EVALUATION OF THE LIPOXYGENASE INHIBITORY EFFECT OF BOSWELLIC ACID ANALOGUES-PART 2

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AIM

To compare the ability of different water soluble preparations of Boswellic acid with varying content of AKBA to inhibit lipoxygenases.

TEST SUBSTANCES

Boswellic acid and acetyl keto boswellic acid containing preparations BSW-017(3%AKBA, total BA 75%),BSAW-170(10%AKBA, total BA 70%) and BSAW-385 (30 %AKBA,total BA 85%) were supplied by Arjuna Natural Extracts, Kochi.

MATERIALS AND METHODS

MATERIALS

All chemicals used were of high purity analytical grade reagents. Histopaque 1077, RPMI-1640, penicillin, streptomycin, tris,bovine serum albumin and linoleic acid were obtained from Sigma Chemicals Co, USA.

METHODS

1. Isolation of monocytes from human blood.

Peripheral blood mononuclear cells were isolated from anticoagulated blood of healthy donors using Histopaque 1077 as per manufacturer's instruction. Briefly anticoagulated blood was layered over equal volume of Histopaque1077. After centrifugation at 400g for 30 minutes at room temperature, mononuclear cells were collected from interface and resuspended in 2ml PBS and mixed by gentle aspiration. Cells were sedimented by centrifugation at 250g for 10 minutes, washed and resuspended in 0.5ml PBS-Tween and was lysed by three freeze thaw cycles. From inflammatory humans also peripheral blood monocytes were isolated and used for assay.

2. Culture of Peripheral Blood Mononuclear Cells

The peripheral blood mononuclear cells isolated from anticoagulated blood were cultured in 35mm culture plates in RPMI medium supplemented with 5% homologous serum and were maintained by incubating the plates in a carbon dioxide incubator at 37^0C in 95% air and 5% carbon dioxide atmosphere. The cells were activated using 40µg of Concanavalin A and the effects of different analogues of Boswellic acid on LOX activity were studied.

3. Assay of LOX activity

Assay of 5-LOX activity

The assay mixture contained 1.1 ml of 0.2M Tris-HCl buffer, pH 9.0, .012 ml of sodium linoleate substrate and 0.025 ml of enzyme. The shift in OD was measured at 234 nm.

Assay of 15-LOX
The assay mixture contained 0.5 ml of 0.2M sodium phosphate buffer, pH 6.5, 0.025 ml of sodium linoleate substrate and 0.012 ml of enzyme. The shift in OD was measured at 280 nm.

4. Protein estimation
Protein was estimated by the method of Lowry et al.

RESULTS AND DISCUSSION

To study the inhibitory effect of different preparations of Boswellic acid containing varying proportion of BA and AKBA on lipoxygenases, human monocytes were used as sources. 5-LOX and 15-LOX activity were analysed in both normal and inflammatory condition and the results are given below.

Effect of different analogues of Boswellic acid on 15-LOX activity in Human Monocytes

To compare the relative efficacy of different analogues of Boswellic acid on 15-LOX activity in human monocytes, cell lysate were preincubated with different concentrations of the three samples of Boswellic acid - (2.08 μM, 4.16 μM, 8.3 μM,) and assayed for 15-LOX activity. The shift in OD was measured at 280 nm. The results are shown in figure 1. Cells treated with Boswellic acid analogues showed a considerable decrease in 15-LOX activity compared to control. The IC_{50} values of the three samples were in the order of BSW 017(3.6 μM) > BSAW 170 (1.9 μM) >BSAW385(1.6 μM).

Effect of different analogues of Boswellic acid on 15-LOX activity in Inflammatory Human Monocytes

To further analyse the relative efficacy of different analogues of Boswellic acid on 15-LOX activity, inflammatory human monocyte lysate were preincubated with different concentrations of the three samples of Boswellic acid. (2.08 μM, 4.16 μM, 8.3 μM,) and assayed for 15-LOX activity. The shift in OD was measured at 280 nm. The results are shown in figure 8. Cells treated with Boswellic acid analogues showed a considerable decrease in 15-LOX activity compared to control. The IC_{50} values of the three analogues were in the order of BSW 017(4.06 μM) >BSAW 170 (1.9 μM) > BSAW 385(1.63 μM) .

Effect of different analogues of Boswellic acid on 5-LOX activity in Inflammatory Human Monocytes

To analyse the relative efficacy of different analogues of Boswellic acid on 5-LOX activity, inflammatory human monocyte lysate were preincubated with different concentrations of the three samples of Boswellic acid – BSW 017,BSAW 170 and BSAW 385. (2.08 µM, 4.16 µM, 8.3 µM,) and assayed for 5-LOX activity. The shift in OD was measured at 234 nm. The results are shown in figure 3. Cell lysates treated with Boswellic acid analogues showed a considerable decrease in 15-LOX activity compared to control. The IC_{50} values of the three analogues were in the order of BSW 017(2.9 µM) > BSAW 170 (1.96 µM) > BSAW 385(1.6 µM).

Effect of different samples of Boswellic acid on 5-LOX activity in *in vitro* activated Human Monocytes in Culture

To study the relative effect of different samples of BA on 5- LOX activity of in vitro activated human monocytes, isolated peripheral blood monocytes were maintained in culture in presence of Con A (40µg/ml) and different samples of BA at 10 micromolar concentration for 24 hours.The cells were harvested, pelleted and the cell lysates were used as enzyme source. Monocyte cells without any treatment served as control. Results are shown in figure 4. Con A induced activation caused about 4.5 fold increase in the activity of 5 Lox . Treatment of these cells with different preparations of BA caused considerable decrease in 5-LOX activity. While the sample BSW 017 caused about 33 % inhibition, BSAW 170 caused about 60% and BSAW 385 caused about 67% inhibition.

Effect of different samples of Boswellic acid on 15-LOX activity in activated Human Monocytes in Culture

To study the relative effect of different samples of BA on 15- LOX activity of *in vitro* activated human monocytes, isolated peripheral blood monocytes were maintained in culture in presence of Con A (40µg/ml) and different samples of BA at 10 micromolar concentration for 24 hours. The cells were harvested, pelleted and the cell lysate were used as enzyme source. Monocyte cells without any treatment served as control. Results are shown in figure 5. Treatment of cells with Con A caused about 4 fold increase in the activity of 15 Lox. When these cells were treated with different samples of BA considerable decrease in 15-LOX activity was observed. While BSW caused about 25% inhibition, BSAW 170 caused 50% inhibition and BSAW 385 caused about 60% inhibition at identical concentrations.

These results indicate that the Boswellic acid and Boswellic acid analogues inhibit 5- LOX and 15-LOX of human monocytes which are key inflammatory cells It is already reported that experimental induction of inflammation in rabbits caused an increase in the activity of lipoxygenases and these lipoxygenases from monocytes of inflammation induced animals were also inhibited by Boswellic acid derivatives. Increase in the activity of these lipoxygenases in blood mononuclear cells under inflammation in humans also occurs as these cells were activated. Lectin induced activation of human peripheral blood mononuclear cells mimic inflammatory monocytes and cause increase in

the activity of these lipoxygenases, particularly 5-LOX. Boswellic acid analogues caused inhibition of these lipoxygenases in activated human mononuclear cells in culture also.Of the different analogues of BA ,AKBA appears to be more potent But it is not clear whether in crease in the relative level of this analogue over BA cause a corresponding effect.

Comparison of the kinetics of inhibition of 5-LOX and 15-LOX showed that in human (table 1) mononuclear cells the concentration of different samples of Boswellic acid required to produce 50% inhibition (IC_{50} value) was in the order of BSAW 385-< BSAW 170 < BSW 017 indicating that increase in the level AKBA to about 10% caused a significant inhibition in both 5 LOX and 15 LOX and further increase in the level of AKBA to 30% did not bring about any further corresponding inhibition. It therefore appears that although AKBA is the most potent form ,increase in its concentration to very high levels over the BA need not produce any further effect . However these investigations do not provide any information in the bioavailability of AKBA and its relative potency in vivo.

SUMMARY AND CONCLUSION

Lipoxygenases are key enzymes involved in the formation of leukotriens, which are the principal mediators of inflammation that trigger the progression of several diseases. These lipoxygenases are therefore potent targets for drugs. Boswellic acid present in the extract of Boswellia serrata is one such compound. Due to the hydrophobic nature of this compound, the bioavailability of Boswellic acid is a major limitation. The biochemical evaluation of acetyl keto derivative (AKBA) of Boswellic acid with respect to its ability to inhibit 5- LOX and 15-LOX in peripheral blood mononuclear cells which are key inflammatory cells was carried out. The ability of different samples of BA (water soluble) containing different amounts of BA and acetyl keto derivative of Boswellic acid (AKBA) (3-30%) to inhibit 5- LOX and 15-LOX were studied and the results are summarized below.

1) 5-LOX and 15-LOX in normal and inflammatory human peripheral blood mononuclear cells were inhibited by these three samples of BA in a dose dependent manner.

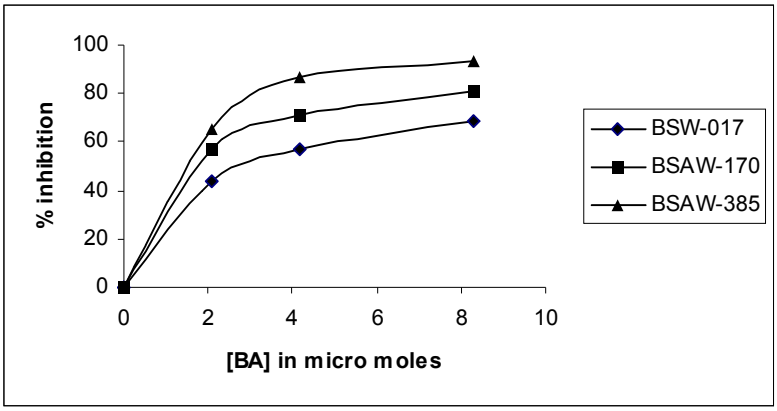
2) To assess the relative efficacy of different analogs of Boswellic acid, the concentration of the drug required to produce 50% inhibition of LOX was determined. The IC_{50} value against 5-LOX of inflammatory monocytes was in the order of BSAW 385=BSAW 170< BSW017;. Although BSAW 385 contained about 30 % AKBA, its IC50 was about 1.6, while that for BSAW 170 containing 10%AKBA was 1.9, indicating that increase in the content of AKBA may not produce any significant additional effect.

3) On comparison of the IC_{50} values, in the case of 15-LOX, in human peripheral blood mononuclear cells, it was found that AKBA containing samples were the most potent. The IC_{50} value was in the order of BSAW 385< BSAW170< BSW 017; although there was not much difference in IC_{50} value for BSAW385 and BSAW170, it was less than 50% of that for BSW 017 indicating that increase in the level of AKBA from 3% to 10% caused a significant effect while further increase to 30% did not produce any such remarkable effect.

Human monocytes activated by lectin, which is a model for inflammatory cells, Boswellic acid caused inhibition of 5-LOX and 15-LOX. Lectin treatment caused about 4 -fold increase in 5-LOX and 15-LOX activity which was reduced to near normal value in cells treated with Boswellic acid . Increase in the level of AKBA up to 10% caused significant inhibition even at 10 micromolar concentrations, but further increase in AKBA to as high as 30%, did not produce any further inhibitory effect.

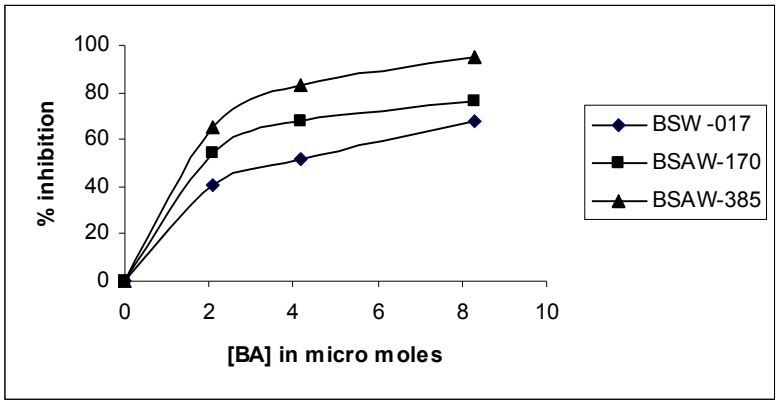
These results suggest that of the different analogues of Boswellic acid, acetyl keto Boswellic acid is most effective in inhibiting 5-LOX in inflammatory cells .However it must be further validated by studies in human subjects.

Figure 1: Effect of different analogues of Boswellic acid on 15-LOX activity in Human Monocytes



The human monocyte lysate was treated with different concentrations of the three analogues of Boswellic acid-BA -017, BA -170, BA-385(2.08µM, 4.16µM,8.3 µM) and assayed for 15-LOX activity. From the graph the IC_{50} values were calculated. IC_{50} for BSW 017- 3.5µM , BSAW -170 – 1.9µM, BSAW 385- 1.6µM .

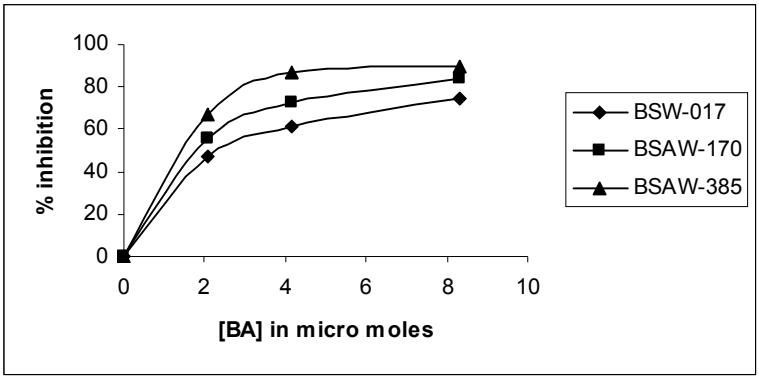
Figure 2: **Effect of different analogues of Boswellic acid on 15-LOX activity in inflammatory Human Monocytes**



The human monocyte lysate was treated with different concentrations of the three analogues of Boswellic acid-BSW -017, BSAW -170, BSAW-385(2.08µM, 4.16µM, 8.3

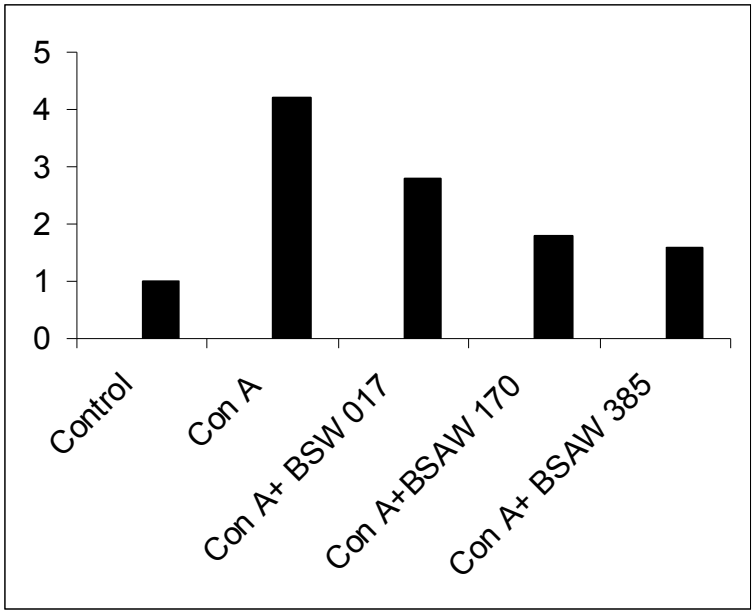
μM) and assayed for 15-LOX activity. From the graph the IC_{50} values were calculated. IC_{50} for BSW 017- 4.06μM , BSAW -170 – 1.9μM, BSAW 385- 1.63μM .

Figure 3: **Effect of different analogues of Boswellic acid on 5-LOX activity in inflammatory Human Monocytes**



The human monocyte lysate was treated with different concentrations of the three analogues of Boswellic acid-BSW -017, BSAW -170, BSAW-385(2.08μM, 4.16μM,8.3 μM) and assayed for 5-LOX activity. From the graph the IC_{50} values were calculated. IC_{50} for BSW 017- 2.9μM , BSAW -170 – 1.96μM, BSAW 385- 1.56μM .

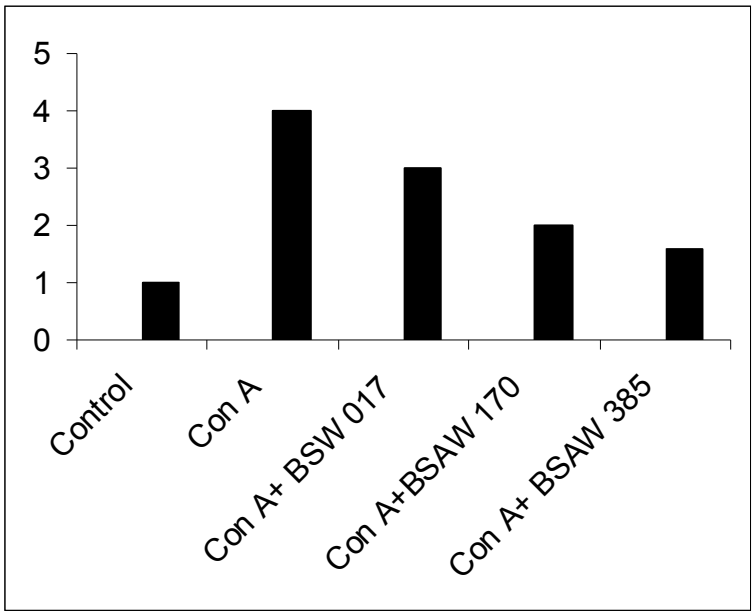
Figure 4: Effect of different samples of Boswellic acid on 5-LOX activity in activated Human Monocytes in Culture



Isolated peripheral blood mononuclear cells were maintained in culture in presence of Con A (40μg/ml) and 10 micromolar concentrations of different BA for 24 hours. The

cells were harvested, pelleted and the cell lysates were used as the 5-LOX enzyme source. Monocyte cells without any treatment served as control. The activity of the control was taken as one and that of Con A activated and BA treated were represented as the degree fold of the control.

Figure 5: Effect of potassium salt of Boswellic acid (K^+ BA) on 15-LOX activity in activated Human Monocytes in Culture



Isolated peripheral blood mononuclear cells were maintained in culture in presence of Con A (40µg/ml) and 10 micromolar concentrations of different BA for 24 hours. The cells were harvested, pelleted and the cell lysates were used as the 15-LOX enzyme source. Untreated culture monocytes served as control. The activity of the control was taken as one and that of Con A activated and BA treated were represented as the degree fold of the control.

Table 1 Comparison of the inhibitory effect of different analogues of Boswellic acid on lipoxygenases activity in human monocytes

Analogues used	IC_{50} for 5- LOX	IC_{50} for 15- LOX	
	Inflammatory Human	Normal Human	Inflammatory Human
BSW-017	2.9 μM	3.5 μM	4.06 μM
BSAW-170	1.96 μM	1.9μM	1.9 μM
BSAW-385	1.56μM	1.6 μM	1.63 μM