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Analgecine, the extracts of *Vaccinia*-inoculated rabbit skin, effectively alleviates the chronic low back pain with little side effect – A randomized multi-center double-blind placebo-controlled phase 3 clinical trial

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ABSTRACT

Background: Chronic low back pain affects daily activities at home and workplaces and causes a huge economic burden. Current therapeutic options are very limited and the effects of available pharmacological agents are less than satisfactory. While NSAIDs might be effective for the short term and opioids might help with urgent pain relief and improving the life quality, their long-term use is associated with significant side effects and drug misuse or abuse. To seek alternative pharmacological agents for effective treatment, we examined the therapeutic potential of the extracts of *Vaccinia variola*-inoculated rabbit skin (*Analgecine*, abbreviated as *AGC*) in patients with chronic low back pain due to degenerative vertebral disorders.

Methods: In this randomized multi-center double-blind placebo-controlled phase 3 clinical trial (Chinese Clinical Trial Registry number 2009L01498), we enrolled patients (aged 26–70 years) with chronic low back pain for at least 3 months due to degenerative spinal (vertebral) disorders from 7 medical centers in China, and randomly allocated 459 participants to receive oral *AGC* or placebo for 28 days to study the efficacy and safety of *AGC*. Randomization was performed according to a centralized randomization schedule, which was blocked by study sites and generated by an unmasked statistician independent of study conduct and data analysis. Both participants and staff at each study site were masked to treatment assignment. The primary efficacy endpoint was the change of the mean pain intensity, based on an 11-point numerical rating scale, between the baseline and the last week of treatment, with the primary efficacy analysis of intention to treat. The ratio between exposed and unexposed groups was designed to be 3:1 in order to increase the likelihood of demonstrating the *AGC* effect upon repeated measures.

Results: 347 patients were assigned to receive *AGC* (4 units/tablet; 2 tablets twice a day) and 112 patients were to take placebo. Among them, 324 patients taking *AGC* and 112 receiving placebo completed the assessment. Patients receiving *AGC* reported significant pain relief at the end of week 2 and 3 compared

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to those taking placebo, with mean reduction of the pain scores as 1.7 *vs.* 0.9 at week 2 ($p < 0.0001$) and 2.8 *vs*. 1.2 at week 3 ($p < 0.0001$). A total of 47 *AGC*-treated patients reported 85 treatment emergent adverse events while 16 patients taking placebo reported 26 events, but no serious side effects were found to be related to *AGC* treatment.

Conclusion: Analgecine (*AGC*, 8 units twice daily) effectively alleviates chronic low back pain due to degenerative vertebral disorders when compared to placebo and is well tolerated by tested individuals, and can be considered as a first-line treatment for chronic low pain due to degenerative vertebral diseases.

1. Introduction

Lower back pain (LBP) is one of the most common reasons for physician consultation in developed countries [1]. Once LBP persists on most days in a 3-month period, it becomes chronic LBP (cLBP) and has detrimental effects over the life quality. Unfortunately, only one-third of LBP patients recover within the first 3 months and 65% of them still suffer from pain one year after onset [2]. Most cases of cLBP result from mechanical (degenerative) factors, *e.g.,* lumbar spondylosis, degeneration of intervertebral discs or facet joints, or spondylolisthesis *etc*.. The cLBP may also result from non-mechanical factors or systemic diseases, including neoplasms, infection, inflammatory, vascular or metabolic disorders. In terms of treatment, prescription of drugs is the first step to reduce the pain intensity, thereby facilitating the implementation of behavior changes and exercise. However, current treatment regimens are less than satisfactory and the choice for appropriate pharmaceutical agents can be confusing. For example, paracetamol is often recommended as the first-line therapy in many guidelines [3,4], but a recent meta-analysis has concluded with its ineffectiveness [5]. Evidences exist for the efficacy of NSAIDs; nevertheless, the long-term effect remains uncertain and the safety profile casts concerns over the prolonged use because of adverse effects [6–10], which increase in the incidence along with the age and dose [10,11]. Opioid drugs, including Tramadol, have also been legitimately used on cLBP and are indeed effective, at least for the short term [3,12–14]. Consequently, they have been increasing prescribed [15] but the long-term use is accompanied by risks for comorbidity, substance abuse and side effects. It has also been estimated that up to 40% of pain patients on chronic opioid therapy display aberrant drug-related behaviors [16] and this number is rising because the prevalence of chronic pain is increasing due to population aging [17]. Hence, US FDA has urged for developing high potency opioid (or alternatives) to address the risks of abuse, misuse, and the exposure of persons who are not opioid-tolerant [18]. The clinical efficacy of antidepressants and anticonvulsants has not been well established, at least when used as monotherapy [3,19], and a recent meta-analysis has invalidated the effect of antidepressants for cLBP [20]. Given the rates of disability from cLBP increase, it is imperative to respond to the growing disease burden by devising effective strategies and drugs.

In addition to the current pain killers in the west, a biological mixture trademarked as *Analgecine* (abbreviated as "*AGC*"; Vanworld Pharmaceuticals Ltd., China) has been clinically used in China as an analgesic. It is manufactured from the extracts of rabbit (*Oryctolagus cuniculus* of the *Leporidae* family) skin that has been inflamed by inoculation of *Vaccinia variola*. Despite the active ingredient remains to be characterized, small-scaled clinical trials have established the effectiveness against various somatic pain and neuropathic pain, [21,22] and importantly, no serious side effects have been reported. Its effect against chronic low back pain has not been examined by a well-designed randomized trial. In this study, we assessed the analgesic efficacy and safety of *AGC* for the treatment of cLBP in patients recruited from 7 medical centers in China.

2. Methods

2.1. Study design and participants

The study was a multi-center, double-blind, placebo-controlled, parallel-group, randomized phase III clinical trial (Chinese Clinical Trial Registry number 2009L01498). The study duration was designed to be 8 weeks, including a screening (wash-out) period (14 days), a treatment period (28 days), and a follow-up period (14 days). Participants were recruited from 7 medical centers in China: (1) Zhongshan Hospital of the Shanghai Fudan University, (2) the 6th People's Hospital of the Shanghai Jiao-Tong University, (3) the 3rd Hospital of the Hebei Medical University, Shijiazhuang, (4) the People's Hospital of Tianjin, (5) the Union Hospital of the Huazhong University of Science and Technology, Wuhan, (6) the Sun Yat-Sen Memorial Hospital of the Sun Yat-Sen University, Guangzhou, and (7) the Xijing Hospital of the 4th Military Medical University, Xian. The trial sites included orthopedic or pain clinics in the hospitals and clinical trial facilities. All patients had persistent chronic low back pain for more than 3 months from degenerative spinal (vertebral) diseases due to herniation of intervertebral discs (HIVD) or lumbar spondylolisthesis of isthmic, degenerative or traumatic types (confirmed by CT scan or MRI, excluding causes of cancer metastasis, metabolic factors or infection). They either had no surgery or had surgical treatment but still suffered from pain for more than 3 months after operation. All participants were aged between 18 and 70 years old and were able to give written consents. The pain severity was evaluated by an 11-point numerical rating scale (NRS) with the anchor points being 0 (no pain) and maximum being 10 (pain as bad as you can imagine) to describe "pain on average in the last 24 h". The baseline mean pain intensity score was calculated from the daily pain scores collected during 5 consecutive days at the end of the screening period (i.e., prior to the day 1 of treatment with AGC or placebo) and all participants had NRS 3 to 8 (both ends included). Those who had a baseline NRS varying by 2.5 points or more in any week during the screening period were excluded to avoid adverse changes in the effect size from enrolled patients with highly variable pain scores at baseline. Written consents were obtained from all participants before the study. The sample size of each group (exposed *vs.* non-exposed) was calculated to be minimally 68 (see "statistical analysis" below). Meanwhile, we increased the number of the exposed patients to maximize the possibility of observing treatment effects and therefore designed to recruit more than 300 patients for the exposure (treatment) group. Recruitment ended with enrollment of 459 patients for randomization and 347 were allocated to receive *AGC* treatment while 112 were to receive placebo (ratio 3:1). The trial protocol was approved by the institutional research ethics committee of each hospital, as well as by the China FDA. The

inclusion and exclusion criteria, and exit criteria were detailed in the Appendix 1.

2.2. Randomization and masking

Randomization was conducted through a centralized randomization schedule, blocked by study site, to one of two treatment groups: *Analgecine* (*AGC*) tablet twice daily or placebo. The randomization schedule was generated by an unmasked statistician at the Yushi Medicinal Technology Co. Ltd (a Contract Research Organization at Guangzhou, China), which was independent of the study conduct and data analysis. The random allocation sequence was implemented by sequentially numbered containers. The tablets of *AGC* or placebo were prepared by a central pharmacy at the InCROM Group, China, as appropriately labeled patient packs and distributed to each clinical site. Both patients and staff at each site were masked to treatment assignment. Study personnel responsible for the operation of the study were also masked to treatment assignment from randomization.

2.3. Procedures

After randomization, patients completed efficacy endpoints, patient-reported outcome measures and then received a treatment pack containing either *Analgecine* (*AGC*) tablets or placebo tablets (identical to *AGC* tablets). We instructed participants to take two tablets (*AGC* 4 units *per* tablet, Appendix 2; or placebo) by mouth twice daily (morning and evening) after a meal. We gave patients a diary card from the first week of treatment to record their daily pain intensity and time of study drug administration. The diary allowed up to 8 days of information to be recorded, for flexibility in scheduling of clinic visits. Doses were then self-administered until the next clinic visit (day 7). On day 7, we collected the diary card and gave patients a new diary card for the treatment of next week. This pattern of clinic visits was continued weekly to collect the final pain diary entry and tertiary efficacy patient-reported questionnaires completed in the evening of the scheduled last day (*i.e.*, day 28). Patients attended their final clinic visit on scheduled day 42 for follow-up pain assessments. The primary efficacy endpoint was the change in the mean pain intensity between baseline and the final week of dosing. Secondary efficacy endpoints were onset and maintenance of effect, as defined by the decrease in the mean pain intensity throughout the entire treatment period, and the proportion of patients achieving 30% or greater reductions in mean pain intensity compared to baseline. In a post-hoc analysis, we calculated the proportion of patients achieving 50% or greater reductions in mean pain intensity compared to baseline. The tertiary efficacy endpoint was the time to 30% decrease in mean pain intensity from baseline. Treatment-emergent adverse events were defined as adverse events that began or worsened in severity after at least one dose of the study drug had been administered. We analyzed the reduction of numerical rating scale (NRS) each week and set a stopping guideline of $P < 0.001$ (between exposed and un-exposed groups) for benefit on the primary endpoint for consecutive 2 weeks. For this purpose, we performed the interim analysis at the end of each week till week 3 and in order to control the overall type 1 error under 0.05, the α spending function was chosen to be 0.001 at week 2 and 3. The blood cell counts and serum biochemistry were also studied at the end of the trial.

2.4. Statistical analysis

The primary efficacy analysis according to the statistical analysis plan included all randomized patients (intention-to-treat principle) and used last observation carried forward imputation. Consequently, in a post hoc primary efficacy analysis, we did baseline observation carried forward imputation and included all randomized patients. For the secondary endpoint of mean weekly pain intensity, we used a mixed model repeated measures analysis to assess the mean profile over time for each treatment group. The fixed effects included in the linear model were: age, sex, treatment, visit, and the interaction between treatment and visit. The random effect was patient. The variance covariance structure for the mixed model was chosen on the basis that it minimized the Akaike information criteria and the Bayesian information criteria. For mean pain intensity over time, the unstructured variance covariance model minimized both sets of criteria, and was therefore used for the mixed model repeated measures analysis. Differences between groups were analyzed with the use of mixed model repeated measures analysis and Dunnett's multiple-comparison post hoc test. With post-hoc sensitivity analysis, we matched the placebo (unexposed) patients to *AGC* (exposed) patients by age and sex in a ratio of 1 to 1 (112:112) based on the logit of the propensity score using a logistic regression model. Cohen's f^2, a measure of local effect size, was calculated using a multiple mixed repeated measures model [23].

Interim analysis was conducted at the end of each week. Furthermore, a stopping guideline was specified when significant statistical difference in the pain reduction was observed between the treatment and placebo groups with $P < 0.001$ for consecutive 2 weeks. A responder was defined as any patient who achieved a 30% or greater (or a 50% or greater reduction in separate analysis) in the mean pain intensity from baseline to the final week, with last observation carried forward for missing observations. We used multiple logistic regression to compare the proportion of patients responding in each treatment group with covariates of age and sex and the pre-specified measurement of the treatment effect was the adjusted odds ratio. We also calculated the unadjusted odds ratio. Finally, we have reported the comparison of the proportion of patients responding in each treatment group as the unadjusted relative risk for ease of interpretation. We estimated the distribution of the tertiary efficacy endpoint of time to 30% decrease in mean pain intensity from baseline with the Kaplan–Meier method. We used a Cox proportional hazards model with age and sex as covariates to test the difference between the treatment groups with respect to the distribution of time to a 30% or greater reduction in weekly mean pain intensity, and we established an adjusted hazard ratio. Data handling and associations were performed using the SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). We calculated the sample size of 68 patients *per* group with the assumption of a mean pain intensity (numerical rating scale) difference between the two groups of 1.00 units (SD 1.60), which provided 95% power to detect such a difference with a two-sample *t* test with a two-sided type I error of 0.05. However, according to ICH guidelines, we increased the sample numbers of the exposed patients and allocated randomly 347 and 112 for the treatment and placebo group, respectively.

2.5. Role of the funding source

Data collection and some analyses reported here were done by an independent contract research organization (Yushi Medicinal Technology Co. Ltd), and most statistical analysis was conducted by an independent statistician at the Kaohsiung Medical University. Blood cell counts and serum biochemistry were analyzed at the hospitals where the trials were conducted.

3. Results

Between Oct 2013 and July 2014, 483 patients from 7 medical

centers were assessed for eligibility and 24 were excluded because of not meeting the inclusion criteria (n = 19) or declining to participate (n = 5). Therefore, 459 participants for randomization and 347 were allocated to receive *AGC* while 112 were to receive placebo treatment. After allocation, 6 in the *AGC* group and 3 in the placebo group withdrew consents. During follow-up, 17 in the *AGC* group and 5 in the placebo group were excluded due to loss for follow-up visits, acute infection or non-adherence to the specified timeline for medication. Therefore, 324 receiving *AGC* and 104 receiving placebo completed the follow-up assessment (Fig. 1).

Analyzes of the participants, including 23 in the AGC group and 8 in the placebo group who were lost for follow-up, showed that there was no difference in the personal characteristics when comparing *AGC*-treated and placebo-treated patients, including age, sex, body height or weight, habits of smoking or alcohol use. None of the participants had concomitant treatment for their cLBP, and no difference either in the percentage of patients receiving treatment for existing chronic diseases (15% *vs.* 13.4% in *AGC*-treated and placebo-treated patients for, *e.g.*, hypertension and/or diabetes, respectively). The proportion of participants between *AGC* and placebo group contributed by any individual center did not differ either. The baseline pain intensity was also similar between groups (Table 1). Analyses of the primary efficacy endpoint demonstrated that the changes of the mean pain intensity (NRS) from baseline at week 2 and 3 were significantly higher with *AGC* treatment in comparison to placebo treatment (5.4–3.7 *vs.* 5.4 to 4.5 at week 2, and 5.4 to 2.6 *vs.* 5.4 to 4.2 at week 3, Table 1), based on last observation carried forward imputation. Consistently, the weekly percentage of NRS reduction was also significantly higher in *AGC* than placebo group (31.6% *vs.* 17.1% and 54.7% *vs.* 23.2% at week 2 and 3 compared to the baseline, respectively; Table 2), indicating a significant time-dependent pain reduction for *AGC*, which was also demonstrated in Fig. 2 showing the time course for change from baseline in weekly pain intensity. Because significant improvement with *AGC* treatment was observed for consecutive 2 weeks ($p < 0.0001$ between *AGC* and placebo at both week 2 and 3, Fig. 2), which fulfilled the pre-specified criterion for early termination, we stopped the trial at the end of the 3rd week. Analyses of the secondary endpoint which was directed at the onset and maintenance of therapeutic effects showed that a greater percentage of patients receiving *AGC* achieved 30% or greater reduction of the pain intensity in week 3 of treatment compared to baseline (Table 2 and Fig. 2). For the *AGC*-treated group, the Kaplan–Meier method estimated a median time to achieve a 30% or greater reduction in mean pain intensity of 14 days (95% CI 14–21); however, for the placebo group the median time could not be estimated because less than 50% of patients achieved a 30% or greater reduction within the 21-day treatment period (Table 2 and Fig. 3). We have also calculated the proportion of patients achieved 50% reduction of the pain intensity, because a response rate of 50% was often used in meta-analysis of pain research. Again, a higher percentage of patients receiving *AGC* achieved 50% or greater reduction than the placebo group (Table 2). Calculation of the numbers needed to be treated (NTT), *i.e.*, the number of patients that need to be treated for one to benefit from the treatment when compared with a control, showed that at week 3 the NTT was 2.20 and 2.15 for 30% and 50% or higher responder rates, respectively (Table 2), implying the presence of therapeutic benefits that might outweigh the risks from potential side effects.

In terms of treatment-emergent adverse events, 47 patients receiving *AGC* reported 85 treatment-emergent adverse events (Table 3). Among 324 tested individuals, 17 (5.2%) had acute upper airway infection complicated with acute bronchitis and/or pneumonia (classified as “major adverse events”) and all recovered with no sequela. The remaining events were regarded as minor and were listed in Table 3. In the placebo group, 16 patients reported 26 treatment-emergent adverse events. Among the 104 placebo-treated individuals, 6 had acute airway infection complicated with bronchitis/pneumonia and 20 had minor events, as similarly described to those in *AGC*-treated group (Table 3). Overall, no significant difference in the frequency of treatment-emergent adverse event was noticed between two groups. Furthermore, no definite cause-effect existed between the occurrence of any adverse event and the treatment (data not shown). Blood cell count and serum biochemistry at the end of the treatment showed that patients in the *AGC*-treated group did not have any abnormal value in either blood cell count or biochemical parameters (*e.g.*, liver and renal functions; Table 4). No abnormality was observed from the routine

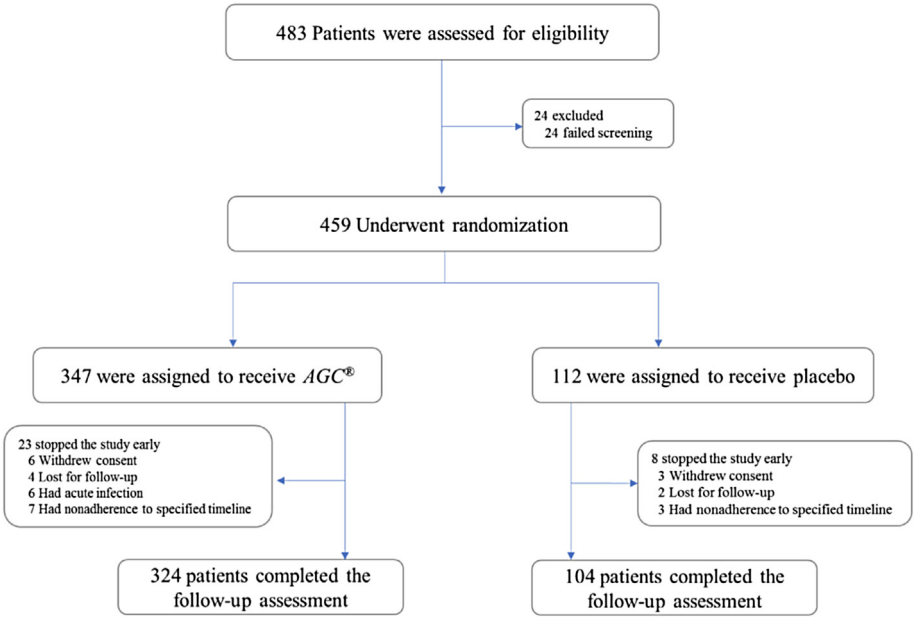

Fig. 1. Enrollment outcome.

Table 1
Characteristics of the study participants.

	AGC	Placebo	P value
	n = 347	n = 112	
Age (SD), years	50.0 (12.1)	50.7 (10.9)	
Sex, n (%)			
Males	124 (35.7)	45 (40.2)	
Females	223 (64.3)	67 (59.8)	
Height (SD), cm	164.1 (7.7)	163.3 (7.6)	
Weight (SD), kg	63.8 (10.5)	63.3 (10.3)	
Body mass index (SD), kg/m^2	23.6 (3.2)	23.7 (3.3)	
Cigarette use, n (%)			
Non-smoker	282 (81.3)	90 (80.3)	
Current smoker	61 (17.6)	20 (17.9)	
Ever smoker	4 (1.1)	2 (1.8)	
Alcohol use, n (%)			
Non-drinker	305 (87.9)	94 (83.9)	
Current drinker	40 (11.5)	18 (16.1)	
Ever drinker	2 (0.6)	0 (0.0)	
History of drug allergy, n (%)			
No	331 (95.4)	102 (91.1)	
Yes	16 (4.6)	10 (8.9)	
Drug treatment of low back pain, other, n (%)			
No	347 (100.0)	112 (100.0)	
Yes	0 (0.0)	0 (0.0)	
Drug treatment of chronic disease, n (%)			
No	295 (85.0)	97 (86.6)	
Yes	52 (15.0)	15 (13.4)	
Center, n (%)			
1	63 (18.2)	20 (17.9)	
2	17 (4.9)	6 (5.4)	
3	72 (20.8)	23 (20.5)	
4	54 (15.6)	18 (16.1)	
5	61 (17.6)	18 (16.1)	
6	71 (20.5)	24 (21.4)	
7	9 (2.6)	3 (2.7)	
Numerical rating scale (SD)			
Baseline	5.4 (1.3)	5.4 (1.2)	0.9390
Week 1	4.6 (1.3)	4.8 (1.3)	0.1306
Week 2	3.7 (1.4)	4.5 (1.4)	<0.0001
Week 3	2.6 (1.6)	4.2 (1.6)	<0.0001
Numerical rating scale change % (SD)			
Week 1 *vs.* Baseline	13.6 (15.4)	9.8 (15.2)	0.0525
Week 2 *vs.* Baseline	31.6 (14.3)	17.1 (15.3)	<0.0001
Week 3 *vs.* Baseline	54.7 (22.3)	23.2 (19.8)	<0.0001

Note: SD, standard deviation; %, percentage. Data of continuous and categorical variables were analyzed by *t* test or Wilcoxon rank-sum test and chi-square test or Fisher's exact test to make comparisons between groups, as appropriate.

urine analyses either (data not shown). Interestingly, the placebo group had a higher incidence of low red blood cell count (10 patients) and lower-than-normal hemoglobin values (12 patients including the 10 with a low red blood cell count; Table 4). All these 12 patients were post-menopause women. No difference was noted in white blood cell or platelet counts. Furthermore, while the incidence for the higher-than-normal serum creatinine level was similar between two groups, the incidence for the higher-than-normal BUN was higher in placebo-treated than *AGC*-treated patients (9.6% of patients receiving placebo *vs.* 4% patients receiving *AGC*; Table 4). The reason remains unknown but there appeared to be no disturbance to the daily activities of these patients (information obtained from interviews at the follow-up period). There was also no significant difference in the incidence of abnormal electrocardiography readings (*e.g.*, arrhythmias, inverted T waves, prolonged PR or QT intervals) between 2 groups (Table 4).

4. Discussion

Treatment for chronic pain, including cLBP, is difficult. Reports for the effectiveness of available drugs, which may not have considered the heterogeneity of cLBP, demonstrate at most modest improvement in lessening the pain with minimal help in the physical and emotional functioning [24]. Evidence indeed exists for the effectiveness of NSAIDs and certain opioid drugs but the associated side effects have significant clinical consequences [20]. The search for an effective pharmacological agent with little side effect is therefore in need. Our study clearly shows that *AGC* can effectively ameliorate cLBP resulting from degenerative vertebral diseases. The pain-killing effect was evident after 2 weeks of treatment ($p < 0.0001$ compared to placebo), a time point when a decrease of 1.7 points in NRS was achieved, in contrast to a decrease of 0.9 in the placebo group (Table 1). In the time-to-event analysis (Table 1), we included 23 patients in the AGC group and 8 patients of the placebo group that stopped the trial early. We have also calculated the total sample size of 459 patients (AGC n = 347 and placebo n = 112) with a change in NRS pain score from baseline to week 3, with a difference of 1.60 units (AGC group mean = −2.76, SD = 0.96; placebo group mean = −1.16, SD = 0.91), which provides >99% power to detect such a difference with a two-sample t test and a two-sided type I error of 0.05. It was interesting to note that treatment for an additional week further reduced the pain intensity. Therefore, the primary efficacy endpoint was achieved irrespective of the data imputation method. Analyses for the secondary efficacy endpoint demonstrated that 83.9% *AGC*-treated patients had at least 30% reduction in the pain score at week 3, whereas only 38.4% of placebo-treated patients had a similar response (Table 2). Consistently, there were significantly more patients in *AGC*-treated group having more than 50% reduction of the pain intensity (54.5% *vs.* 8.04% in the placebo group; Table 2). The NNT (a treatment-specific measure reliably describing the difference between a treatment and a control in achieving a particular clinical outcome) for *AGC* treatment to achieve >30% reduction in pain intensity was 2.2 and that to achieve >50% reduction was 2.15 (Table 2), indicating potent effects of *AGC* treatment. We have further made stratification and analyzed the effect of gender, age and body mass index (BMI) over the primary efficacy endpoint and found that none of these factors affected the therapeutic effect of AGC treatment (*vs* placebo; Appendix Table 1).

The further reduction of the pain intensity at week 3 of *AGC* treatment is interesting. We do not have satisfactory explanations at the moment because pharmacokinetic data of *AGC* are not available. As a matter of fact, the studies on the pharmacokinetics or pharmacodynamics would be hardly possible because *AGC* is a biological mixture with unknown active ingredient(s). In spite of this, the efficacy of *AGC* to alleviate cLBP is established by this trial. We have also performed a post-hoc analysis to match the placebo (unexposed) patients to *AGC* (exposed) patients by age and sex in a ratio of 1 to 1 (112:112) based on the logit of the propensity score using a logistic regression model. Consistently, there was no difference in the personal characteristics and *AGC* treatment exhibited significant improvement compared to the placebo group at both week 2 and 3 (Appendix Table 2).

Despite ~25–26% of patients reporting treatment-emergent adverse events, which was not higher than placebo patients, there was no evidence for a higher incidence of direct drug-related side effects associated with *AGC* treatment (Table 3). *AGC* treatment therefore appeared safe and well tolerated. This is unsurprising because *AGC* has been clinically used in China for decades without reported undesirable effects. Furthermore, the chronic toxicology study of *AGC* in rats and dogs for continuous 3-month treatment revealed no major side effects either (data not shown). In this sense, *AGC* treatment is superior to the use of NSAIDs and opioids. Taking intestinal function for an example, NSAIDs treatment causes a higher relative risk of gastrointestinal diseases. [6] meanwhile, constipation could happen in 15–90% patients receiving opioid treatment and may occur within weeks of use [25–27]. In contrast,

Table 2
Summary of efficacy parameters.

	AGC n = 347	Placebo n = 112	Difference of least square means (SE; 95% CI)	P value	RR or AHR
Change in numerical rating scale (*per* week) from baseline to week 3	−0.92 (0.03)	−0.38 (0.06)	−0.63 (0.09; −0.80 to −0.45)	<0.0001	
Effect size (Cohen's f^2)[a]	–	–	0.13		
Patients with a ≥30% or ≥50% reduction in numerical rating scale from baseline to week 3					
≥30%	291 (83.9%)	43 (38.4%)	–	<0.0001	2.18 (1.94–2.46)[b]
≥50%	189 (54.5%)	9 (8.04%)	–	<0.0001	6.78 (4.94–9.31)[c]
NNT to achieve ≥30% reduction in pain at week 3 based on the numerical rating scale (95% CI)	2.20 (1.83 −2.83)	–			
NNT to achieve ≥50% reduction in pain at week 3 based on the numerical rating scale (95% CI)	2.15 (1.89 −2.62)	–			
Median time (days) to at least 30% decrease of the numerical rating scale from baseline (95% CI)	14 (14–21)	–		0.0004	0.50 (0.35–0.74)

Data are least square means (standard error (SE)) or n (%), unless otherwise indicated.
RR = relative risk; AHR = adjusted hazard ratio; NNT = number needed to treat.
The tertiary efficacy endpoint of time (week 2). For comparison between *AGC* and placebo treatment, the Kaplan–Meier method was used to estimate event curves for each group, and the log-rank test was used to test the homogeneity between event curves.
[a] Cohen's f^2, a measure of local effect size, calculated using a multiple mixed repeated measures model. According to Cohen's (1988) guidelines, $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively.
[b] The unadjusted odds ratio was 8.34 (95% CI 5.18–13.42; $p < 0.0001$), and the adjusted odds ratio was 8.54 (5.27–13.86; $p < 0.0001$).
[c] The unadjusted odds ratio was 13.69 (95% CI 6.71–27.94; $p < 0.0001$), and the adjusted odds ratio was 14.08 (6.85–28.91; $p < 0.0001$).

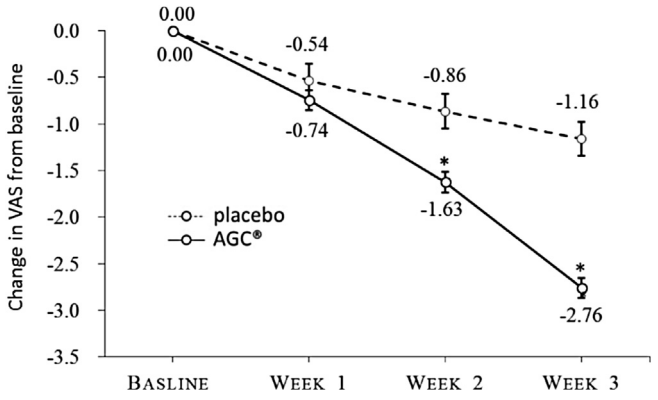

Fig. 2. The mean change in the numerical rating scale from baseline by week of treatment. Data are the mean change from baseline in average weekly numerical rating scale *per* week of treatment using the mixed model repeated measures analysis. *AGC vs.* Placebo (*P < 0.0001 at week 2 and 3).

no higher incidence of gastrointestinal complications was associated with *AGC* treatment (Table 3). *AGC* treatment did not lead to changes in the blood cell counts or important biochemical parameters (for liver and renal functions), or abnormal EKG readings either (Table 4). Intriguingly, however, more placebo-treated patients had lower-than-normal RBC counts and hemoglobin (Hb) at the end of the trial, as well as higher-than-normal levels of blood urea nitrogen (BUN; Table 4). The reason is unclear and we noticed that those having low RBC and Hb were all post-menopause women and those having high BUN were all male (in both *AGC* and placebo groups), and none of them had abnormal blood cell counts or BUN levels prior to treatment (data not shown). Whether or not this implies that AGC may contain other molecules with additional biological functions deserves further investigation. In any case, no serious side effects could be attributable to *AGC* treatment.

Early stopping of a clinical trial for evidence of benefit has been widely debated in the medical literature [28–31]. The treatment period of this study was initially designed to be 4 weeks but the trial was stopped one week earlier because the results at week 2 and 3 were consistent with the pre-specified stopping guideline. Furthermore, the level of statistical significance observed at week 2 and 3 minimized the concerns that the findings could be reversed or simply reflected the play of chance. Moreover, after matching the placebo patients to *AGC* patients by age and sex in a ratio of 1:1 using a logistic regression model, the conclusion still stood. Finally, the results were consistent with previous trials of *AGC*, despite being in a small scale, for treating other types of pain [21,22]. An additional potential limitation of this trial, as seen in most trials for pain evaluation, is the use of the NRS to measure the pain severity which, similar to the visual analog score (VAS), evaluates only a particular component of the pain intensity and therefore does not capture the complexity and idiosyncratic nature of the pain experience from symptom fluctuations. Nevertheless, such potential imprecision could have been disregarded by the remarkable difference in the NRS reduction between the exposed and non-exposed groups at the end of week 2 and 3.

We have compared the effects of AGC with the results from trials studying the effects of other drugs, although the treatment period between different trials varied and the parameters for determining efficacy might differ. We used the I^2 statistics to assess the heterogeneity between trials. The I^2 index has been proposed to quantify the degree of heterogeneity in a meta-analysis [32], and values higher than 50% were defined to identify high heterogeneity. A meta-analysis with $I^2 = 0$ means that all variability in effect size estimates is due to sampling error within studies (homogeneity hypothesis). Furthermore, percentages of around 25% (I2 = 25), 50% (I2 = 50), and 75% (I2 = 75) are regarded to represent low, medium, and high heterogeneity, respectively. In the meta-analysis, we calculated weighted mean differences and 95% confidence intervals and used the random effects model to pool estimates for each analysis obtained with Comprehensive Meta-Analysis version 2.2.048 (Englewood, NJ, 011). The comparison clearly revealed that in terms of the magnitude in pain relief (*vs.* placebo), *AGC* is more effective than paracetamol, regardless for immediate (<2 weeks) or short term efficacy (>2 weeks but < 3 months); furthermore, for the short term efficacy, *AGC* (3 weeks *vs.* placebo; mean difference −1.61, 95% CI −1.96 to −1.26) is equivalent to oxycodone (12 weeks *vs.* placebo; mean difference −1.20, 95% CI −1.89 to −0.51) (Table 5 & Appendix Table 3). In terms of the change in

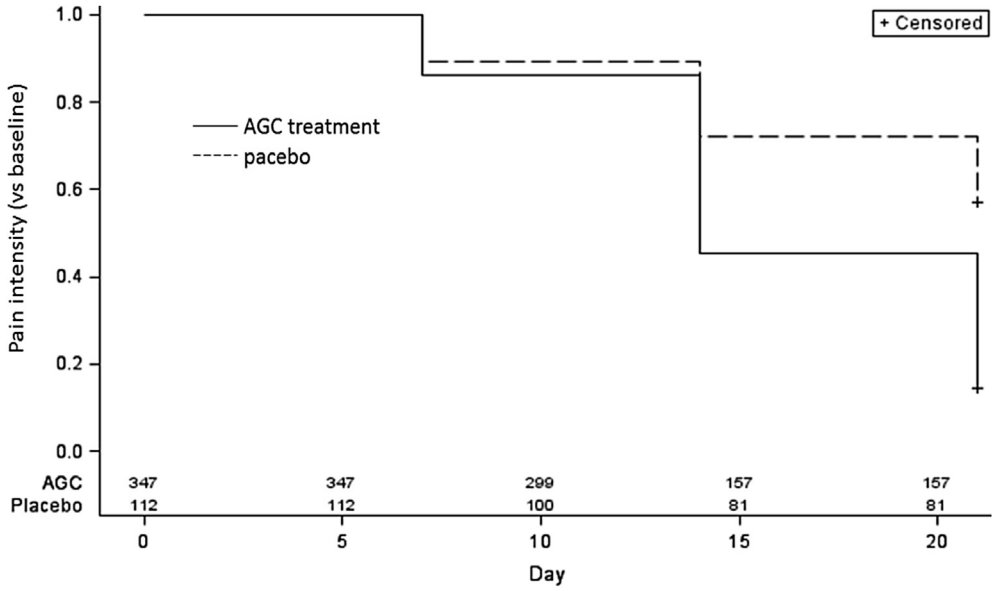

Fig. 3. The Kaplan–Meier plot for the tertiary efficacy endpoint of time vs pain intensity from the baseline. The number of patients in each group at indicated time points is given along the time axis.

Table 3
Total and specific treatment-related adverse events.

	AGC	Placebo	P
	N = 324	N = 104	
Total treatment-emergent adverse events, n	85	26	
Patients reporting one or more treatment emergent adverse events, n (%)	47 (14.5%)	16 (16.3%)	0.8738
Treatment-emergent adverse events by preferred term and organ system, n (% of total patients)			
Major events			
Acute bronchitis/pneumonia	17 (5.2%)	6 (5.8%)	0.5936
Minor adverse events	68 (21%)	20 (19.2%)	0.7810
Gastrointestinal system			
Stomachache	12 (3.7%)	4 (3.8%)	1.0000
Nausea	11 (3.4%)	3 (2.9%)	1.0000
Abdominal discomfort	10 (3.1%)	3 (2.9%)	1.0000
Nervous system			
Headache	9 (2.8%)	3 (2.9%)	1.0000
Skin			
Itching	19 (5.9%)	6 (5.8%)	1.0000
Dermatitis	7 (2.2%)	1 (1%)	0.6859

Note: Data of categorical variables was analyzed by Fisher's exact test to make comparisons between groups.

Table 4
Percentage of patients with out-of-range blood cell count and biochemistry at the end of the trial (week 3).

Clinical and biochemistry parameters	*AGC*	Placebo	P value*
	n = 324 (%)	n = 104 (%)	
Blood cell count			
Red blood cell (RBC)	12 (3.7)	10 (9.6)	0.018#
White blood cell (WBC)	16 (4.9)	4 (3.8)	0.793
Platelet	11 (3.4)	5 (4.8)	0.553
Serum biochemistry			
Hemoglobin (Hb)	12 (3.7)	12 (11.5)	0.003#
Bilirubin (total)	20 (6.2)	6 (5.8)	0.881
Alanine transaminase (GPT)	13 (4.0)	3 (2.9)	0.771
Aspartate transaminase (GOT)	5 (1.5)	4 (3.8)	0.230
Blood urea nitrogen (BUN)	13 (4.0)	10 (9.6)	0.028#
Creatinine	8 (2.5)	2 (1.9)	1.000
Electrocardiography (abnormal readings)[a]	39 (12.0)	16 (15.4)	0.334

Note: *Categorical variables were analyzed by chi-square test or Fisher's exact test to make comparisons among groups, as appropriate.
#$p < 0.05$.
[a] Including arrhythmias, prolonged PR intervals, ST depression, or T wave inversion.

Table 5
Mean differences of *AGC*, Paracetamol, NSAIDs, Oxycodone, Buprenorphine and Duloxetine for the treatment of chronic nonspecific low back pain according to the reduction of the pain intensity *vs.* placebo or the change in the pain intensity *vs.* baseline[a].

	Mean difference (95% CI)
Parameter 1. Reduction of the pain intensity (*vs.* placebo)	
Immediate term	
AGC (2 weeks)	−0.77 (−1.07, −0.48)
Paracetamol (2 weeks) (1 trial)	0.10 (−0.12, 0.32)
Short term	−1.61 (−1.96, −1.26)
AGC (3 weeks)	
Paracetamol (4 weeks) (1 trial)	0.10 (−0.19, 0.39)
Oxycodone (12 weeks) (1 trial)	−1.20 (−1.89, −0.51)
Parameter 2. Change in the pain intensity (*vs.* baseline)	
Short term	
AGC (3 weeks)	−1.60 (−1.80, −1.40)
NSAIDs (4–12 weeks)[a] (4 trials, Mata-analysis $I^2 = 0.0\%$; $P < 0.0001$)	−1.20 (−1.50, −0.91)
Buprenorphine (8–24 weeks)[a] (2 trials, Mata-analysis $I^2 = 0.0\%$; $P = 0.0009$)	−0.75 (−1.18, −0.31)
Oxycodone (12 weeks) (2 trials, Mata-analysis $I^2 = 0.0\%$; $P < 0.0001$)	−0.84 (−1.19, −0.50)
Duloxetine (7–13 weeks) (2 trials, Mata-analysis $I^2 = 0.0\%$; $P = 0.0001$)	−0.94 (−1.04, −0.35)

Note: Immediate term = follow-up ≤2 weeks; short term = follow-up >2 weeks but ≤3 months; CI = confidence interval.
[a] Clinic trials, see Appendix Table 3.

pain intensity from baseline, the effect of *AGC* (3 weeks *vs.* baseline; mean difference −1.60, 95% CI −1.80 to −1.40) is equivalent to that of NSAIDs (12 weeks *vs.* baseline; mean difference −12.04, 95% CI −15.00 to −9.08), whereas *AGC* is more efficacious than opioid drugs, including Buprenorphine, Oxycodone and Duloxetine [mean difference −1.60, 95% CI −1.80 to −1.40 for *AGC* (3 weeks); mean difference −7.46, 95% CI −11.80 to −3.11 for Buprenorphine (24 weeks); mean difference −0.84, 95% CI −1.19 to −0.50 for Oxycodone (12 weeks), and mean difference −0.94, 95% CI −1.04 to −0.35 for Duloxetine (13 weeks)] (Table 5 & Appendix Table 3). Collectively, *AGC* exhibits potent pain-killing capacity against cLBP and the effect is comparable to or superior to most of existing pharmacological agents, at least for the immediate and short terms.

In summary, the extracts of *V. variola*-infected rabbit skin (*Analgecine*, *AGC*) efficaciously ameliorate the chronic low back pain caused by degenerative vertebral disorders and, more importantly, it causes little side effect. Given its potency and safety, *AGC* can be considered in the future clinical practice as the first line treatment for cLBP, not only for the sake of patient benefits but also for reducing the prescription of opioids and the resultant drug misuse or abuse. Undoubtedly, *AGC* contains molecule(s) that possess analgesic properties, and it is likely to contain additional molecules or compounds that display other biological functions. *AGC* therefore represents a novel source of animal-derived products deserving investigation for drug discovery. This study also warrants the identification of the active component(s) in *AGC* contributing to the power pain-killing effect.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.conctc.2015.11.002.

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