysis of four clinical trials was conducted to determine the effect size of HupA for the treatment of AD. As patients in the placebo group were generally treated with concomitant treatment, we included trials comparing HupA with placebo or with other non-positive treatment. A published review has shown no evidence of the efficacy of vitamin E for people suffering from AD and mild cognitive impairment (Isaac et al. 2008). In addition, subjects in the control group of study 4 (Zhang et al. 2006) received *Salvia miltiorrhiza* tablets, which is a natural drug used in China to prevent or treat cardiovascular and cerebrovascular diseases, mainly coronary heart disease and angina, by activating blood circulation to dissipate blood stasis. *Salvia miltiorrhiza* tablets are thought to be effective in the treatment of AD by some doctors in China, but there is no convincing evidence regarding its efficacy. Thus, *Salvia miltiorrhiza* was considered as placebo, and the estimates of treatment difference across all trials were combined in this meta-analysis.

Our results confirmed that HupA (300–500 μg per day) could significantly improve the MMSE score of AD patients, with mean difference of change as 3.5157 ($P < 0.05$). The effect of HupA on the ADLs of AD patients was detected using the mean difference of change (mean difference: -4.5028; $P < 0.05$). For ADL and MMSE scales, the statistic Q was very large, indicating that there was heterogeneity among the studies. There were some obvious differences among the four studies, such as the duration of treatment, mean age, mean dose, mean MMSE scores before treatment and publication year, all of which might contribute to heterogeneity among the studies. The estimated regression line of the treatment difference of MMSE on treatment duration showed that longer duration would result in better efficacy ($P < 0.05$). In addition, the estimated regression line for ADL showed a similar trend but did not reach statistical significance. The results indicated that, within the range of 6–24 weeks, longer treatment duration might lead to better effects of HupA.

Table 4 Estimated differences of mean changes on MMSE and ADL scores at weeks 8, 16 and 24 using the meta-regression model

Treatment	Scale	Week 8		Week 16		Week 24	
		Mean	StdErr	Mean	StdErr	Mean	StdErr
Overall (HupA – placebo)	MMSE	1.7541	0.2882	3.4692	0.3102	5.1844	0.4534
	ADL	−0.7090	0.9378	−1.4179	1.8756	−2.1269	2.8134

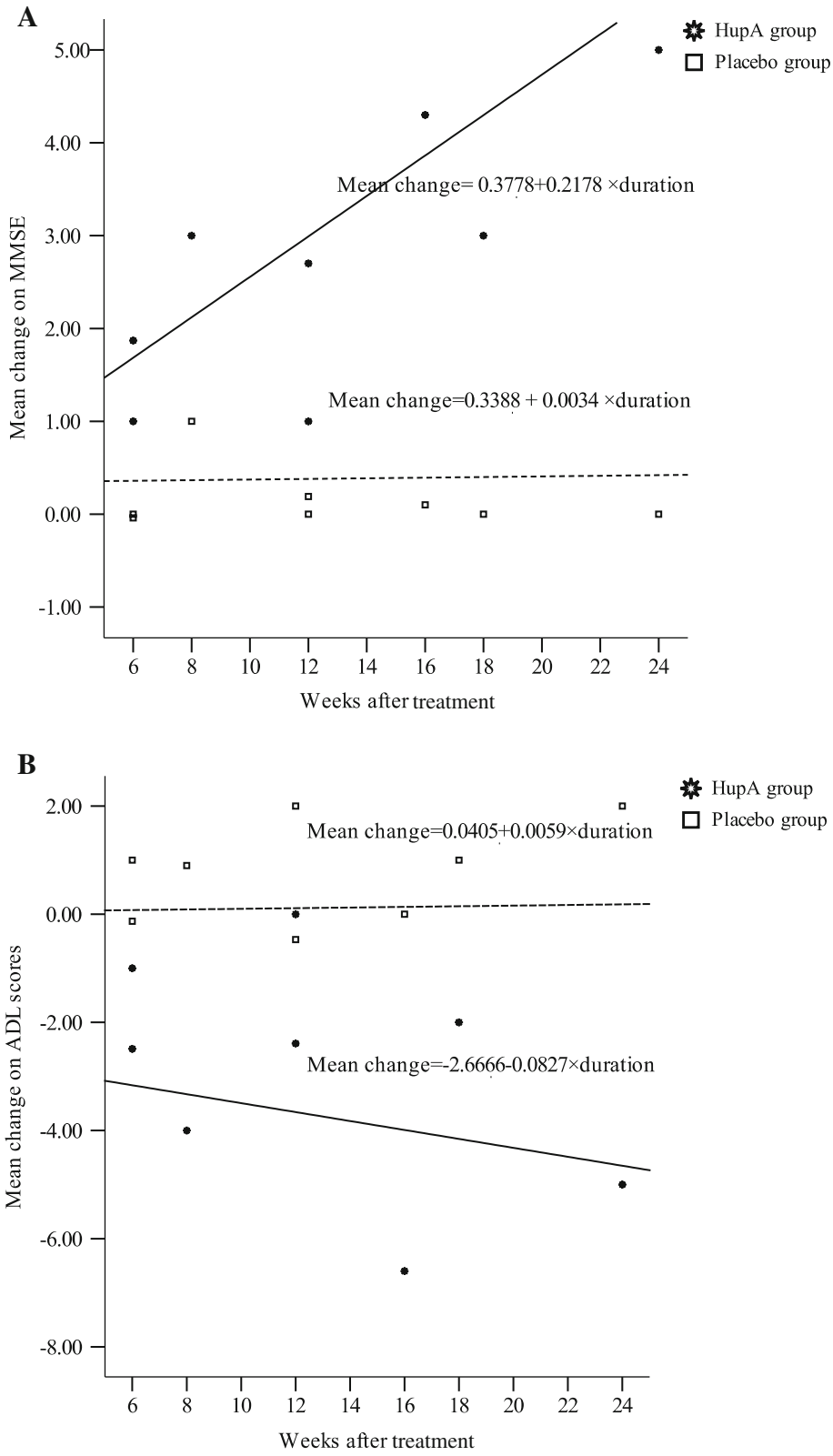


Fig. 2 Mean change from baseline versus duration of treatment for HupA group and placebo group on **a** MMSE and **b** ADL. The *dashed line* is the regression line for placebo group. The *solid line* is the regression line for HupA group

And the overall estimated difference of mean change in MMSE and ADL between HupA and placebo at 8, 16 and 24 weeks post-treatment provided a fuller picture of HupA treatment effect in AD.

As yet, no human trials have directly compared the efficacy of HupA with other ChEIs. The pooled effect size of HupA versus placebo was 3.52 points on the MMSE scale. In comparison, the effect size of donepezil versus

Table 5 Treatment-emergent adverse events

Adverse event	Number of subjects (pooled occurrence)		Odds ratio (fixed) 95% CI
	HupA ($n = 235$)	Placebo ($n = 239$)	
Agitation	3 (1.29)	3 (1.26)	1.06 (0.20, 5.54)
Ankles edema	1 (0.43)	0 (0.00)	2.04 (0.07, 61.55)
Anorexia	12 (5.16)	6 (2.51)	1.88 (0.69, 5.14)
Bradycardia	0 (0.00)	1 (0.42)	0.50 (0.02, 15.08)
Chest tightness	0 (0.00)	1 (0.42)	0.50 (0.02, 15.08)
Diarrhea	5 (2.15)	2 (0.84)	2.83 (0.52, 15.33)
Dizziness	9 (3.87)	11 (4.60)	0.81 (0.32, 2.07)
Festinating gait	0 (0.00)	1 (0.42)	0.50 (0.02, 15.08)
Headache	2 (0.86)	4 (1.67)	0.66 (0.13, 3.33)
Hyperactivity	5 (2.15)	3 (1.26)	1.85 (0.42, 8.19)
Hypopraxia	0 (0.00)	1 (0.42)	0.50 (0.02, 15.08)
Indigestion	3 (1.29)	1 (0.42)	3.43 (0.35, 34.04)
Insomnia	6 (2.58)	6 (2.51)	1.09 (0.33, 3.60)
Mild bellyache	3 (1.29)	0 (0.00)	5.63 (0.27, 117.02)
Nasal obstruction	4 (1.72)	4 (1.67)	1.07 (0.25, 4.51)
Nausea or vomiting	12 (5.16)	5 (2.09)	2.21 (0.77, 6.31)

Zero counts were changed to 0.5 before calculating overall odds ratio

placebo was 1.26–1.36 points on the MMSE scale (Rogers et al. 1998a, b). However, it is difficult to compare effect sizes across trials, particularly trials conducted in different countries and different languages, and with different inclusion/exclusion criteria and methods.

The recommended dose of HupA in China is 150–250 μg b.i.d (300–500 μg per day). A US open-label trial of HupA for the treatment of AD has shown that it is well tolerated at doses up to 200 μg b.i.d. and is effective in enhancing cognition, as measured by the MMSE. The addition of HupA 100 μg b.i.d. to prior treatment regimens (including donepezil and tacrine) could improve the MMSE by 1.5, 1.75 and 2.2 points at 1, 2 and 3 months, respectively (Mazurek 2000). Now, a multicenter, double-blind, placebo-controlled phase II study in 210 patients with mild to moderate AD has been completed in the US. Preliminary results have indicated that there is no statistical difference in the mean change in AD Assessment Scale-Cognitive (ADAS-Cog) scores after 16-week treatment with HupA 200 μg b.i.d. compared with placebo ($P = 0.81$). However, the higher dose tested, 400 μg b.i.d., was associated with cognitive enhancement versus placebo on the ADAS-Cog. The maximum cognitive improvement was observed at week 11 of treatment ($P = 0.001$). These results suggested that larger doses were needed for significant clinical effects in AD patients. Until recently, supplement companies in US sold HupA as a cognitive enhancer with a small doses of 50 μg b.i.d for people whose cognitive abilities fell into the normal range.

As expected from the results of randomized controlled trials completed to date, HupA was shown to be well tolerated. Most adverse effects were related to the well-known cholinergic activity of this class of drug, and were mainly gastrointestinal in nature. Such adverse effects were generally of mild to moderate severity and transient. Unlike tacrine, HupA was not associated with dose-limiting hepatotoxicity. There were no clinically significant differences in abnormal laboratory parameters, vital signs or cardiovascular parameters between the study groups. Two US Phase I studies have been conducted to determine the safety and tolerability of HupA. Adverse symptoms included tachycardia, low energy levels, dry mouth, and hypertension at multiple dose ranges; bradycardia, headache, and intense dreams at a dose of 400 μg b.i.d.; muscle cramps at 400 μg b.i.d.; arthralgia at 300–400 μg b.i.d.; and nausea, drowsiness and diarrhea. Most of these adverse effects were rated as mild (Mazurek 2000).

In our opinion, there are some limitations to this meta-analysis. These include the small amount of available data for inclusion in the meta-analysis, the small sample size of individual trials, treatment duration shorter than 24 weeks, which has been suggested as a key time point for medication assessment, and some unreported variance estimates of effect imputed based on the suggestions of Dean Follmann et al. 1992, all of which influence the robustness of the estimate of treatment effect. In addition, only MMSE and ADL scales were used to evaluate the efficacy of HupA because the other scales used in the studies were too divergent for a meta-analysis. MMSE has good reliability and validity when used for screening for dementia but is not considered to be an ideal outcome measure for AD drug trials because it is not designed to measure more subtle changes in cognition (Schneider 2001). Also, as the number of studies is quite small, there is a danger of over-fitting in meta-regression. The associations derived from meta-regression are observational, thus more evidence is needed to reach reliable conclusions (Thompson and Higgins 2002). Finally, the data for meta-analysis came solely from published scientific literature, so there might be a publication bias. The effect of publication bias was not evaluated owing to the low levels of data.

Compared with the previous meta-analysis (Li et al. 2008), we excluded one center report (Liu et al. 1995) and two trials with less than 20 participants in each arm (Dong et al. 2002; Zhou et al. 2004), and included one randomized controlled trial in this study (Zhang et al. 2006). The results of this meta-analysis indicated that administration of HupA for at least 8 weeks might lead to a significant improvement in the cognitive function and ADLs of patients with AD, and longer duration of treatment might have better

efficacy within the duration of 6–24 weeks. Most adverse events were cholinergic in nature and were generally of mild severity and brief in duration. More convincing evidence could be expected from the phase II trial in the US which has not yet been published.

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