Received: 12 January 2021 | Accepted: 19 January 2021

DOI: 10.1002/prp2.727

ORIGINAL ARTICLE

Mouse strain specificity of DAAO inhibitors-mediated antinociception

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81374000 and 81673403; Shanghai Industrial Translational Project, Grant/Award Number: 15401901300; Teaching Research Project of Shanghai Xuhui Central Hospital, Grant/Award Number: FDXH2112; Zhejiang Provincial Natural Science Foundation, Grant/Award Number: LQ21H090002; Ningbo Science and Technology Program, Grant/Award Number: 202003N4117

Abstract

D-Amino acid oxidase (DAAO) specifically catalyzes the oxidative deamination of neutral and polar D-amino acids and finally yields byproducts of hydrogen peroxide. Our previous work demonstrated that the spinal astroglial DAAO/hydrogen peroxide (H_2O_2) pathway was involved in the process of pain and morphine antinociceptive tolerance. This study aimed to report mouse strain specificity of DAAO inhibitors on antinociception and explore its possible mechanism. DAAO inhibitors benzoic acid, CBIO, and SUN significantly inhibited formalin-induced tonic pain in Balb/c and Swiss mice, but had no antinociceptive effect in C57 mice. In contrast, morphine and gabapentin inhibited formalin-induced tonic pain by the same degrees among Swiss, Balb/c and C57 mice. Therefore, mouse strain difference in antinociceptive effects was DAAO inhibitors specific. In addition, intrathecal injection of D-serine greatly increased spinal H_2O_2 levels by 80.0% and 56.9% in Swiss and Balb/c mice respectively, but reduced spinal H_2O_2 levels by 29.0% in C57 mice. However, there was no remarkable difference in spinal DAAO activities among Swiss, Balb/c and C57 mice. The spinal expression of glutathione (GSH) and glutathione peroxidase (GPx) activity in C57 mice were significantly higher than Swiss and Balb/c mice. Furthermore, the specific GPx inhibitor D-penicillamine distinctly restored SUN antinociception in C57 mice. Our results reported that DAAO inhibitors produced antinociception in a strain-dependent manner in mice and the strain specificity might be associated with the difference in spinal GSH and GPx activity.

KEYWORDS
antinociception, D-amino acid oxidase, glutathione, glutathione peroxidase, hydrogen peroxide

Abbreviations: CAT, catalase; CNS, central nervous system; DAAO, D-amino acid oxidase; FAD, flavin adenine dinucleotide; GPx, glutathione peroxidase; GSH, glutathione; GSSH, Oxidized Glutathione; H_2O_2, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; ROS, reactive oxygen species.

Hao Liu and Yu-Cong Zhou represent the co-first author.

1 | INTRODUCTION

As a flavin adenine dinucleotide (FAD)-dependent peroxisomal flavoenzyme, D-amino acid oxidase (DAAO) catalyzes oxidation of D-amino acids to H_2O_2, a stable reactive oxygen species (ROS). In the CNS, its expression is concentrated in the lower brainstem, cerebellum, and spinal cord, with reduced levels in the cerebral area. Previous studies demonstrated that mutation or down-regulation of spinal DAAO diminished formalin-induced tonic pain but not acute phase nociception,[1,2] and that DAAO inhibitors (such as benzoic acid, AS057278, and CBIO, as shown in Figure 1A) could prevent morphine antinociceptive tolerance in both mice and rats[3] efficiently and dose-dependently.[4] In addition, DAAO inhibitors probed by CBIO could interact with morphine on antinociception in an additive manner both in the acute nociception settings (the formalin acute flinching response, hot-plate test and tail immersion test) and in the formalin-induced pain model.[4]

However, our findings indicated that DAAO inhibitors (benzoic acid, CBIO and SUN) did not exhibit antinociceptive effect in the formalin-induced pain model in C57 mice. Subsequently, two classic analgesic drugs (gabapentin and morphine) were employed to investigate their strain-dependent antinociceptions. Selection of the formalin test was based on the well-accepted notion that subcutaneous injection of formalin produces biphasic behavioral effects in animals with the early phase reflecting an acute nociceptive state and the following tonic pain caused by ongoing activation of sensory afferents and tissue damage as well as inflammation reflecting a state of central sensitization.[5–8] Thus this study aimed to report mouse strain specificity of DAAO inhibitors on antinociception and explore its possible mechanism.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Benzoic acid, gabapentin, formalin, and morphine hydrochloride injection were from Sinopharm Group Chemical Reagent Co., Ltd. CBIO was from Maybridge Chemicals. Compound SUN was synthetized in ENNO Bioscience. D-penicillamine, D-serine, and D-alanine were purchased from Sigma-Aldrich Chemical Company. All the drugs and reagents were dissolved or diluted in sterile normal saline.

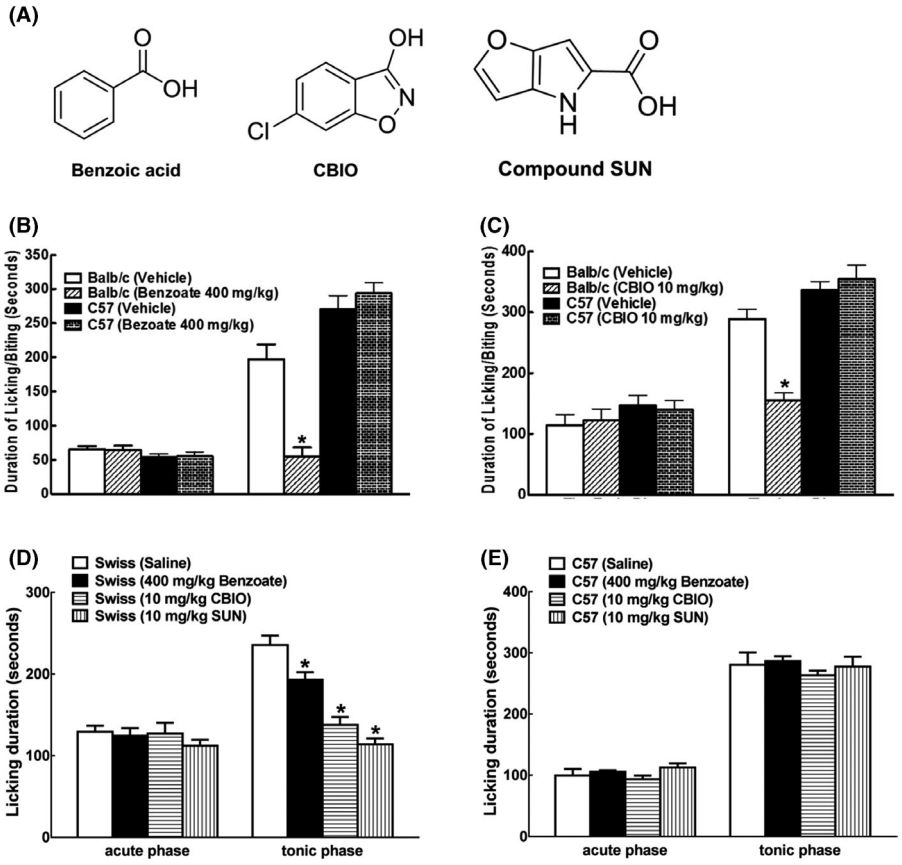

FIGURE 1 The molecular structures of DAAO inhibitors applied in this study (A). Mouse strain difference in DAAO inhibitors (benzoic acid, CBIO, and SUN) in 5% formalin-induced biphasic pain model (B–E). Nociceptive behavior was quantified by licking/biting duration (0–5 min considered as acute pain, 20–40 min as tonic pain). Data were presented as means ± SEM. ($n = 6$). * denotes statistically significantly different from the saline control group ($p < .05$, analyzed by one-way ANOVA followed by the *post hoc* Student–Newman–Keuls test)

2.2 | Animals

Male adult Swiss, Balb/c, and C57BL/6 mice (age >8 weeks, 20–25 g) were ordered from the Shanghai Experimental Animal Institute for Biological Sciences. The mice were adopted in a temperature- and humidity-controlled environment on a 12-hour light/dark cycle with free excess to food and water ad libitum. The mice were acclimatized to the animal house environment at least 3 days before the experiments. The animal protocols were approved by the Animal Care and Welfare Committee of Shanghai Jiao Tong University, in accordance with the animal care guidelines of NIH.

2.3 | Hydrogen peroxide (H_2O_2) detection

The concentration of spinal H_2O_2 was determined using a commercial H_2O_2 quantitative assay kit (Sangon Biotech). In brief, an aliquot of supernatant (20 mg tissue in 200 μl double-distilled H_2O) was mixed with the reaction solution (200 μl) containing ferrous iron and incubated at room temperature for 20–30 min. The OD values were detected at the wavelength of 590 nm on a Varioskan Flash spectral scanning multimode reader (Termo Labsystems). The concentration of H_2O_2 was calculated using a standard curve and was normalized to tissue protein as mentioned before.[3]

2.4 | Formalin-induced pain model

The mice were acclimated individually in the observation cage for a few hours in former days before the formal test. The formalin test in mice was performed by subcutaneous injection of 10 μl 5% formalin in saline on the dorsal side of the left hindpaw.[4] In short, a characteristic bi-phasic licking/biting response was manually quantified in the pooled durations at 0–5 min (acute phase) and 20–40 min (tonic phase). The behavioral scores were determined by one observer blindly.

2.5 | IC_{50} determination of spinal DAAO

According to the previous method with minor modifications,[9] 60 μl of D-alanine (1.0 M, 89 mg dissolved in 1 ml 0.1 M Tris-HCl buffer, pH 8.2) was mixed with 20 μl of 0.1 M Tris-HCl buffer and 20 μl of supernatants (20 mg tissue in 150 μl 0.1 M Tris-HCl) in a total 100 μl reaction solution (1150 *g* with final concentration of 600 mM D-alanine and incubated at 37°C for 30 min. Trichloroacetic acid (50%; 10 μl) was used to stop the reaction. The whole solution was mixed, and centrifuged (12 000*g*, 5 min). The supernatant (90 μl) was mixed with 9 μl of 10 mM 2,4-dinitrophenylhydrazine (in stock solution of HCl) and incubated at 37°C for 10 min. Lastly, 18 μl of 15 M sodium hydroxide was added, mixed, and incubated at 37°C for 10 min. The absorbance (OD value) was read at 450 nm on an ELx800 universal microplate reader (BioTek Instruments, Winooski, VT) against a blank sample consisting of the same homogenates without D-alanine. The activity of DAAO in the homogenates was quantified against the standard curve of pyruvic acid,[10] and expressed as pyruvic acid production per milligram of protein per minute.

Twenty microliter supernatants (20 mg tissue in 150 μl 0.1 M Tris-HCl) and 10 μl DAAO inhibitors (CBIO and SUN) with different concentration gradients (2.5×10^{-2}, 1×10^{-2}, 2.5×10^{-3}, 1×10^{-3}, 5×10^{-4}, 2.5×10^{-4}, 1×10^{-5} mg/ml) were premixed for 5 min first, then additional 10 μl of 0.1 M Tris-HCl buffer and 60 μl of D-alanine (1.0 M, Dissolve in 0.1 M Tris-HCl buffer, pH 8.2) were added to a final 100 μl reaction solution, shake and centrifuge at low speed (700 rpm), and incubate at 37°C for 10 min. As previously described, the enzymatic activity was then stopped by trichloroacetic acid and the pyruvate content was finally determined.

2.6 | Determination of catalase (CAT) activity

This experiment was introduced briefly according to the former method with slight modifications.[11] The mice were subjected to cervical dislocation, and about 20 mg of spinal tissue was placed on ice. The tissue was homogenized in 200 μl PBS buffer containing 1% Triton X-100 (50 mM, pH 7.0), followed by centrifuge at 10 000 rpm, 4°C for 10 min. One hundred microliters of the supernatant was incubated with 2 μl of absolute ethanol (final concentration 1%, 0.17 M) for 30 min on ice, then diluted 100 times to a total volume of 10 ml with PBS buffer (50 mM, pH 7.0), as a spinal enzyme source for further reaction.

In addition, 30 mM H_2O_2 was prepared by adding 34 μl of 30% H_2O_2 solution into 10 ml of PBS buffer solution (50 mM, pH 7.0) as a reaction substrate. In room temperature, 120 μl of spinal enzyme source was mixed immediately with 60 μl of 30 mM H_2O_2 in a quartz 96-well plate. The absorbance (OD value) at 0 s and 30 g during the reaction were measured by microplate reader at 240 nm in a range of around 0.7–1.0. The absorbance (OD value) of H_2O_2 standard solutions in gradient concentrations was measured to calculate a standard curve, and the total consumption of H_2O_2. The concentration of protein in the supernatant was determined by Bicinchoninic Acid Protein Assay. The final data of CAT activity were represented as the consumption rate of H_2O_2 in a unit of nmol/mg protein/min.

2.7 | Determination of glutathione peroxidase (GPx) activity

An aliquot of spinal tissue (20 mg) was extracted and homogenized in 200 μl tissue extraction buffer. The supernatant of homogenate was used as the enzyme source, followed by centrifugation at 10 000 rpm, 4°C for 10 min. This experiment was based on the

protocol of the Shanghai Biyuntian Total Glutathione Peroxidase Detection Kit.[12]

2.8 | Determination of reduced/oxidized glutathione (GSH/GSSG) content

The spinal tissue was premixed with protein removal reagent M (10 mg +100 μl), homogenized for 10–20 s at high speed, and placed on ice for 10–15 min, followed by centrifugation at 4°C for 5 min (10 000 rpm), the precipitate was discarded and the sample solution was saved for measurement. The specific steps of this experiment were mainly followed with the instruction of the Shanghai Biyuntian GSH and GSSG test kit.[13] The physiological level of GSH was analyzed according to the consumption rate of NAPDH in the enzymatic reaction of glutathione reductase, since GSH level was the key factor of this reaction. The final concentration of GSH would be calculated according to the standard curve of the consumption rate of NAPDH in gradient concentration of GSH.

2.9 | Statistical analysis

For dose–response curve analysis, the parameters, that is, minimum effect, maximum effect (E_{max}), half-effective dose (ED_{50}), and Hill coefficient (n), were determined from individual dose–response curves. To calculate these parameters of dose–response curves, values of response (Y) were fitted by nonlinear least-squares curves to the relation: $Y = a + b\chi$, where $\chi = [D]^n/(ED_{50}{}^n + [D]^n)$, to give the ED_{50} and E_{max} values yielding a minimum residual sum of squares of deviations from the theoretical curve.[10,14,15]

All data were presented as means ± SEM. The distribution of continuous variables was assessed using the Shapiro–Wilk normality test and the data in this study were found to be distributed normally (usually defined as $p > .1$). Thus no data transformation was exerted. Statistical significance, assessed using unpaired and two-tailed Student t-test or one-way analysis of variance followed by post hoc Student–Newman–Keuls test, was defined as p value less than .05. During the statistical analysis, Bartlett's homogeneity test of sample variance was performed automatically by software. Welch's t-test would be introduced if unequal variance occurred (usually defined as $p < .1$), which was not applicable in all the data of this study. The dose–response curve and statistical analyses were executed using the GraphPad Prism software 7.00.

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[16] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[17,18]

3 | RESULTS

3.1 | Mouse strain difference in DAAO inhibitors on antinociceptive effects under the formalin-induced pain model

Our previous work verified that in both mice and rats, DAAO inhibitors (such as CBIO, benzoic acid, etc.) had significant antinociceptive effects on the formalin-induced tonic pain.[4,10]

Effects of DAAO inhibitors (CBIO, benzoic acid, and SUN) were further compared between Swiss, Balb/c, and C57 mice in formalin test (**Figure 1B–E**). The results showed that the DAAO inhibitors significantly inhibited formalin-induced tonic pain in Balb/c and Swiss mice, but had no effect in C57 mice.

3.2 | Antinociceptive effects of other drugs in formalin test

In order to confirm whether the mouse strain difference in DAAO inhibitors on antinociception is specific, two classic analgesic drugs (50 mg/kg gabapentin and 3 mg/kg morphine) were employed to investigate their potential antinociceptive effects in different strains of mice. The results showed that gabapentin, a GABA derivative, could effectively alleviate tonic pain in the second-phase, whereas morphine could diminish both the acute and chronic pain in two-phases. Their antinociceptive effects were significant with no obvious difference between the Swiss, Balb/c, and C57 mice (Figure 2A and B). Hence it was affirmed that mouse strain difference in antinociceptive effects was DAAO inhibitors specific.

3.3 | Mouse strain difference in DAAO inhibitor CBIO on inhibiting DAAO activity

The spinal tissues of three strains were extracted for homogenization, and the supernatant was taken as a DAAO enzyme stock solution for comparative study. The results showed that IC_{50} values of CBIO in three strains were similar with no significant difference (Figure 2C).

3.4 | Mouse strain difference in spinal DAAO activity and H_2O_2 level in formalin test

This study is to further investigate DAAO activity and downstream byproducts H_2O_2 levels in three mouse strains under the formalin-induced pain models. In physiological condition, the spinal DAAO activity in C57 mice was 29.4% higher compared to Swiss mice ($p < .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test). In tonic phase (30 min), DAAO activities in all three strains were significantly increased by 22.9%, 25.1% and 20.1%, respectively ($p < .05$, analyzed by one-way ANOVA followed

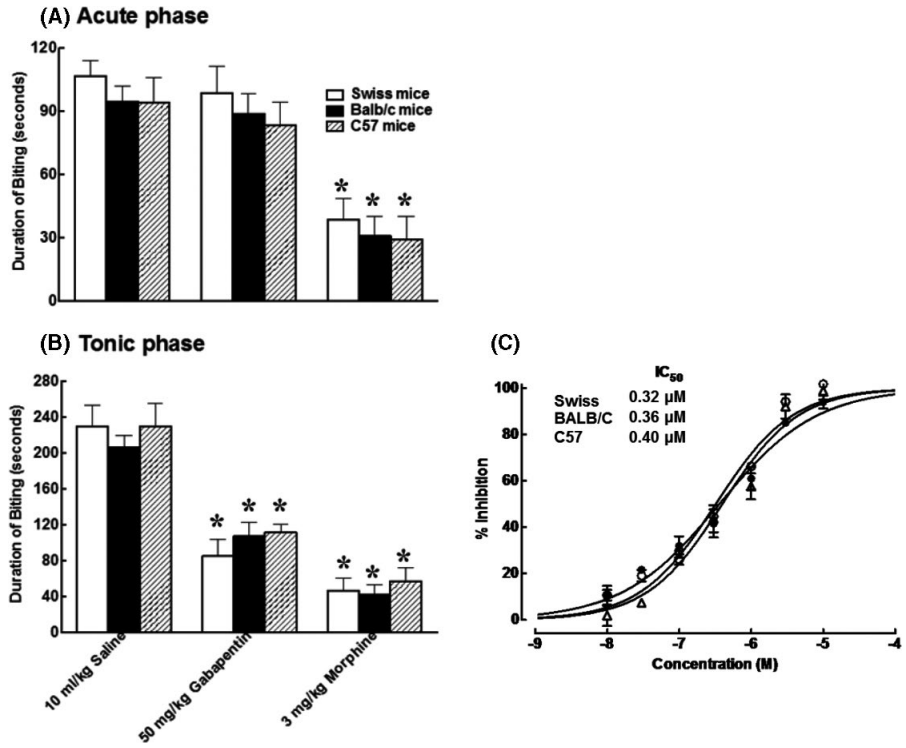

FIGURE 2 The antinociceptive effects of other drugs in formalin test (A–B). Nociceptive behavior was quantified by licking/biting duration (0–5 min considered as acute pain, 20–40 min as tonic pain, $n = 6$). Mouse strain difference in DAAO inhibitor CBIO on inhibiting DAAO activity (C). DAAO activity of spinal homogenates was assayed using the pyruvate production assay. All the tests were done in triplicates. Concentration–response analysis of DAAO inhibitors on DAAO activity was fitted by the nonlinear least-squares method. Data were shown as means ± SEM ($n = 10$). * denotes statistical significance ($p < .05$) from Swiss control group, analyzed by one-way ANOVA followed by the *post hoc* Student–Newman–Keuls test

by the post hoc Student–Newman–Keuls test, Figure 3A). The DAAO activities in C57 mice were significantly higher compared to Swiss mice at 0, 30 and 60 min by 30.0%, 27.0% and 27.4%, respectively ($p < .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test).

Spinal H_2O_2 levels in three mouse strains were also tested under the formalin-induced pain models. In tonic phase (30 min), spinal H_2O_2 levels in Swiss and Balb/c strains were significantly increased by 34.6% and 21.4%, respectively ($p < .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test, Figure 3B), which was consistent with DAAO activity as one byproduct. However, the spinal H_2O_2 levels in C57 mice did not increase during all phases in the formalin-induced pain model. It was speculated to be related to two cases: (1) The basal level of the DAAO enzyme substrate (D-serine, etc.), is extremely low in the spinal cord of C57 mice. (2) There may be a high-efficient metabolic pathway for H_2O_2 and other reactive oxygen species in C57 mice.

3.5 | Mouse strain difference in spinal CAT and GPx activities and GSH levels in formalin test

The changes in spinal CAT activity in different strains of mice under the formalin-induced pain model were studied first. The results indicated that there was no significant difference in spinal CAT activity during the whole nociception process between each strain ($p > .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls Test; Figure 3C).

Subsequently, the changes in total GPx activity in different strains of mice under the formalin-induced pain model were studied. The results showed that under physiological conditions, the GPx activity in C57 mice was significantly higher by 87.2% compared to Swiss or Balb/c mice. In this pathological model (30 min), the GPx activity was significantly increased in all strains by 60.9%, 44.5% and 52.7%, respectively ($p < .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test. Figure 3D). It could be learned that GPx activities in C57 mice were significantly higher compared to Swiss and Balb/c mice at 30 min by 77.3% and 99.6%, respectively ($p < .05$, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test).

As another substrate corresponds to the GPx catalyzed reaction, GSH is a very important endogenous antioxidant and free radical scavenger. Under the catalytic activity of GPx, GSH can quickly eliminate various free radicals, relieve the oxidative damage of cell tissues and play an anti-aging effect.[13] Commonly, most of the glutathione in the body exists in the form of reducing GSH. GSH and GSSG can be transformed mutually. The results showed that the spinal GSH level in C57 mice was 33.9% higher

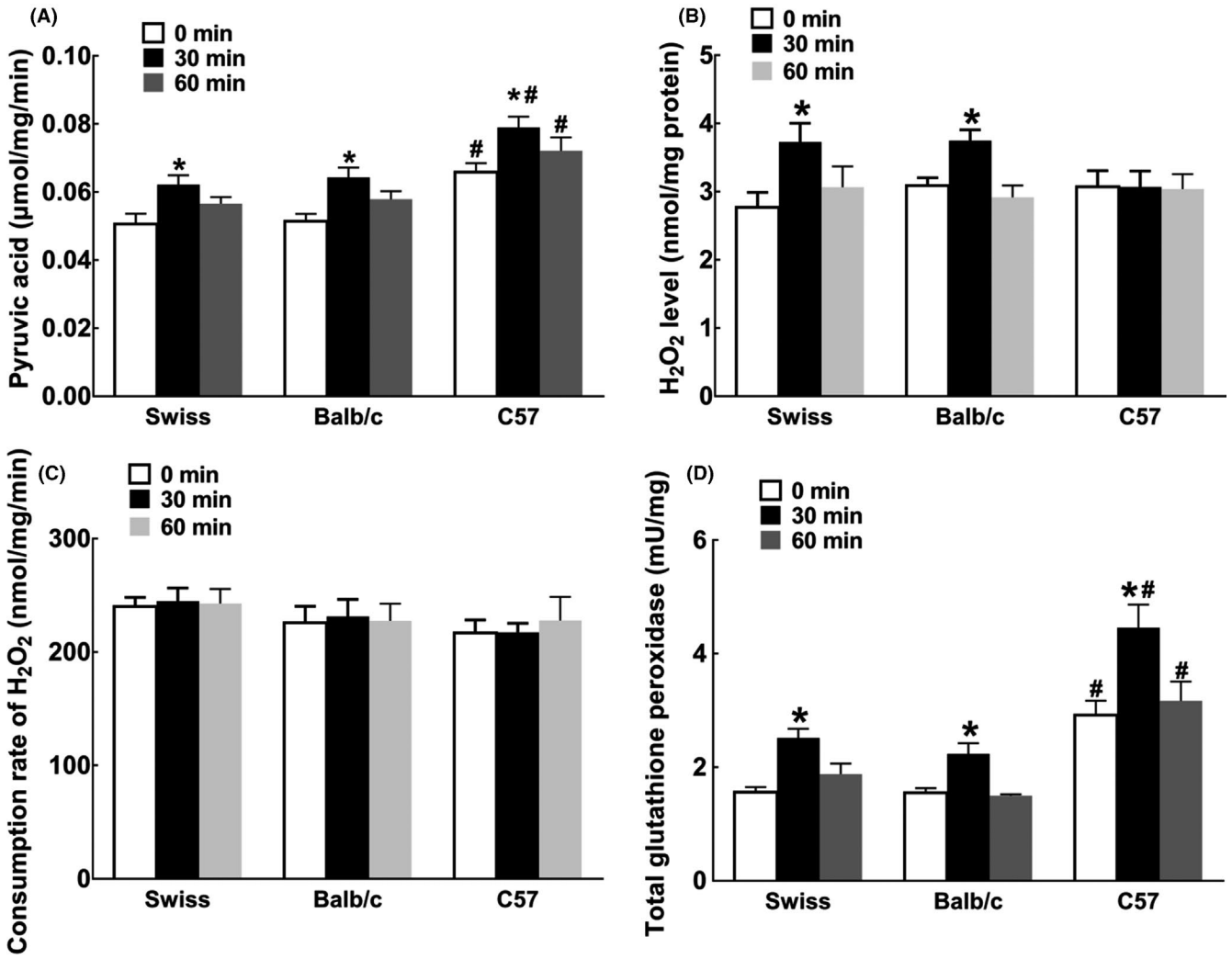

FIGURE 3 Mouse strain difference in spinal DAAO activity (A) and H_2O_2 level (B) in the formalin test. Mice were sacrificed at different time points (0, 30, 60 min) in the formalin test. The DAAO activity in spinal homogenates was studied using the pyruvate production assay (n = 6). The spinal H_2O_2 was measured using the H_2O_2 quantitative assay kit (water-compatible, n = 9). Mouse strain difference in spinal CAT (C) and GPx (D) activities was measured across all phases in formalin test (n = 5–9). The spinal tissue was extracted and the CAT activity was measured by adding exogenous H_2O_2 as substrate. Data were presented as means ± SEM. * denotes statistical significance (p < .05) compared with basal group; $^{\#}p$ < .05, significantly different from the Swiss group by analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test

than that of Swiss or Balb/c mice under physiological conditions (p < .05, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test; Figure 4A). The levels of GSSG in different strains of mice were basically similar, with no significant difference.

Our results reported that both GPx activity and GSH level in C57 mice were significantly higher compared to Swiss and Balb/c mice, which meant that the scavenging effect on free radicals and antioxidant capacity in the spinal cord of C57 mice might be significantly higher compared to others. The role of free radical scavenging system GSH/GPx in antinociceptive effect was identified in behavioral test as well (Figure 4B). Single intraperitoneal injection of GPx inhibitor D-penicillamine or(and) DAAO inhibitor SUN was performed in the formalin-induced pain model. The result indicated that the specific GPx inhibitor D-penicillamine distinctly restored SUN antinociception in C57 mice, which implied that free radical scavenging system GSH/GPx was at least partly involved in the mouse strain specificity of DAAO inhibitor-induced antinociception.

3.6 | Effect of exogenous D-serine on spinal H_2O_2 levels in three mouse strains

After intrathecal injection of 100 μg/10 μl D-serine for 1 h, the spinal samples in three mouse strains were homogenized. The results showed that the spinal H_2O_2 levels in Swiss and Balb/c mice were greatly increased by 80.0% and 56.9%, respectively. However, the spinal H_2O_2 level in C57 mice decreased oppositely by 29.0% (p < .05, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test. Figure 4C).

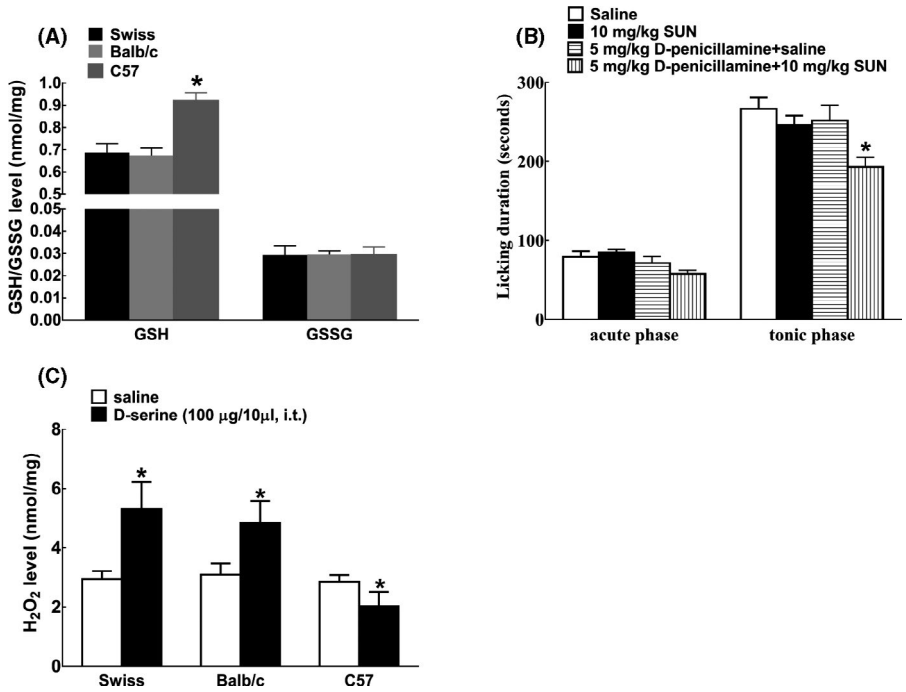

FIGURE 4 Mouse strain difference in spinal GSH levels (A) was measured across all phases in the formalin test (n = 5–9). The role of free radical scavenging system GSH/GPx in antinociceptive effect was identified in behavioral test (n = 7–9) (B). Both GPx inhibitor D-penicillamine and DAAO inhibitor SUN were injected intraperitoneally. Nociceptive behavior was quantified by licking/biting duration (0–5 min considered as acute pain, 20–40 min as tonic pain). Effect of exogenous D-serine on spinal H_2O_2 levels in three mouse strains (C). Mice were sacrificed and spinal H_2O_2 level was measured 1 h after intrathecal injection of 100 μg/10 μl D-serine using the H_2O_2 quantitative assay kit (water-compatible, n = 6). Data were presented as means ± SEM. * denotes statistical significance (p < .05) compared with basal group, analyzed by one-way ANOVA followed by the post hoc Student–Newman–Keuls test

4 | DISCUSSION

In our former study, antinociceptive effects of a series of chemical structure-unrelated DAAO inhibitors including CBIO, "Compound 8", AS057278, and benzoic acid were substantiated in the formalin-induced pain model.[10] Intrathecal injections of DAAO inhibitors specifically blocked formalin-induced tonic pain but not acute nociception, with the potency rank of CBIO > "Compound 8" > AS057278 > benzoic acid in the DAAO activity assay. The anti-hyperalgesic potencies of these DAAO inhibitors were highly correlated with their inhibitions of spinal DAAO activity. Maximum inhibition of formalin-induced tonic pain by these compounds was approximately 60%, suggesting that a larger portion of formalin-induced tonic pain was "DAAO-sensitive," whereas the remaining 40% of tonic pain and acute nociception was "DAAO-insensitive". In addition, both intrathecal CBIO and genetic ablation, by intrathecal gene silencers siRNA/DAAO and shRNA/DAAO, almost completely prevented morphine antinociceptive tolerance and the increment of spinal H_2O_2 following chronic morphine treatment.[3] Intrathecal administration of nonselective H_2O_2 scavenger PBN and the specific H_2O_2 catalyst CAT also abolished morphine antinociceptive tolerance and pain hypersensitivity. These findings demonstrated that the spinal dorsal horn astroglial DAAO/H_2O_2 pathway contributed to the initiation and maintenance of central sensitization-mediated pain hypersensitivity and morphine antinociceptive tolerance, and provided a pharmacological basis for DAAO inhibitors in combination with morphine to clinically manage pain. DAAO might be a potential molecular target for the treatment of pain hypersensitivity and morphine antinociceptive tolerance.

In this study, the behavioral test demonstrated that DAAO inhibitors had no antinociceptive effect in C57 mice. The antinociceptive effects of other drugs (gabapentin and morphine) were also studied in the formalin-induced pain model. The results showed that gabapentin (a GABA derivative) could effectively alleviate tonic pain in the second-phase, whereas morphine could diminish both the acute and chronic pain in two phases. Their antinociceptive effects were persistent between Swiss, Balb/c and C57 mice. Thus mouse strain difference in antinociceptive effects was DAAO inhibitors-specific. The metabolism of H_2O_2 might be the pivotal difference between the three mouse strains. The spinal H_2O_2 levels in C57 mice did not increase during all phases in formalin test. It was speculated to be related to two cases: 1) The basal level of the DAAO enzyme substrate (D-serine, etc.), is extremely low in the spinal cord of C57 mice. 2) There may be a high-efficient metabolic pathway for H_2O_2 and other reactive oxygen species in C57 mice.

The GSH expression in different strains of mice had been studied a lot in the past decades of years. The GSH level in liver homogenates of C57BL/6 was 36.6–42.9% higher compared to normal DBA/2 mice with no difference in CAT level.[19,20] The activity of hepatic GPx in C57BL/6 mice was 48.7% or 60% higher compared to normal

DBA/2 or BTBR mice.[19,21] It indicated in this study that GPx activities in C57 mice were significantly higher compared to Swiss and Balb/c mice at 30 min by 77.3% and 99.6%, respectively ($p < .05$). Besides, as another substrate corresponds to the GPx catalyzed reaction, GSH is a very important endogenous antioxidant and free radical scavenger. The spinal GSH level in C57 mice was 33.9% higher than that in Swiss or Balb/c mice under physiological conditions ($p < .05$). Both GPx activity and GSH level in C57 mice were significantly higher compared to Swiss and Balb/c mice, which meant that the scavenging free radicals and antioxidant capacity in the spinal cord of C57 mice might be significantly higher compared to others.

In brief, DAAO inhibitors significantly inhibited formalin-induced tonic pain in Balb/c and Swiss mice, but had no antinociceptive effect on C57 mice. DAAO inhibitors produced antinociception in a strain-dependent manner in mice and the strain specificity might be associated with the difference in spinal GSH and GPx activity.

ACKNOWLEDGMENTS

The authors thank Dr. Le Ma and Dr. Usman Ali for their assistance in these studies. This study was supported by the National Natural Science Foundation of China [Grants 81374000, 81673403]; the Shanghai Industrial Translational Project [Grant 15401901300], Teaching Research Project of Shanghai Xuhui Central Hospital [FDXH2112], Zhejiang Provincial Natural Science Foundation (LQ21H090002), and Ningbo Science and Technology Program (202003N4117).

CONFLICT OF INTEREST

The authors declare no competing interests in this work.

AUTHOR CONTRIBUTIONS

Liu, Zhou, Y.X. Wang, and M.X. Ou participated in research design. Liu, Zhou, Z.Y. Wang, Gong, Lu, Apryani, and Han conducted the experiments. Liu, Zhou, and Y.X. Wang performed data analysis. Liu, Y.X. Wang, and M.X. Ou wrote or contributed to writing of the manuscript.

DATA AVAILABILITY STATEMENT

Additional information and requests for data should be directed to the corresponding author M.X. Ou (oumx2oo7@163.com).

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How to cite this article: Liu H, Zhou Y, Wang Z, et al. Mouse strain specificity of DAAO inhibitors-mediated antinociception. *Pharmacol Res Perspect*. 2021;9:e00727. https://doi.org/10.1002/prp2.727