Effect of a Novel, Orally Active Matrix Metalloproteinase-2 and -9 Inhibitor in Spinal and Trigeminal Rat Models of Neuropathic Pain

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Aims: To study the effects of a novel matrix metalloproteinase-2 (MMP-2) and MMP–9 inhibitor, AQU-118, on mechanical allodynia in the spinal nerve ligation (SNL) model of neuropathic pain and the chronic constriction injury of the infraorbital nerve (CCI-IoN) model of neuropathic orofacial pain. **Methods:** Five groups of SNL rats were given daily oral doses of AQU-118 (5, 10, 20 mg/kg), gabapentin (100 mg/kg), or vehicle (0.5% methylcellulose) and then paw withdrawal threshold was measured with von Frey filaments (VF). Three groups of CCI-IoN rats were given daily oral doses of either AQU-118 (40 mg/kg), gabapentin (100 mg/kg), or vehicle (0.5% methylcellulose) and then mechanical allodynia was measured with facial VF and non–reflex-based orofacial stimulation test (OFST) assay. Naïve rats were also tested for the effect of AQU-118 (40 mg/kg) on basal sensitivity to mechanical stimulation/locomotive activity. **Results:** Mechanical allodynia in SNL rats was attenuated by gabapentin (100 mg/kg) and AQU-118 (in a dose-dependent manner). Mechanical allodynia in CCI-IoN rats was also attenuated (in an equipotent manner) by both AQU-118 (40 mg/kg) and gabapentin (100 mg/kg) as measured by both facial VF and OFST assay. Upon cessation of either AQU-118 or gabapentin, VF-related responses in both models and OFST assay times reverted to levels observed in vehicle-treated rats. No statistically significant change was observed in locomotive activity/paw withdrawal threshold by AQU-118 (40 mg/kg) in naïve rats. **Conclusion:** The results demonstrated that oral AQU-118 attenuates mechanical allodynia in both neuropathic pain models and with efficacies that mirror gabapentin at the 40 mg/kg dose used in the CCI-IoN model but without effect on basal sensitivity to mechanical stimulation/locomotive activity. These findings support a possible role for MMP-2/-9 in the etiology of neuropathic pain and also suggest that inhibition strategies represent a viable treatment option. *J Oral Facial Pain Headache 2015; 29:286–296. doi: 10.11607/ofph.1350*

Keywords: *allodynia, inhibitor, matrix metalloproteinase, MMP-2, MMP-9, operant, orofacial pain*

Neuropathic pain conditions affect both the spinal and trigeminal systems and can be resistant to management with currently available medications. This lack of efficacy dictates the need for new treatment approaches based on mechanisms that contribute to the development of neuropathic pain. Use of rodent models has implicated various inflammatory proteins such as cytokines and in particular certain matrix metalloproteinases (MMPs) in the development of neuropathic pain after nerve injury.[1–6] MMPs are a family of structurally related zinc-containing enzymes that have been reported to mediate the breakdown of connective tissue in normal physiologic processes such as embryonic development, reproduction, and tissue remodeling. Of particular interest is the observation that the parenteral administration by epineurial (EP), intrathecal, (IT) and intraperitoneal (IP) routes of injection of hydroxamate-containing MMP inhibitors can reduce mechanical allodynia in various surgically induced rodent models of neuropathic pain.[7–9] These include the chronic constriction injury (CCI), spinal nerve ligation (SNL), and the L5-spinal nerve crush (L5-SNC)

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Table 1 Protocol for SNL Study Using Male Sprague-Dawley Rats

Group	Rats (n)	Route	Dose* (mg/kg)	Compound	Dosing days	VF testing days‡
1	8	Oral	NA	Vehicle†	4–10	3,5,6,8,10-12,14
2	8	Oral	100	Gabapentin	4–10	3,5,6,8,10-12,14
3	8	Oral	5	AQU-118	4–10	3,5,6,8,10-12,14
4	8	Oral	10	AQU-118	4–10	3,5,6,8,10-12,14
5	8	Oral	20	AQU-118	4–10	3,5,6,8,10-12,14

*Once per day dosing via gastric gavage.
†0.5% methyl cellulose.
‡A baseline measure was taken via VF before surgery and then on day 3 after surgery. Testing was done on the same time each day and 1 hour after dosing on dosing days.
SNL = spinal nerve ligation; NA = not applicable; VF = von Frey filaments.

rodent models.[7–9] The reduction in mechanical allodynia produced by these MMP inhibitors is currently thought to be due to the inhibition of MMP-9 and/or MMP-2, both of which have been observed to be elevated after surgery.[8–10] MMP-9 knock-out mice show reduced sensitivity to mechanical allodynia after SNL and sciatic nerve crush. Additionally, membrane-type 5 MMP (MT5-MMP/MMP24, which specifically activates the inactive zymogen form of MMP-2) knock-out mice do not develop mechanical allodynia after sciatic nerve injury.[11] Taken together, these findings suggest the possible role of MMP-2 and MMP-9 in the generation of neuropathic pain and also that strategies to inhibit these MMPs may be effective in reducing neuropathic pain symptoms.

The aim of the present study was to study the effects of a novel MMP-2 and MMP-9 inhibitor, AQU-118, on mechanical allodynia in the SNL model of neuropathic pain and the CCI injury of the infraorbital nerve (IoN) model of neuropathic orofacial pain. AQU-118 (3-(1H-Indol-3-yl)-2-[5-(4-trideuteromethyl-phenylethynyl)-thiophene-2-sulfonylamino]-propionic acid) is a potent small molecule inhibitor of both MMP-2 and MMP-9 with an in vitro IC50 of 3 nM and 9 nM, respectively, and an in vivo oral bioavailability in rats of 44% at 20 mg/kg.[12] This study with AQU-118 encompassed several goals. The first goal was to evaluate the oral efficacy of AQU-118 in the SNL-rat model, because this model is able to differentiate between compounds that are useful in combating neuropathic pain in humans (gabapentin) from those that are not (indomethacin).[13,14] The second goal was to test AQU-118 in a common rodent model of orofacial neuropathic pain where the CCI lesion is performed with chromic suture to the IoN of the trigeminal system.[15,16] The lesion produces mechanical allodynia as commonly detected by von Frey filament (VF) stimulation of the vibrissal pad and so mimics the tactile allodynia reported by humans in experimental and clinical pain studies.[17,18] Lastly, it was important to include a non–reflex-based pain readout in the evaluation of orally administered AQU-118. Some recently published reviews have questioned the ability of animal models that rely solely on reflex-based readouts (ie, paw withdrawal, tail flick, and writhing behavior) to accurately predict human efficacy.[19,20] Thus, an animal model was included that uses an "operant" measure of pain sensitivity.[20,21] This operant model is the orofacial stimulation test (OFST) assay, which measures the time spent retrieving a sweetened milk reward while encountering a self-applied mechanical stimulus to the vibrissal pad in animals with a CCI-IoN lesion.[22–24] In addition, mechanical allodynia of the rodent's vibrissal pad was assessed with VF. This design allowed the use of the same positive control (oral gabapentin, 100 mg/kg) and rat species in an evaluation of oral AQU-118 efficacy on mechanical allodynia in both the CCI-IoN and SNL-rodent models.

Materials and Methods

Testing in the SNL Rat Model of Neuropathic Pain

The use of animals was approved by the Institutional Animal Care and Use Committee at PsychoGenics, Inc. Sprague-Dawley rats (200 to 225 g) from Harlan (Indianapolis, IN) were used in the study. Each treatment group incorporated an equal but mixed distribution of animals to be orally dosed with vehicle, gabapentin, and AQU-118 (Table 1). The doses of AQU-118 (5, 10, 20 mg/kg, QD) were chosen based on initial oral pharmacokinetic studies that showed linear oral bioavailability with doses up to 10 mg/kg then reaching saturation at the 20-mg/kg dose.

Spinal nerve ligation (SNL) surgery. Surgery was performed with aseptic procedures under general anesthesia with continuous inhalation of isoflurane. The skin at the area of the lower lumbar and sacral level of the rat was shaved and disinfected. A longitudinal incision at the lumbar level left of the vertebral column was made and the left paraspinal muscles

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Table 2 Protocol for CCI-IoN Study Using Male Sprague-Dawley Rats

Group	Rats (n)	Route	Dose* (mg/kg)	Compound	Dosing days	VF testing days‡	OFST testing days‖
1	12	Oral	NA	Vehicle†	7–15	7,8,10,15,16,18,21	7,14,21
2	10	Oral	100	Gabapentin	7–15	7,8,10,15,16,18,21	7,14,21
3	10(9)¶	Oral	40	AQU-118	7–15	7,8,10,15,16,18,21	7,14,21

*Once per day dosing via gastric gavage.
†0.5% methyl cellulose.
‡A baseline measure was taken before surgery and then on day 7 after surgery. Testing was done on the same time each day and 1 hour after dosing on dosing days, except on day 7 on which dosing occurred after baseline testing.
‖A baseline measure was taken before surgery and then on day 7 after surgery. Testing was done on the same time each day and 1 hour after dosing on dosing days, except on day 7 on which dosing occurred after baseline testing.
¶One rat was excluded (to make n = 9) during the OFST testing because it exhibited certain postures that avoided mechanical stimulation.
CCI-IoN = chronic constriction injury of the infraorbital nerve; NA = not applicable; VF = von Frey filaments; OFST = orofacial stimulation test.

were separated. The transverse process of L6 was removed and the L5 and L6 spinal nerves exposed. 4-0 silk thread was used to ligate the left L5 spinal nerve. The wound was closed by suture and staples. All rats received an analgesic (buprenorphine, 0.05 mg/kg, subcutaneously) immediately before and 6 hours after surgery. After recovery from anesthesia, animals were then single-housed for the duration of the study.

Monofilament (VF) mechanical stimulation assay. Withdrawal from a mechanical stimulus was measured by applying VF (Stoelting) of ascending bending force to the plantar surface of both hind paws, ipsilateral and contralateral to the surgical manipulation. Filaments ranged from 0.69 to 60 g (0.692, 1.202, 1.479, 2.041, 3.63, 6, 8, 10, 15, 26, and 60 g). Each filament was applied three times to determine withdrawal. A positive response was defined as withdrawal from the VF. Confirmation of the paw withdrawal threshold (PWT) was tested by assessing the response to the filament above and below the withdrawal response. Rats were brought to the experimental room and allowed to habituate in the room for 1 hour prior to testing and to acclimate to the observation chambers for at least 15 minutes prior to taking PWT measurements.

Preoperative baseline testing. Prior to surgery, all rats were tested using the VF test. Rats that had an ipsilateral PWT of less than 12 g were excluded from the study.

Postoperative testing. Three days following surgery, VF-related responses were obtained and animals were balanced and assigned to treatment groups (n = 8 per group) based on their postoperative PWT values. On days 5, 6, 8, and 10 postsurgery, rats were given vehicle, gabapentin, or AQU-118 and tested within 1 hour of administration (see below). PWT values were measured without compound on days 11, 12, and 14. All measurements were taken at approximately the same time every day by observers blinded to the treatment.

Compound administration. Gabapentin (100 mg/kg, QD; Toronto Research Chemicals) was dissolved in saline and administered orally on days 4 to 10, prior to testing, at a dose volume of 1 mL/kg. The gabapentin dose was prepared fresh daily. AQU-118 (5, 10, 20 mg/kg, QD) was dissolved in 0.5% methylcellulose (400 cP and administered orally on days 4 to 10, prior to testing, at a dose volume of 1 mL/kg. The AQU-118 dose was prepared fresh daily. The vehicle was administered at a dose volume equivalent to the test compound administered. Compounds or vehicle were administered in the afternoon at approximately the same time each day.

Statistical analyses. Data at all time points postsurgery were analyzed by two-way repeated measures analysis of variance (RM ANOVA) with time as the within-subjects factor and treatment as the between-subjects factor. This was followed by Fisher protected least significant difference (PLSD) post-hoc comparisons where appropriate. Presurgery baseline paw withdrawal data were analyzed by one-way ANOVA. An effect was considered significant at $P < .05$. Data are presented as the mean ± standard error of the mean (SEM).

Testing in the CCI-IoN Model of Orofacial Neuropathic Pain

The use of animals was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio. Thirty-two male Sprague-Dawley rats (175 to 200 g; Charles River Laboratories) were used in this study (Table 2).

Orofacial stimulation test assay. Animals were acclimated for 1 week after arrival. Animal training with the orofacial stimulation test (OFST) assay was started in the second week and included two sessions in which animals learned to retrieve sweetened milk (30% sweetened condensed milk; HEB), diluted in tap water, as a reward by placing their head through an opening in the box and licking milk from a bottle spout. Training continued during week 3 and included three consecutive daily sessions in which the animal again retrieved a reward but with a mechanical

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insert placed into the opening. The mechanical insert consisted of an array of 16 individual 0.006-inch-diameter nickel titanium wires that protruded 5 to 7 mm beyond the edge of the metal insert frame (Fig 1) and was different from the one provided by the manufacturer of the OFST assay (Ugo Basile). The mechanical insert provides mechanical stimulation to the center of the vibrissal pad while the animal is retrieving the sweet milk reward. The tip of the milk bottle spout was placed 8 mm from the insert wall to provide maximum contact of wires to the vibrissal pad while the milk reward was being retrieved. Each training session consisted of a 10-minute acclimation period in which animals were placed in the box in the absence of milk reward followed by a 20-minute period during which reward was available. Animals were then food-deprived overnight (with access to water) for 18 hours and tested the next day with the OFST assay for a 10-minute period while the total time associated with reward retrieval with the mechanical insert in place as a baseline measurement was recorded. The cumulative time spent retrieving the reward (feeding time) was determined automatically by recording the length of time that a beam of infrared light was interrupted by the snout of the rat that only occurred while it retrieved the reward. The OFST assay was also performed 7 days following placement of the CCI-IoN lesion (see below); 14 days after the lesion, at which time the animals had been treated with eight daily doses of AQU-118, gabapentin, or vehicle; and again on day 21, 6 days after drug therapy had been stopped (on day 15). All OFST assay sessions were performed with the use of the same mechanical insert. Each animal undergoing testing with the OFST assay was carefully observed to ensure that reward retrieval events were associated with an interruption of the infrared beam while mechanical stimulation of the vibrissal pad was occurring. Baseline measures were also obtained for VF mechanical stimulation of the vibrissal pad (see below).

Monofilament (VF) mechanical stimulation assay. All animals were also tested for baseline behavioral response following VF mechanical stimulation to the vibrissal pad (see Table 2). The animals were placed into a plastic box for a 10-minute acclimation period, after which testing began with Semmes-Weinstein monofilaments (Touch-Test Sensory Evaluator, North Coast Medical Inc). The middle of the left vibrissal pad was successively tested with monofilaments of 0.4, 0.6, 1.0, 1.4, 2.0, 4, 6, 8, 10, 15, and 26 g until threshold behavior was recorded. Two minutes were allowed between testing with each filament. Each filament was applied three times and threshold was defined when stimulation with two consecutive filaments of increasing size resulted in two of the three stimulations with each monofilament that produced a behavioral response characterized by any one or combination of the following behaviors: head withdrawal, bite/attack filament, or asymmetric facial grooming directed to the side of the CCI-IoN lesion. The first (lowest) of these two consecutive monofilaments that produced consecutive threshold responses was defined as the threshold monofilament. Animals were tested prior to the CCI-IoN lesion (baseline measure), and again 7 (immediately before the initiation of drug/vehicle therapy), 8, 10, 15 (last day drug given), 16, 18, and 21 days after the lesion.

Fig 1 Orofacial stimulation test assay mechanical insert. The mechanical insert placed into the opening of the box consists of an array of nickel titanium wires that contact the vibrissal pad while the animal is retrieving a sweetened milk reward.

Infraorbital nerve lesion. Three days after baseline measures were obtained with the OFST assay and VF stimulation, animals were anesthetized with an intramuscular injection of 75 mg/kg ketamine (Putney) and 0.5 mg/kg dexmedetomidine (Pfizer). Once they were pain-free, the left infraorbital nerve (IoN) was exposed just distal to the IoN foramen by way of a midline incision over the snout. The IoN was fully exposed as visualized with the use of a surgical microscope (Zeiss). Two 4-0 chromic gut sutures (Ethicon) were placed around the IoN just distal to the foramen, and each suture was loosely constricted onto the nerve. The superficial incision was closed with 3-0 black silk suture (Ethicon).

Compound administration. Three groups of nerve-lesioned animals were used in the study: animals treated with vehicle (n = 12; lesion with vehicle), animals treated with gabapentin (Toronto Research Chemicals; n = 10; lesion with gabapentin), and animals treated with AQU-118 (n = 10; lesion with AQU-118). An AQU-118 dose of 40 mg/kg was selected based on the results of the SNL rat study that showed that AQU-118 at the 20 mg/kg dose exhibited approximately half the efficacy of gabapentin. It was of interest to see if doubling the dose of AQU-118 to 40 mg/kg would give equipotent reductions in responses to vibrissal pad stimulation as compared to gabapentin. All animals received once daily administration of compound or vehicle on days 7 to 15 after placement of the nerve lesion. Postoperative dosing

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periods after CCI-IoN were different than after SNL, since onset of mechanical allodynia may be more rapid after SNL.[14,15] All three nerve-lesioned groups received either compound or vehicle by way of gastric lavage feeding tubes (18 gauge/75 mm length, Solomon Scientific). Vehicle was 0.5% methylcellulose (Sigma) in water, and this vehicle was used to deliver AQU-118. Vehicle-treated animals received 2 mL of vehicle and AQU-118-treated animals received a 40-mg/kg dose of AQU-118 in 2 mL of vehicle. Gabapentin-treated animals received a 100-mg/kg dose of gabapentin dissolved in saline at a concentration of 100 mg/mL of saline. On the days that the animals received the AQU-118, gabapentin, or vehicle and were tested with either the OFST assay (14 days after lesion placement) or VF testing (8, 10, 15 days after lesion placement), testing was initiated one hour after AQU-118, gabapentin, or vehicle was administered to each animal.

Statistical analyses. Statistical analyses were accomplished with the use of GraphPad Prism 5.0. Differences between groups were evaluated with two-way ANOVA with Bonferroni's multiple comparison post-hoc tests and were considered significant at $P < .05$ (*), $P < .01$ (**), and $P < .001$ (***). No animals were excluded from the VF analysis, whereas one animal in the AQU-118 group was excluded from the OFST assay analysis because the animal consistently retrieved milk reward while avoiding mechanical stimulation of the vibrissal pads by turning of the head.

Testing in Naïve Rats

Sixteen male naïve Sprague-Dawley rats (200 to 300 g; obtained from Charles River Laboratories) were randomly assigned to one of two experimental conditions (n = 8 each): administration of AQU-118 (40 mg/kg) or vehicle (0.5% methylcellulose).

Measurement of mechanical allodynia. To assess the effect of AQU-118 on responses to basal mechanical stimulation, a test was conducted 1 to 2 days before dosing (day –1, baseline) and then on day 1 and day 3 following consecutive daily dosing with AQU-118 (see below). Animals were placed in a Plexiglas chamber (20 × 10.5 × 40.5 cm) and habituated for 10 minutes to derive PWT scores. The chamber was positioned on top of a mesh screen so that mechanical stimuli could be administered to the plantar surface of both hind paws. Mechanical PWT sensitivity for each hind paw was measured using the up-and-down method with eight VF (0.39, 0.58, 1.00, 1.87, 4.00, 7.88, 13.79, and 25.62 g). Each trial began with a VF force of 1.00 g delivered to each hind paw for approximately 1 second. If there was no withdrawal response, then the next highest force was delivered. If there was a response, the next lowest force was delivered. This procedure was repeated until no response was detected at the highest force (25.62 g) or until five total stimuli were administered. This procedure was performed three times, and the 50% withdrawal values of each were averaged to determine a mean 50% threshold to tactile stimulation for the right and left paws of each animal.

Measure of locomotor activity. Motor activity was measured using a circular open field. The open field consists of a circular base (100-cm diameter) with an aluminum sheet-metal wall (height of 45 cm). Each animal was individually placed in the center of the apparatus, and the total horizontal distance traveled during a 60-second test was recorded and calculated using a Med-Associates Ethovision tracking system.

Dosing and testing. Before dosing, the rats had baseline (pre-drug) measures of PWT and locomotor activity. After 24 to 48 hours, animals were randomly assigned to receive an oral administration of either vehicle (0.5% methylcellulose, n = 8) or AQU-118 (40 mg/kg in 0.5% methylcellulose, n = 8) at a volume of 1 mL/kg. Sixty minutes after AQU-118 (or vehicle) administration, animals were tested for PWT and locomotor activity. AQU-118 administration occurred for 3 days, with locomotive and VF testing on day 1 and day 3 after drug administration. The compound was made up fresh for each of the three drug-administration days.

Statistical analysis. Mixed repeated measures ANOVAs were conducted with condition as the between-subject factor (drug or vehicle) and time as the within-subject factor.

Results

Attenuation of Mechanical Allodynia by Oral Administration of AQU-118 in SNL Rat Model

The study was designed to have in addition to a vehicle (n = 8) and a positive control (n = 8, gabapentin, 100 mg/kg), three doses of AQU-118 (5, 10, and 20 mg/kg, n = 8/dose) (see Table 1). By the third day postsurgery, SNL rats displayed significant ipsilateral mechanical allodynia as compared to preoperative testing (Fig 2a). Oral dosing of AQU-118 beginning on day 4 caused an increase in the PWT at the 10-mg/kg ($P < .01$ on day 8) and 20-mg/kg ($P < .001$ on day 8) dose groups as compared to the vehicle control group. The AQU-118 dose dependently increased PWT. The PWT returned to postsurgical levels upon cessation of AQU-118 after day 10. With oral dosing of AQU-118, no statistically significant effect on contralateral PWT was observed, which was comparable to both the vehicle and positive control (gabapentin) arms (Fig 2b).

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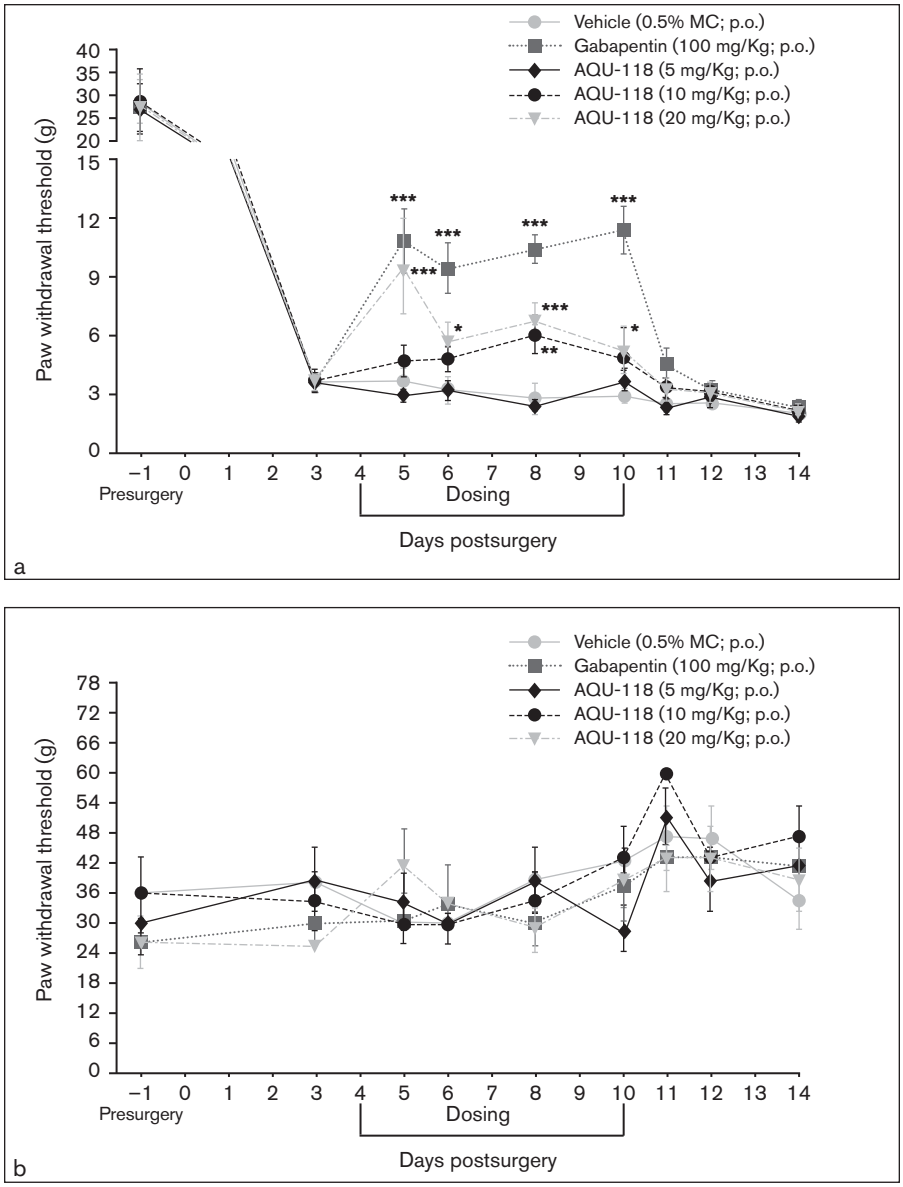

Fig 2 Mechanical response threshold in SNL rats. **(a)** Paw withdrawal thresholds following SNL surgery for ipsilateral hind paws (n = 8 for all groups). **P* < .05, ***P* < .01, ****P* < .001 as compared to vehicle ipsilateral threshold. **(b)** Contralateral paw withdrawal thresholds following surgery and administration of AQU-118, gabapentin, or vehicle (n = 8). Data are presented as mean; error bars are SEM.

Attenuation of Mechanical Allodynia by Oral Administration of AQU-118 in CCI-IoN Rat Model

Before CCI-IoN surgery, mechanical stimulation of the left vibrissal pad with VF showed no significant difference between the animals included in the three nerve-lesioned groups (vehicle, 100 mg/kg gabapentin, and 40 mg/kg AQU-118) in gram force needed to elicit a behavioral threshold response (vibrissal pad response threshold) (Fig 3; see Table 2). On the seventh day after CCI-IoN surgery, the measurement of vibrissal pad response threshold indicated a marked increase in mechanical sensitivity for all groups as compared to preoperative baseline measures (Fig 3). The vehicle group showed a gradual and progressive reduction in the force needed for a threshold response (increase in mechanical sensitivity) that continued from day 7 after the lesion to day 15 and which was still present on day 21, the last day tested. This decreased force needed for threshold behavioral response indicates that mechanical allodynia of the vibrissal pad resulted from the CCI-IoN lesion. Eight consecutive daily oral doses with AQU-118 (40 mg/kg)

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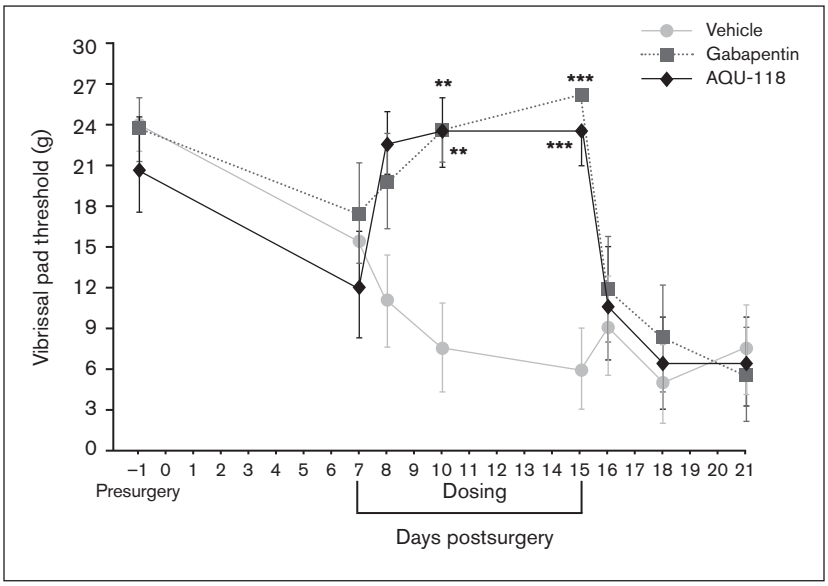

Fig 3 Mechanical response threshold in CCI-IoN rats. Vibrissal pad response threshold following CCI-IoN surgery and daily oral administration of AQU-118 (40 mg/kg), gabapentin (100 mg/kg), and vehicle (0.5% methylcellulose). Dosing began on day 7 immediately after day 7 testing and continued once daily through day 15. Data are presented as mean; error bars are SEM. $^{**}P < .01$, $^{***}P < .001$ as compared to vehicle.

or gabapentin (100 mg/kg), which began 7 days after the CCI-IoN, and after vibrissal pad response threshold measurement was obtained on day 7, continued until day 15. Testing with VF showed a statistically significant reversal of mechanical allodynia in both the AQU-118 and gabapentin groups when compared to the vehicle group, at both 10 days (4 consecutive days of drug) and 15 days (9 consecutive days of drug) after lesion placement. This reversal of mechanical allodynia observed in both drug-treatment groups was rapidly lost at day 16, 24 hours after the last administration of each agent, at which time there was no significant difference between any of the three groups (Fig 3). This lack of significant difference among the three groups remained at 18 and 21 days (after lesion placement).

Effect of Oral Administration of AQU-118 After CCI-IoN Surgery in the OFST Assay

Use of the OFST assay to measure the retrieval of a sweetened milk reward while the animal encountered a self-applied mechanical stimulation of the vibrissal pads showed changes in this behavior as a result of the CCI-IoN lesion in the different groups (vehicle, 40 mg/kg AQU-118, and 100 mg/kg gabapentin) (see Table 2). These differences were observed in the cumulative feeding time (Fig 4a) and the cumulative feeding time as a percent difference from baseline for each animal (Fig 4b).

All feeding times were observed to decrease 7 days after lesion placement and before initiation of drug/vehicle administration (Fig 4). Cumulative feeding times were significantly increased in both the gabapentin and AQU-118 groups when compared to the vehicle group at 14 days after the CCI-IoN lesion following eight daily doses of compound/vehicle administration (Fig 4a). This same difference was apparent when cumulative feeding time was evaluated as a percent difference from each animal's baseline measure (Fig 4b). The cumulative feeding times in the drug-treated groups were reduced at 21 days, which were not different from the feeding times in the vehicle group and where these times approached baseline levels in all three groups (Fig 4).

Effect of Oral Administration of AQU-118 in Naïve Sprague-Dawley Rats

Mechanical sensitivity as measured with PWT showed no significant effect of time, $F(2,28) = 2.84$, $P = .08$, on PWT scores in both AQU-118 and vehicle groups, and with PWT scores that remained stable over time. There was also no significant main effect of treatment condition (AQU-118 as compared to vehicle), $F(1,14) = .003$, $P = .96$, nor a significant interaction of time with treatment condition interaction, $F(2,28) = .62$, $P = .55$ (Fig 5a). Results on locomotive activity showed no significant effect of time, $F(2,28) = 2.45$, $P = .10$, or condition, $F(1,14) = .21$,

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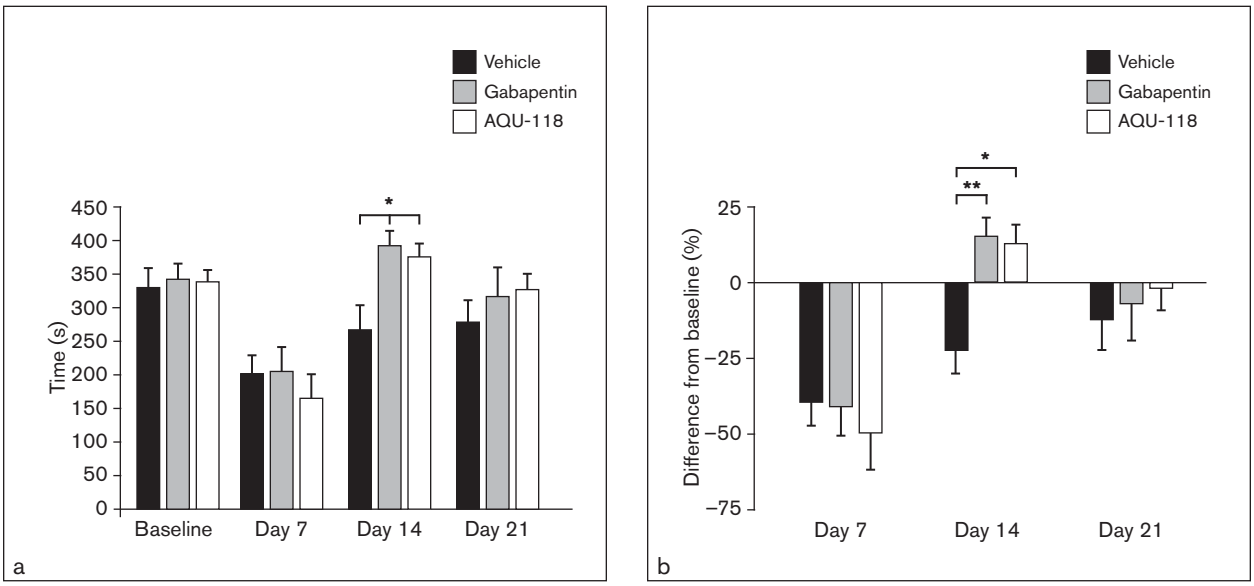

Fig 4 Cumulative feeding time. Data are presented as mean; error bars are SEM. **(a)** Cumulative feeding time before CCI-IoN lesion (Baseline); 7 days after CCI-IoN lesion (Day 7); effect of once daily oral administration of AQU-118 (40 mg/kg), gabapentin (100 mg/kg), and vehicle (0.5% methylcellulose) for 8 consecutive days as tested 14 days after lesion (Day 14); and 6 days after cessation of oral dosing (Day 21). Dosing began on day 7, immediately after day 7 measurement, and continued through day 15. **P* < .05 as compared to vehicle. **(b)** Average percent difference in cumulative feeding time from baseline (presurgical levels) for each animal 7 days after lesion placement (Day 7), after 8 consecutive days of oral dosing (Day 14), and 6 days after cessation of oral dosing (Day 21). **P* < .05, ***P* < .01 as compared to vehicle.

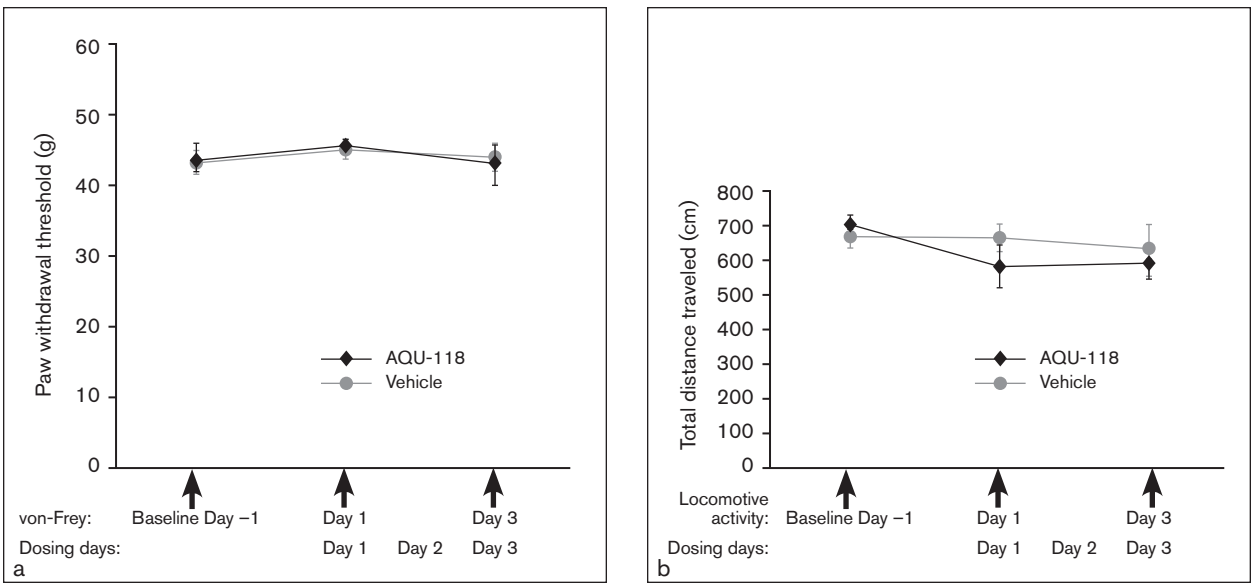

Fig 5 **(a)** Paw withdrawal threshold and **(b)** locomotive activity in naïve rats (n = 8 for all groups) at day 1 (baseline, before dosing) and at day 1 and day 3 after dosing with either AQU-118 (40 mg/kg) or vehicle (0.5% methylcellulose). Once per day dosing occurred started on day 1 and continued through day 3.

P = .66, and no significant interaction of time with condition, F(2,28) = 1.37, *P* = .27 (Fig 5b). These results indicate that oral dosing with AQU-118 at 40 mg/kg for multiple days in naïve rats does not alter basal sensitivity to mechanical stimulation or induce changes in locomotive activity that could be due to sedative effects.

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Discussion

Current treatments for neuropathic pain are often either not effective or only partially effective.[25,26] Opioids have limited potential for alleviating neuropathic pain and can cause the unwanted side effects of dependency, tolerance, nausea, drowsiness, and constipation.[27,28] Many of the most common treatments, such as the use of gabapentin and more recently pregabalin, generally give less than desired results while still producing significant dose-limiting side effects.[29] In the last few years, no oral drug has been approved for pain that is truly novel. Since no current single drug treatment is effective in more than 50% of patients, novel therapeutic approaches are urgently needed. Studies showing that MMP-2 and/or MMP-9 become elevated after CCI or SNL surgeries and that inhibiting these MMPs can reduce mechanical allodynia in rodents presents a novel approach by which neuropathic pain may be treated.[8–10] Up until now, however, no translational research has been reported to substantiate this approach. The present results show that a dual active MMP-2/MMP-9 inhibitor can attenuate mechanical allodynia when given orally in both the SNL and CCI-IoN rat models of neuropathic pain when compared to vehicle control. A dose response was observed in the SNL study whereby increasing the daily oral dose of AQU-118 increased the paw withdrawal VF threshold of rodents. The greater reduction in PWT seen with gabapentin as compared to AQU-118 in the SNL rat model could be due to the different effective molar concentrations associated with the doses used for each drug. At 100 mg/kg, gabapentin has an effective molar concentration that is more than 14 times higher than that of AQU-118 at the 20-mg/kg dose (0.58 mol/L for gabapentin versus 0.04 mol/L for AQU-118). However, the finding that the actual difference in PWT between the animals treated with AQU-118 (20 mg/kg) and gabapentin (100 mg/kg) was only approximately 2× suggests that AQU-118 may have greater efficacy or target tissue penetration than gabapentin. Moreover, these findings also raise the possibility that a higher dose of AQU-118, such as the 40-mg/kg dose used in the CCI-IoN study, could have produced even greater reductions in PWT scores in SNL animals, although additional studies will be needed to test for this possibility.

The finding that oral dosing with an MMP-2/-9 inhibitor can attenuate mechanical allodynia in the CCI-IoN rat model is significant, since it was unknown whether a MMP inhibitor could attenuate mechanical allodynia in this rodent model. The present study helps to settle this efficacy question by showing decreased mechanical sensitivity of the vibrissal pad that was maintained with daily oral dosing with AQU-118 (40 mg/kg) for 8 days, but with a rapid return of mechanical sensitivity seen 24 hours after dosing was terminated. The results obtained with AQU-118 mirror those observed with the positive control (100 mg/kg, gabapentin) in both the extent of the decrease in the vibrissal pad response threshold as well as the duration of action after its cessation 8 days later. A comparison of the effects of AQU-118 to the effects of gabapentin showed greater efficacy of AQU-118 in the CCI-IoN study when compared to the SNL study. Although other explanations are possible, this result could be due to the higher dose of AQU-118 used in the CCI-IoN study (40 mg/kg) as compared to the 20-mg/kg dose used in the SNL study. A common finding in both studies was that the positive effects of AQU-118 on mechanical allodynia were maintained throughout the dosing period and apparently without the development of tolerance after a week of treatment. Although the acute effects of AQU-118 on mechanical allodynia were not specifically evaluated, rapid effects were seen after only 1 day of compound administration, and these observations suggest that AQU-118 may possess acute analgesic properties.

Because of concerns that VF-related reflex-based readouts may not be a predictor of clinical efficacy, evaluation of AQU-118 was augmented with an operant OFST assay that measured the time spent retrieving a sweetened milk reward while encountering a self-applied mechanical stimulation of the vibrissal pads. The assay showed that after 8 days of oral dosing with either AQU-118 or gabapentin, the average cumulative feeding time and the cumulative feeding time as calculated by the percent difference from baseline for each animal both showed significant increases in the AQU-118 and gabapentin-treated groups as compared to vehicle group. This assay has been used by others[22–24] and also utilized a mechanical insert that varied in design from the one provided by the manufacturer, thus suggesting that the design of the insert is an important factor for this assay. Since the IoN innervates the vibrissal pad and the vibrissal pad is contacted by mechanical stimuli while the animal retrieves the reward, the change in feeding times seen in animals treated with AQU-118 or gabapentin is interpreted as a reduction of mechanical sensitivity due to this treatment. The VF results also help support this possibility.

Although the results obtained in the CCI-IoN animals with the use of the OFST assay were mostly similar to the results obtained with the VF test, some differences were noted. Similarities included the same positive reduction with both AQU-118 and gabapentin of the mechanical allodynia in both assays during the active administration phase. In contrast, some differences were noted between the two assays that involved a complete and rapid return of

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mechanical allodynia 1 day after drug administration was terminated, as shown by the VF assay, whereas effects of drug termination in the OFST assay were more modest, since cumulative feeding times remained higher than seen 7 days after lesion placement. One possible explanation for this observation is that the increased cumulative feeding times observed after dosing was discontinued may be an example of conditioned place preference/learned behavior that enhances an animal's pain tolerance.[30,31] However, more studies would be needed to help substantiate this possibility. The fact that the cumulative feeding time for the AQU-118 group was significantly greater on day 21 than on day 7 and greater than the vehicle control group on day 21 does suggest that discontinuation of AQU-118 does not cause an increase in pain sensitivity (ie, rebound effect) as one might expect with morphine.[32,33] In fact, preliminary experiments involving the testing of AQU-118 in the naloxone-precipitated mouse model of morphine withdrawal showed no apparent increase in the level of MMP-2 and MMP-9 after AQU-118 discontinuation.[34]

Even though these results are very encouraging in predicting clinical efficacy for AQU-118 in reducing mechanical allodynia, the exact mechanisms of action responsible for this reduction are uncertain. The fact that oral treatment with AQU-118 is capable of reducing mechanical allodynia in both the SNL and CCI-IoN models suggests a similar mechanism of action in both animal models. Earlier studies in rodents have found that following injury to dorsal root ganglion primary sensory neurons, MMP-9 induced early neuropathic pain via interleukin-1β cleavage to its active form and microglia activation and MMP-2 induced delayed neuropathic pain via IL-1β cleavage to its active form and astrocyte activation.[8] In other rodent studies, MMP-9 has been observed to promote Schwann cell–mediated myelin basic protein degradation and macrophage infiltration in the spinal nerve and astrocyte activation in the spinal cord.[9] Together, these studies suggest possible mechanisms of action of MMP-2/-9 inhibitors in reducing mechanical allodynia by actions on macrophages and Schwann cells within the injured peripheral nerve and/or actions on glial cells within the central nervous system, yet additional work is needed to define the specific mechanisms involved. In addition, even though investigators were blinded in the SNL study, investigators were not blinded in the CCI-IoN study. Although this represents a possible limitation/bias when interpreting behavioral responses following mechanical stimulation of the vibrissal pad in the drug-treated animals, the bias from lack of blinding was minimized with the use of the automated OFST assay. Another possible limitation included the lack of sham animals in both groups (SNL and CCI-IoN), but results showed analgesic-like effects in both groups of drug-treated animals when compared to vehicle-treated animals.

Although there is no definitive clinical study proving that elevated levels of MMP-9 and/or MMP-2 can directly cause neuropathic pain, there have been a few biomarker studies correlating elevated levels of these MMPs in patients suffering from certain types of pain conditions. For example, MMP-9 plasma levels have been found to be elevated in patients suffering from migraine when compared to controls.[35] Elevated levels of MMP-2 and/or MMP-9 have also been observed in chronic inflammatory demyelinating polyneuropathy and nonsystemic vasculitic neuropathy.[36,37] There have also been clinical studies relating elevated levels of MMP-2 and/or MMP-9 with certain diseases known to be responsible for producing specific types of neuropathy. For example, elevated serum levels as well as zymographic activity have been found for both MMP-2 and MMP-9 in type 2 diabetic patients as compared to nondiabetics.[38] Elevated levels and activity of MMP-2 have also been found in the urine and plasma of type 1 diabetic patients as compared to healthy control subjects.[39,40] Together, these findings suggest a possible role for MMP-9/MMP-2 in the development of certain pain conditions that include neuropathic pain and provide a basis for the clinical use of inhibitors of these enzymes as a new class of analgesics.

Conclusions

The results of this study demonstrate for the first time that attenuation of mechanical allodynia can be produced by oral dosing of a dual-active MMP-2/MMP-9 inhibitor, AQU-118, in both the SNL rat model and the CCI-IoN rat model of neuropathic pain. In both animal models, the effectiveness of AQU-118 paralleled that of the positive control, gabapentin, providing additional evidence that this novel compound may prove to be clinically effective in the treatment of various neuropathic pain conditions.

Disclosures

Michael Henry, DDS, PhD, is co-inventor of the orofacial stimulation test assay that was used in this study. This assay is commercially available from Ugo Basile and licensed to J.C. Fehrenbacher, M.A. Henry, and K.M. Hargreaves, with provisional patent application 61/235,590. Irving Sucholeichi, PhD, is a stockholder in Aquilus Pharmaceuticals, Inc, a privately held biotech company interested in clinically developing the matrix metalloproteinase inhibitor, AQU-118, used in this study.

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