

Quinazoline alkylthio derivative targets norepinephrine and calcitonin gene-related peptide to improve behavior and radiographic score in the rat model of cervical vertigo

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The present study investigated the effect of quinazoline alkylthio derivative (QAD) on cervical vertigo in the rat model and explored the underlying mechanism. Treatment of the cervical vertigo rats with QAD led to a significant ($P < 0.05$) improvement in balance beam score compared to the model group. Cervical vertigo-mediated reduction in CGRP level in rat plasma samples was effectively inhibited on treatment with QAD. Treatment with QAD led to a significant ($P < 0.05$) reduction in cervical vertigo-induced increase in ET-1 and NE levels in rats. An increase in NO production by cervical vertigo induction showed a significant ($P < 0.05$) decrease in rats by QAD treatment. The QAD treatment of the rats significantly ($P < 0.05$) inhibited cervical vertigo-induced increase in radiographic score on day 56. The radiographic score in cervical vertigo rats was decreased to 0.42 on treatment with QAD compared to 8.2 in the model group. Therefore, QAD treatment of the cervical vertigo rats improves behavioral score and inhibits radiographic score. It up-regulates CGRP expression and suppresses ET-1, NE and NO levels in a rat model of cervical vertigo. Thus, QAD can be used for the treatment of cervical vertigo however, further investigations are required to study the mechanism in detail.

Keywords: cervical vertigo, endothelin, norepinephrine, nitric oxide, chemotherapy

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Abbreviations: ANOVA, One Way analysis of variance; CGRP, Calcitonin gene-related peptide; ELISA, Enzyme-linked immunosorbent assay; ET-1, Endothelin; NE, norepinephrine; NO, Nitric oxide; QAD, Quinazoline alkylthio derivative

INTRODUCTION

Vertigo, a commonly diagnosed disease is a highly prevalent and most common complaint of patients received by doctors. Among various types of vertigo, cervical vertigo is induced by hyperplasia of the cervical bone and degeneration of the cervical disc which causes pain in the neck, dizziness, numbness, nausea and blurred vision (Huang *et al.*, 2020). In adults, the incidence of cervical vertigo is around 10% but it is more commonly diagnosed in elderly and middle-aged people throughout the world (Fernandez *et al.*, 2015). Changing people's lifestyle such as the development of intelligent mobile devices has led to the onset of cervical vertigo in the younger age group and a gradual increase in its

incidence rate (Kovacs *et al.*, 2019). Moreover, cervical vertigo has a serious impact on daily activities of people and put a heavy burden on the health care facilities (Kovacs *et al.*, 2019). The treatment available currently for cervical vertigo involves the prescription of drugs and surgical intervention. However, this treatment strategy has very poor efficacy and induces adverse side effects in the patients (Yin *et al.*, 2017).

Quinazoline as well as its synthetic derivatives have been found to be highly useful compounds in medicinal chemistry. These compounds have been found to possess distinct and diverse biopharmaceutical activities. Medicinal Chemists have prepared a wide range of compounds from quinazoline over the past decades which were investigated for pharmaceutical activities. Studies have revealed that quinazoline derived nitrogen-containing heterocyclic compounds possess many therapeutic properties including, anti-inflammatory, analgesic, anti-oxidant, anti-hypertensive, anti-diabetic activity (Alagarsamy *et al.*, 2007; Jatav *et al.*, 2008; Saravanan *et al.*, 2010). The present study investigated the effect of quinazoline alkylthio derivative (Fig. 1) on cervical vertigo in a rat model and explored the underlying mechanism. The study assessed the effect of quinazoline alkylthio derivative on behavioural changes, level of calcitonin gene-related peptide (CGRP), endothelin (ET-1), norepinephrine (NE), nitric oxide (NO) and X-ray score in rats.

MATERIALS AND METHODS

Reagents

Benzylpenicillin sodium and quinazoline alkylthio derivative were obtained from the North Chinese Pharmaceutical Co., Ltd. China and chloral hydrate from Sinochem Chemicals Co., Ltd., Shanghai, China. Sibelium tablets (5 mg/pill) were obtained from the Xi'an Janssen Pharmaceutical, Xi'an, China. The other common chemicals were procured from Sigma-Aldrich.

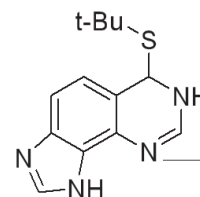


Figure 1. Chemical structure of quinazoline alkylthio derivative (QAD).

Rats, grouping and treatment

Sixty male Sprague-Dawley rats (7–8 weeks old; body weight 210 ± 10 g) were supplied by the Animal Center belonging to the Shandong First Medical University, Jinan, Shandong, China. The rats were acclimatized to the laboratory conditions for one week before starting the experiments. All the rats were kept in sterile cages at ~55% humidity, under $22 \pm 2^\circ\text{C}$ and exposed to 12 h light/dark cycles. The rats were given access to tap water and a standard rodent diet freely. All the experimental protocols on rats were conducted as per the guidelines issued by the Animal Ethics Committee for Care and Use of laboratory animals, Shandong First Medical University, Jinan, Shandong, China. The study approval was obtained from the Animal Ethics Committee of the Central Hospital Affiliated to Shandong First Medical University, Shandong, China. The rats were separated randomly into four groups of 15 each: control, model (cervical vertigo), QAD treatment and sibelium administered groups. The rats in the treatment group were given 2 mg/kg doses of QAD daily from the 8th to the 14th day of surgery in physiological saline through the intragastric route. Sibelium (Xi'an Janssen Pharmaceutical, Xi'an, China; Cat No.H10930003) was administered to rats at 0.45 mg/kg doses daily from the 8th to the 14th day of surgery. The rats in the control and model groups received only double distilled water in equal volumes.

Cervical vertigo induction in rats

Rats in the model, QAD treatment and sibelium administered groups were subjected to cervical spine surgery for induction of cervical vertigo. The previously reported method consisting of cervical instability development with little modification for reducing operative risk and chances of trauma was used for the establishment of cervical vertigo in rats (Song *et al.*, 2004). In brief, the cervical muscles of the rat on the posterior side were excised followed by the removal of inter transverse and interspinous ligaments at the C5–C6 level. The joint capsule between C5–C6 was wiped and put using scissors, and vertebral bodies on the upper and lower side were damaged to induce cervical spine instability. After wound suture, the rats were injected with penicillin antibiotic (40 000 U/kg; Phoenix Scientific) to prevent infection. After 7 days of surgery, rats were put onto the balance beam to confirm successful induction of cervical vertigo. Development of cervical vertigo in rats was confirmed if rats were unable to move or stand on the beam, stopped on the beam, failed to cross the beam or abruptly stopped on the beam or shook on the beam. Blood samples were taken from the rats on days 14 and 28 of the surgery and stored in centrifuge tubes at -80°C for subsequent analysis.

Balance Beam Test

To determine behavioral changes in rats a balance beam apparatus having 2×80 cm dimensions was designed and suspended above the ground (Gu *et al.*, 2018). Before placing the rats on a wooden beam for assessment of their ability to traverse it, the head of the rats was moved up, down, left and right. Performance of rats on the beam was scored using the criteria: (i) no movement of rat on the beam, the body of rat shook on the beam, the rat moved slightly but couldn't traverse the beam, stooped and changed direction abruptly "was assigned 0 scores"; (ii) body of rat shook, rat stopped and abruptly changed the direction but traversed the beam

"was assigned 1.5 scores"; (iii) rat moved smoothly to traverse the beam "was assigned 3 scores". This test was conducted on days 7th, 14th and 28th of the surgery to assess the behavioral score.

Determination of CGRP level in rat plasma

The enzyme-linked immunosorbent assay kit was used for the detection of CGRP level in rat plasma samples on days 14th and 28th of the surgery. The assay uses a double-antibody sandwich technique for the detection of CGRP level in rat plasma in accordance with the instruction of the manufacturer.

Assessment of ET-1 Level in rat plasma

In rat plasma samples, the level of ET-1 was detected on days 14th and 28th of the surgery using an enzyme-linked immunosorbent assay (ELISA) kit according to the instructions of the manufacturer.

Assessment of NE Level in rat plasma samples

To a 40 μl sample of plasma was added anti-luteinizing hormone antibodies (10 μl) and streptavidin-HRP (50 μl). The ELISA kits were used for the assessment of NE level in the plasma samples according to the manufacturer's instructions.

Determination of NO level in rat plasma

The nitrate reductase method was used for the determination of NO level in the plasma samples of the rats. Briefly, a plasma sample (100- μl) was taken in a test tube containing nitrate reductase, mixed thoroughly and then incubated at 37°C in a water bath. The solution was then allowed stand for 10 min at room temperature and subsequently centrifuged to collect the supernatant. Development of color was followed by supernatant mixing and measurement of absorbance at 550 nm to calculate the NO level.

Determination of cervical spine X-ray score

Sodium pentobarbital (1% solution, 40 mg/kg) was injected into the rats for anesthetization before recording the X-ray. The cervical spine X-ray score was calculated using the following criteria: (i) development of physiological lordosis: appearance of cervical physiological lordosis was assigned score 0, physiological lordosis straightening was assigned score 1, disappearance of cervical physiological lordosis was assigned score 2, disappearance of cervical physiological lordosis completely was assigned score 3; (ii) Narrowing of disc space: normal spacing was assigned score 0, mild narrowing of space (10% narrowing) was assigned score 1, moderate space narrowing (30% narrowing) was assigned score 2 and severe space narrowing (50% narrowing) was assigned score 3; (iii) Osteophytes in vertebral bodied: no osteophytes was assigned score 0, mild osteophytes was assigned score 1, presence of moderate osteophyte was assigned score 2 and large osteophytes was assigned score 3; (iv) Formation of sclerosis and osteophytes in uncovertebral joints and facet joints: no changes was score 0, joint blurring was assigned score 1, mild formation was scored 2, evident and high changes was scored 3.

Statistical Analysis

The data are expressed as the mean \pm S.D. of triplicate measurements. All the data were analyzed using the SPSS 19.0 software. Differences between the groups

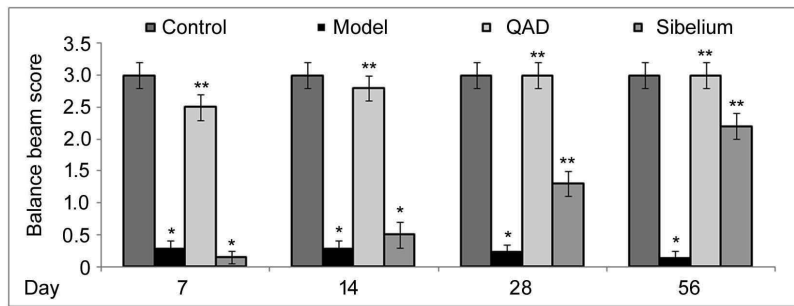


Figure 2. Effect of QAD on balance beam score in the cervical vertigo rat model.

A balance beam test was used to calculate the balance beam score in rats after 7 days of surgery (without drug administration), following 14, 28 and 56 days of QAD and Sibelium administration. * $P<0.05$, ** $P<0.01$ vs. model group.

were determined statistically using One-Way analysis of variance (ANOVA) followed by Bonferroni tests and Student's *t*-test. The differences at $P<0.05$ were taken significant statistically.

RESULTS

QAD treatment increases balance beam score in the cervical vertigo rat model

The performance of the rats in the balance beam test was measured after 7 days of surgery without administration of the drugs (Fig. 2). Rats in the model, QAD and Sibelium groups showed significantly ($P<0.05$) lower balance beam scores after 14 days of surgery compared to the control group. However, after 14, 28 and 56 days of QAD and Sibelium administration rats showed a significant ($P<0.05$) increase in balance beam score compared to the model group. Moreover, the increase in balance beam score by QAD treatment was greater compared to the Sibelium-administered group.

QAD treatment increases plasma CGRP level in the cervical vertigo rat model

The model group of cervical vertigo showed significantly ($P<0.05$) lower CGRP level in the plasma samples on days 28 and 56 of the surgery compared to the control group (Fig. 3). Cervical vertigo mediated reduction in CGRP level in rat plasma samples was effectively prevented on treatment with QAD. Treatment with QAD raised CGRP level in cervical vertigo rat plasma samples close to that of the control group on days 28 and 56 of the surgery. Sibelium administration also elevated CGRP level significantly ($P<0.05$) in cervical vertigo rat plasma samples on days 28 and 56 of the surgery.

QAD treatment decreases plasma ET-1 level in the cervical vertigo rat model

The ET-1 level in rat plasma samples was determined on days 28 and 56 of the surgery using an ELISA assay (Fig. 4). The plasma samples of model rats showed significantly ($P<0.05$) elevated ET-1 levels on days 28 and 56 of the surgery compared to the control group. However, QAD-treatment led to a significant ($P<0.05$) reduction in cervical vertigo-induced elevation in ET-1 level in rat plasma samples on days 28 and 56. Administration of sibelium also suppressed cervical vertigo-induced upregulation of ET-1 level in rat plasma samples compared to the model group.

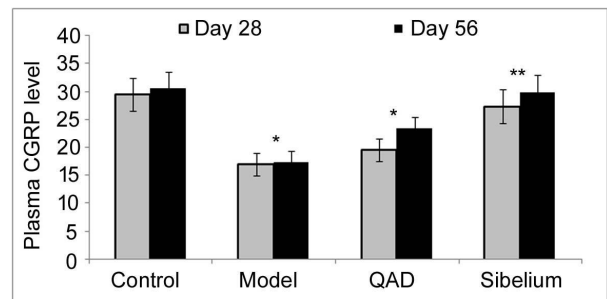


Figure 3. Effect of QAD on CGRP level in the cervical vertigo rat model.

The CGRP level in model, control, QAD-treated and sibelium administered rat plasma samples was determined on days 28 and 56 of the surgery using the ELISA method. * $P<0.05$, ** $P<0.01$ vs. model group.

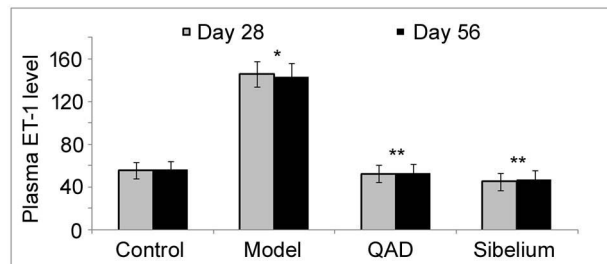


Figure 4. Effect of QAD on ET-1 level in the cervical vertigo rat model.

The ET-1 level in model, control, QAD-treated and Sibelium administered rat plasma samples was determined on days 28 and 56 of the surgery using the ELISA method. * $P<0.05$, ** $P<0.01$ vs. model group.

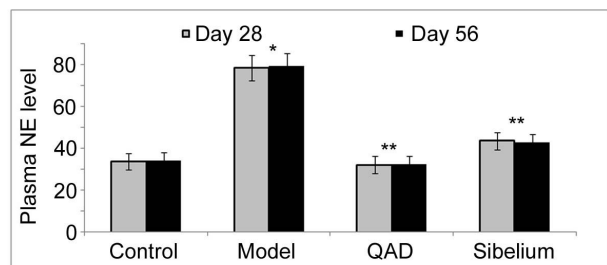


Figure 5. Effect of QAD on NE level in the cervical vertigo rat model.

The NE level in model, control, QAD-treated and sibelium administered rat plasma samples was determined on days 28 and 56 of the surgery using the ELISA method. * $P<0.05$, ** $P<0.01$ vs. model group.

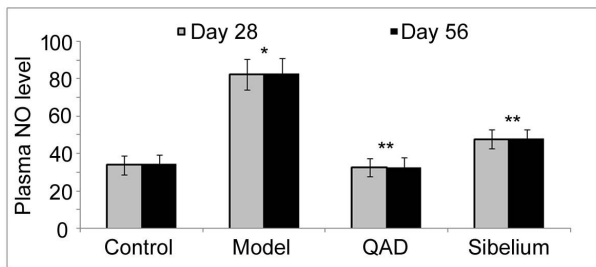


Figure 6. Effect of QAD on NO level in the cervical vertigo rat model.

The NO level in model, control, QAD-treated and sibelium administered rat plasma samples was determined on days 28 and 56 of the surgery using the ELISA method. * $P < 0.05$, ** $P < 0.01$ vs. model group.

QAD treatment suppresses plasma NE level in the cervical vertigo rat model

The model group of cervical vertigo showed significantly ($P < 0.05$) higher NE level in the plasma samples on days 28 and 56 of the surgery compared to the control group (Fig. 5). Cervical vertigo-induced increase in NE level in rat plasma samples was effectively inhibited by QAD treatment. Treatment with QAD reduced NE level in cervical vertigo rat plasma samples close to that of the control group on days 28 and 56 of the surgery. Sibelium administration also suppressed the NE level significantly ($P < 0.05$) in cervical vertigo rat plasma samples on days 28 and 56 of the surgery.

QAD treatment decreases plasma NO level in the cervical vertigo rat model

The level of NO showed a significant increase in plasma samples of the model cervical vertigo rat group on days 28 and 56 of the surgery compared to the control group (Fig. 6). Treatment with QAD significantly ($P < 0.01$) inhibited cervical vertigo-induced increase in NO level in the plasma samples on days 28 and 56 of the surgery. In QAD-treated cervical vertigo rats, the level of NO in the plasma sample was close to that of the control group. Again, administration of sibelium to the cervical vertigo rats reduced NO level in the plasma samples but the effect was less effective compared to QAD treatment.

QAD treatment decreases cervical spine X-ray score in the cervical vertigo rat model

The radiographic scores were compared in the model, control, QAD treated and Sibelium administered rats after 56 days of the surgery (Table 1). The model group showed a significantly ($P < 0.05$) raised radiographic score on day 56 of the surgery compared to the control rats. Treatment with QAD significantly ($P < 0.05$) prevented cervical vertigo-induced increase in radiographic score in rats on day 56. Sibelium administration also reduced cervical vertigo-induced increase in radiographic score in rats but the effect was less compared to the QAD-treatment group.

DISCUSSION

The present study induced cervical vertigo in rats using a previously known method of disturbing the cervical spine stability. This method of cervical vertigo induction is generally preferred because of having a high rate of

Table 1. Effect of QAD on cervical spine X-ray score in the cervical vertigo rat model. Cervical spine X-ray score in the model, control, QAD-treated and Sibelium administered rats was determined on day 56 of the surgery.

S. No	Group	Radiographic score
01	Control	0.00±0.00
02	Model	8.2±1.2
03	QAD	0.42±0.11
04	Sibelium	1.68±0.43

success and simple procedure (Ma *et al.*, 2020). Vertigo is commonly treated using the drug sibelium which prevents vasospasm of the vertebral basilar artery (Swartz *et al.*, 2005). Thus, the present study used sibelium, chemically known as flunarizine hydrochloride as the positive control.

Cervical vertigo has been demonstrated to be related to the cervical spine straightening or cervical kyphosis which leads to instability in the cervical spine during physiological load (He *et al.*, 2011). Instability in the cervical spine leads to cervical sympathetic excitation followed by vertebral basal artery spasm resulting in the development of cervical vertigo. Many humoral factors including CGRP, NE, ET-1 and NO are believed to play a crucial role in the pathogenesis and development of cervical vertigo (Fan *et al.*, 2019). The two important and powerful members of endogenously produced vasoconstrictor and vasodilator families are ET and CGRP (Parlapiano *et al.*, 1999). It is reported that ET and CGRP function together for the regulation of blood flow and maintaining vascular function (Parlapiano *et al.*, 1999). Studies have found that cervical vertigo development is closely associated with the imbalance in the production of ET-1 and CGRP and these two factors serve as important pathogenetic factors for the disorder (Casani *et al.*, 2021; Wang *et al.*, 2007). It has been found that regulation of ET-1 and CGRP levels in plasma dilates blood vessels of the brain as well as the inner ear thereby exhibiting a therapeutic effect on vertigo (Zhou *et al.*, 2018). Additionally, inhibition of plasma ET level and its receptors has also been shown to regulate the pathogenesis of cervical vertigo (Wang *et al.*, 2013). In the present study, QAD treatment led to a prominent improvement in behavioral score in the rat model of cervical vertigo compared to the model group. This was evident by the fact that after 7, 14 and 28 days of QAD treatment rats showed a significant increase in balance beam score in comparison to the model group. Although sibelium administration also improved behavioral score in the rat model of cervical vertigo but the effect was less efficient compared to the QAD treatment. Moreover, QAD treatment also inhibited cervical vertigo-mediated reduction in CGRP level in rat plasma samples efficiently. In QAD-treated rats cervical vertigo-mediated reduction in CGRP level in rat plasma samples was inhibited more effectively compared to the sibelium-administered group. The QAD treatment also led to a significant suppression in cervical vertigo-induced upregulation of ET-1 level in rat plasma. Although cervical vertigo-induced upregulation of ET-1 level in rat plasma samples was also inhibited by sibelium administration but the effect was less efficient compared to the model group. Therefore, QAD treatment improves behavioral score in cervical vertigo rats through up-regulation of CGRP level and suppression of ET-1 production in plasma.

Vertigo has different symptoms which depend mainly on changes induced in the sympathetic nervous system

activity (Savitz & Caplan, 2005). Development of lesions in the cervical spine results in sympathetic nerve excitation followed by artery spasm and posterior circulatory ischemia which ultimately causes dizziness and vertigo (Gu *et al.*, 2016). Moreover, excitation of the sympathetic nervous system is associated with the development of emotional problems (Gu *et al.*, 2016). It is reported that sympathetic activities are linked with the expression of NE (Gu *et al.*, 2018). Vasoconstriction is induced by NE through stimulation of $\alpha 1$ receptor present in the blood vessels and therefore NE serves as an indicator for sympathetic activation. Reduction in NE level reveals that abnormality in sympathetic activity is controlled while vasoconstriction is suppressed. In the present study cervical vertigo-induced increase in NE level in rat plasma samples was effectively inhibited by QAD treatment. Treatment with QAD reduced NE level in cervical vertigo rat plasma samples close to that of the control group on days 28 and 56 of the surgery. Sibelium administration also suppressed the NE level significantly in cervical vertigo rat plasma samples on days 28 and 56 of the surgery.

Blood vessels are dilated by NO thereby decreasing resistance and increasing blood supply under the conditions of acute cerebral ischemia. It is reported that cervical vertigo increases the level of NO in the blood plasma of the rats (Dumlu *et al.*, 2014). In the present study level of NO showed a significant increase in plasma samples of the model cervical vertigo rat group on days 28 and 56 of the surgery. However, treatment with QAD significantly inhibited cervical vertigo-induced increase in NO level in the plasma samples on days 28 and 56 of the surgery. Again, administration of sibelium to the cervical vertigo rats reduced NO level in the plasma samples but the effect was less effective compared to QAD treatment. Treatment with QAD significantly ($P < 0.05$) prevented cervical vertigo-induced increase in radiographic score in rats on day 56. Sibelium administration also reduced cervical vertigo-induced increase in radiographic score in rats but the effect was less compared to the QAD-treatment group.

CONCLUSION

In summary, the study demonstrates that QAD treatment of cervical vertigo rats improves behavioral score and promotes radiographic score. The mechanism involves up-regulation of CGRP expression and suppression of ET-1 level in the cervical vertigo rat plasma by QAD treatment. Moreover, the level of NE and NO was also inhibited by QAD treatment in the plasma of cervical vertigo rats. Therefore, QAD may be investigated further as a therapeutic agent for the treatment of cervical vertigo.

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