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Rapid communication

Mathematical analysis of involvement ratio between central and peripheral COX-2 in rat pain models with two types of COX-2 inhibitors with different distribution, celecoxib and CIAA

Takako Okumura*, Ayano Sakakibara, Yoko Murata, Yasuhiro Kita

Discovery Biology Research, Nagoya Laboratories, Pfizer Global Research and Development, Pfizer Inc., 5-2 Taketoyo, Aichi, 470-2393, Japan

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Abstract

The purpose of this study is to clarify involvement ratios between central and peripheral cyclooxygenase (COX)-2 in rat inflammatory pain models, by evaluating celecoxib and [6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-yl]acetic acid (CIAA) on carrageenan-induced mechanical and thermal hyperalgesia. Celecoxib and CIAA exhibited ID_{30} values with 1.5 and 7.7 mg/kg on mechanical hyperalgesia, respectively, and ID_{25} values with 0.54 and 36 mg/kg on thermal hyperalgesia, respectively. By solving quadratic functional analysis with prostaglandin E_2 (PGE_2) inhibitory activities, it was calculated that involvement ratios between central and peripheral COX-2 involvement were 0.47 and 0.53 on mechanical hyperalgesia, and 0.97 and 0.03 on thermal hyperalgesia, respectively. These data suggest that central and peripheral COX-2 are equally involved in mechanical hyperalgesia, while central COX-2 is predominantly involved in thermal hyperalgesia.

Keywords: COX-2; Hyperalgesia; Involvement ratio; CNS; Periphery

In pain pathways, cyclooxygenase (COX)-2 is known to have important roles in both central nervous system (CNS) and peripheral tissues. It has been reported that COX-2 is expressed in both the spinal cord (Beiche et al., 1996; Ichitani et al., 1997) and peripheral inflammatory tissue (Seibert et al., 1994), and it was shown that central and peripheral COX-2 were relevant to inflammatory pain by experiments with i.t. injected COX-2 inhibitor (Nishiyama, 2006; Yaksh et al., 2001) and the i.v. injected monoclonal antibody to prostaglandin E_2 (PGE_2) (Zhang et al., 1997), respectively. These reports indicated that central and peripheral COX-2 was involved in pain pathways, however there are no related reports to mathematically analyze involvement ratios between central and peripheral COX-2 on pain.

Celecoxib and originally discovered [6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-yl]acetic acid (CIAA) are novel and selective COX-2 inhibitors, and these compounds have differential drug distributions after oral administration. It was reported in our previous study (Okumura et al., 2006) that celecoxib was a more potent compound centrally; on the other hand, CIAA was more potent in the periphery. Furthermore, we found that the ratios of ID_{50} value of PGE_2 production in lipopolysaccharide (LPS)-injected rat brain and ID_{50} value of PGE_2 production in carrageenan-injected rat paw of celecoxib and CIAA were well correlated to the brain/plasma concentration ratios, respectively, and it was confirmed that celecoxib and CIAA could separate the central and peripheral function of COX-2, pharmacologically.

The purpose of this study is to clarify mathematically the involvement ratios between central and peripheral COX-2 on inflammatory pain, carrageenan-induced mechanical and thermal hyperalgesia, utilizing celecoxib and CIAA which have different main sites of action between brain and periphery.

All procedures used in *in vivo* assays were approved by the Animal Ethics Committee at the PGRD Nagoya Laboratories according to the Laboratory Animal Welfare guidelines. Male Sprague-Dawley rats (135–150 g) were purchased from Charles River (Japan), and housed under a 12-h light/dark cycle with free access to food and water. The rats were fasted for 16–18 h before experimental use. Celecoxib (0.3, 1, 3, 10, 30 mg/kg) or CIAA (1, 3, 10, 30, 100 mg/kg) were orally given suspended

* Corresponding author. Discovery Biology Research, Nagoya Laboratories, Pfizer Global Research and Development, Pfizer Inc., 5-2 Taketoyo, Aichi, 470-2393, Japan. Tel.: +81 569 74 4529; fax: +81 569 74 4350.
E-mail address: Takako.Okumura@pfizer.com (T. Okumura).

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with 0.1 % methylcellulose (Wako chemical) in a volume of 10 ml/kg, respectively.

To induce inflammatory pain, 0.1 ml of 1 % (w/v) λ-carrageenan (Picnin A, Zushikagaku laboratories) was injected into the right hind paw in overnight fasted rats. To determine mechanical hyperalgesia, paw withdrawal threshold (g) was measured using an analgesy meter (Ugo Basile). To determine thermal hyperalgesia, rats were placed in plastic cages of plantar test apparatus (Ugo Basile), and the mobile radiant heat source was focused on the right hind paws of the rats (IR=50) to measure paw withdrawal latency (s) to the thermal stimulation. Paw withdrawal threshold and paw withdrawal latency was measured before, 4 and 7 h after the carrageenan injection. Rats were separated into appropriate number of groups (n=12 in each group) according to two values of paw withdrawal threshold or paw withdrawal latency before and 4 h after carrageenan injection. Tested drug was administered at 5 h after the carrageenan injection under a blind manner.

Celecoxib and CIAA were synthesized by Pfizer Global Research and Development. The inhibitory activity of each dose of the test compound was shown as % inhibition when the values in groups with disease and normal were expressed as 0% and 100 % inhibition, respectively. ID_{25} and ID_{30} were calculated by computed linear regression analysis with between 2 data points including the target inhibition.

Dose-response relationships of celecoxib and CIAA were evaluated on carrageenan-induced mechanical and thermal hyperalgesia. Before a carrageenan injection paw withdrawal threshold or paw withdrawal latency were 119.6±7.7 g and 18.9±1.8 s, respectively and they decreased significantly 4 h after a carrageenan injection (paw withdrawal threshold: 29.8±3.6 g and paw withdrawal latency: 5.6±6.1 s, respectively). These hyperalgesic responses were maintained 7 h after carrageenan-injection. Celecoxib and CIAA inhibited established mechanical and thermal hyperalgesia in a dose-dependent manner when administered at 5 h after carrageenan injection. Maximum efficacies of these compounds on mechanical and thermal hyperalgesia reached approximately 60% and 50% inhibition, respectively; so ID_{30} values of mechanical hyperalgesia and ID_{25} values of thermal hyperalgesia were calculated as a dose which showed half efficacy. As shown in Table 1, ID_{30} values of celecoxib and CIAA on mechanical hyperalgesia were 1.5 and 7.7 mg/kg, p.o., respectively, and ID_{25} values of celecoxib and CIAA on thermal hyperalgesia were 0.54 and 36 mg/kg, p.o., respectively.

In the previous study (Okumura et al., 2006), we investigated inhibitory effects of celecoxib and CIAA on PGE_2 production in lipopolysaccharide (LPS)-injected brain with ID_{50} values of 0.14 and 14 mg/kg, respectively; and carrageenan-injected paw with ID_{50} values of 2.6 and 2.9 mg/kg, respectively. By analyzing quadratic functional equations, the results were obtained as X=0.47, Y=0.53 on mechanical hyperalgesia and X=0.97 and Y=0.03 on thermal hyperalgesia as shown in Table 1. These results suggested that central and peripheral COX-2 were equally involved in mechanical hyperalgesia, while central COX-2 was predominantly involved in thermal hyperalgesia. Thus, central and peripheral COX-2 had different involvement ratios in mechanical and thermal hyperalgesia.

Table 1
Pharmacological efficacies of celecoxib and CIAA, and their calculated involvement ratios of COX-2 in CNS and periphery on mechanical and thermal hyperalgesia

	Celecoxib	CIAA	X	Y
Mechanical hyperalgesia	1.5[a]	7.7[a]	0.47	0.53
Thermal hyperalgesia	0.54[b]	36[b]	0.97	0.03
Brain PGE_2	0.14[c]	14[c]	–	–
Paw PGE_2	2.6[c]	2.9[c]	–	–

For carrageenan-induced mechanical hyperalgesia, the following formulas were created;

$$1.5 = 0.14X_1 + 2.6Y_1$$
$$7.7 = 14X_1 + 2.9Y_1$$
$$X = X_1/(X_1 + Y_1)$$
$$Y = Y_1/(X_1 + Y_1)$$

For carrageenan-induced thermal hyperalgesia, the following formulas were created;

$$0.54 = 0.14X_2 + 2.6Y_2$$
$$36 = 14X_2 + 2.9Y_2$$
$$X = X_2/(X_2 + Y_2)$$
$$Y = Y_2/(X_2 + Y_2)$$

[a]ID_{30} value (mg/kg) on mechanical hyperalgesia.
[b]ID_{25} value (mg/kg) on thermal hyperalgesia.
[c]ID_{50} value (mg/kg) on PGE_2 production in LPS-injected brain or carrageenin-injected paw (Okumura et al., 2006).
X: normalized involvement ratio of COX-2 in CNS.
Y: normalized involvement ratio of COX-2 in periphery.
X and Y values were obtained following quadratic functional equations which were created using ID_{50} values on brain PGE_2 production and ID_{50} values on paw PGE_2 production as values expressing central function and peripheral function of these compounds, respectively. X and Y were normalized from X_1 and Y_1, or X_2 and Y_2, expressed involvement ratios in CNS and periphery, respectively.

This is the first report which mathematically analyzed involvement ratios between central and peripheral COX-2.

In conclusion, we have established a mathematical equation to clarify involvement ratios between central and peripheral COX-2 on mechanical and thermal hyperalgesia using celecoxib and CIAA, which have different main sites of action. It is proposed this analysis method can be applied to clarify the involvement ratios between central and peripheral COX-2 on COX-2 related phenomena.

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