Analgesic activity of acetyl-11-keto-beta-boswellic acid, a 5-lipoxygenase-enzyme inhibitor

Acetyl-11-keto-beta-boswellic acid (AKBA) is one of the four major pentacyclic triterpenic acids present in the acidic extract of the *Boswellia serrata* gum resin that is used for a variety of inflammatory disorders, such as rheumatoid arthritis, osteoarthritis, and cervical spondylosis.[1] AKBA is a novel, highly specific inhibitor of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis. It inhibits 5-LOX either directly interacting with the enzyme itself, or interacting with 5-lipoxygenase activating proteins (FLAP).[2]

Leukotriene, especially the LTB_4, as well as the peptidoleukotrienes (LTC_4 LTD_4, LTE_4) results in an increase in vascular permeability and chemotaxis of polymorphonuclear leukocytes, as well as release of mediators from the leukocytes, which sensitize nociceptors.[3, 4] On the basis of the possible role of the leukotrienes in sensitization and provocation of nociceptors, the study was undertaken to assess the analgesic activity of AKBA (5-LOX inhibitor) in different pain models.

Laca mice (20–30 g) of either sex, bred in the central animal house of Panjab University, Chandigarh, maintained at 12 h light/dark cycle was used for the study. Animals were housed under standard laboratory conditions, with free access to food and water. All the experiments were carried out between 0900 and 1700 h. All the experimental protocols were approved by the Institutional Animal Ethics Committee of the university. Standard (nimesulide [2 mg/kg]), and test (AKBA [50, 100 and 200 mg/kg]) drugs were prepared in 0.5% w/w carboxy-methyl-cellulose and administered p.o., half an hour before the onset of pain stimulus in the different models of nociception. Both the chemicals were obtained from M/s. Panacea Biotech, Ltd., Lalru, Punjab.

Antinociceptive activity was assessed by two different models of nociception. Abdominal constrictions were induced by 1% v/v acetic acid solution (1 ml/kg, i.p.) in mice pretreated with saline solution or one of the test substances. The number of abdominal writhing was measured over 20 min after the injection of acetic acid, and the animals treated with nimesulide (2 mg/kg) were used as positive controls.[4] Results were expressed as percent inhibition of abdominal constrictions.

The central antinociceptive effect was determined using the tail flick test. The response was elicited every 30 min after the treatment with test sample. Five experimental groups were used. Results were expressed in % maximum possible effect (%MPE).

$$\%MPE = (\text{post treatment latency} - \text{pre treatment latency}) \times 100/\text{pre treatment latency}$$

Animals from the control group showed 61±1.6 abdominal writhings for 20 min after the acetic acid injection. Animals pretreated with AKBA (50,100 and 200 mg/kg) showed diminished writhing in a dose-dependent manner. Animals pretreated with nimesulide (2 mg/kg, p.o.) showed 32.4±5.1 writhings in 20 min. [Table1] Animal pretreated with AKBA (50 and 100 mg/kg) showed dose- dependent, as well as time-dependent increase in the tail flick latency up to 60 min as compared to the vehicle-treated animals. After 60 min, the effect declined as shown by the decreased %MPE at 120 min. There was no significant change in the %MPE at different time points when the dose was doubled. When compared to nimesulide (2 mg/kg), AKBA (100 and 200 mg/kg) showed a considerable increase in the % MPE at 60 and 120 min. [Table 2] Statistical significance was analyzed using one-way ANOVA

Table 1

Effect of AKBA at different doses on acetic acid induced writhing test in mice

Drug treatment (mg/kg, p.o.)		*Number of wriths*	*%Inhibition with respect to control*
Control		61.0± 1.58	-
Nimesulide (2)		32.4± 5.11[a, b]	47.54
AKBA (50)		41.33± 1.80[a]	32.25
AKBA (100)		36.0± 4.31[a, b]	40.98
AKBA (200)		27.33± 1.58[a, b, c]	55.19
One-way ANOVA	F	6.42	
	P	< 0.05	

Values are mean±SEM. n=6 in each group. df = 25, 4. [a]P <0.05 as compared to control; [b]P <0.05 as compared to AKBA (50 mg/kg); [c]P <0.05 as compared to AKBA (100 mg/kg).

Table 2

Effect of AKBA at different doses on tail flick latency in mice

Drug treatment (mg/kg, p.o.)		*%MPE 30 min*	*%MPE 60 min*	*%MPE 120 min*
Nimesulide (2)		62.59±3.52[a]	136.42±5.4[a,b]	58.34±9.62[a,b]
AKBA (50)		29.35±2.54	46.78±5.63[a]	9.17±3.33
AKBA (100)		58.37±6.58[a]	97.28±4.89[a]	29.86±3.56[a]
AKBA (200)		58.74±3.54[a]	114.79±7.8[a,b]	36.77±6.66[a]
One-way ANOVA	F	54.24	62.21	88.20
	P	<0.05	<0.05	<0.05

Values are mean±SEM. n=6 in each group. df = 25, 4. [a]P <0.05 as compared to AKBA (50 mg/kg) values. [b]P <0.05 as compared to AKBA (100 mg/kg) values. MPE = Maximum Possible Effect

followed by Dunnett's test ($P<0.05$ was considered significant).

There are different evidences supporting the role of leukotrienes in acute pain. LTB_4 and HETE are potent chemotactic factors for the polymorphs, which in turn lower the firing threshold of pain fibers and therefore stimulate the nociceptors directly.[3-9] Capsaicin receptors (VR-1) that are cloned recently are reported to be activated by noxious heat and acid, suggesting their role in thermal and chemical pain.[5] One of the reports had also confirmed that the metabolic products of the lipoxygenase enzyme are the able candidates for the endogenous activation of capsaicin receptor.[6] In addition to this, the inhibition of epinephrine-induced hyperalgesia (which act directly on primary afferent nociceptors) by different selective and non-selective 5- and 12-lipoxygenase inhibitors, also support the notion that lipooxygenase products might be involved in the nociceptor sensitization.[7]

5-lipoxygenase products of arachidonic acid metabolism also act as second messenger, downstream to protein kinase A and protein kinase C, and modulate the action of these kinases in primary afferent nociceptors to mediate their sensitization to the mechanical and chemical stimuli. 5 and 12 lipooxygenase products act synergistically with PGE_2 to produce mechanical hyperalgesia.[8] In the present study, there was a dose-dependent increase in the analgesic activity of AKBA in acetic acid-induced writhing. In case of tail flick test there was no difference in the analgesic effect of 100 and 200 mg/kg dose of AKBA. AKBA showed antinociceptive activity as early as 30 min which was increased up to 60 min and after that the effect declined, as showed in Table 2. The effect of AKBA at 200 mg/kg, p.o. was more pronounced in tail flick test rather than acetic-acid–induced writhing as compared to nimesulide (2 mg/kg, p.o.). AKBA showed lesser duration of action.

In conclusion, further evaluation and toxicity test are needed to prove that AKBA possesses antinociceptive property.

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