Immunology

Comparison of Anti-Inflammatory Effects of Boswellin

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Boswellia serrata **resins have long history in medicine. However, the recent commercial samples are rarely tested. The aim of this report was to directly compare four different commercial samples of boswellin. Our study confirmed the anti-inflammatory effects of boswellin. However, as only one of the four samples tested showed significant effects, it seems that highly active boswellin extracts represent precisely isolated and well-characterized boswellic acid, and not the crude boswellin offered commercially.**

Boswellin | Inflammation | Cancer | Liver | Colitis

Introduction

Gum resin of *Boswellia serrata* has a long history in both perfume production and medicinal use. It is used mostly in India as a part of the Ayuverdic system of medicine in treatment of inflammatory problems. Some studies suggested effects in reduction of peritumoral brain edema in patients with glioblastomas [1] and improvements in colitis [2] In clinical trials of bronchial asthma patients, Boswellin was found to have positive effects via inhibition of leukotriene biosynthesis [3]

Some studies tried to isolate the responsible molecule. Safayihi's group isolated isomers of boswellic acids and their acetyl derivates from the gum resins. These derivatives decreased the formation of leukotriene B_4 in peritoneal macrophages [4]. Subsequent studies showed that pentacyclic triterpene acetyl-11-keto-β-boswellic acid induced apoptosis in human cell lines and inhibited topoisomerase I [5].

A different group isolated boswellic acid acetate from *Boswellia carteii* and found that it will induce differentiation and apoptosis in leukemia cell lines [6]. Similarly, three types of boswellic acid had a strong inhibitory activity against human leukemia cells [7].

Boswellin, a methanol extract of the gum resin exudate has anti-arthritic activity [8]. Topical application on the back of mice inhibited skin inflammation, epidermal proliferation and tumor promotion in anthracene-treated animals [9].

With the ever growing interest in biological effects of natural products in general, and boswellin in particular, we decided to directly compare 4 different products containing boswellin.

Material and Methods

Material

RPMI 1640, Lipopolysaccharide (from *Escherichia coli*) were purchased from (Sigma, St. Louis, MO, USA), fetal calf serum (FCS) from Hyclone (Ogden, UT, USA), Biotrak cell proliferation kit from (GE Healthcare Bio-Sciences, Pittsburg, USA).

Samples

Four samples were used in our study: Sample #1 was Boswellia serrata powder purchased from Orcas Naturals (Landing, NJ, USA), Sample #2 was Boswellin HBD from Sabinsa (Sabinsa Corp., East Windsor, NJ, USA), Sample #3 was Bosmeric-SR from Sanjevani (Albuquerque, NM, USA), and Sample #4 was Easy-Flex 2 from Daily Manufacturing (Rockwell, NC, USA).

Cells

Mouse macrophage cell line RAW 264.7 and human breast cancer cell line ZR-75-1 (American Type Culture Collection, Manassas, VA, USA) were maintained in culture in RPMI 1640 medium supplemented with 10% FCS at 37°C in a humidified atmosphere supplemented with 5% CO_2.

Cell Proliferation

A hundred l of cells ($1x10^6$/ml in RPMI 1640 medium with 5% FCS) were added into each well of a 96-well plate in triplicates. After 72 hr. incubation in RPMI 1640 medium with 10% FCS, proliferation was evaluated using Biotrak cell proliferation ELISA system according to instructions given by the manufacturer.

Animals

Female, 8 week old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were sacrificed by CO_2 asphyxiation followed by cervical dislocation.

Hepatoprotective Activity

Hepatotoxicity was induced by an ip. injection of 100 ng/kg body weight of lipopolysaccharide (LPS) as described by [10]. Mice were randomly divided into several groups and administered orally by gavage during 14 days. At the end of the study, blood was collected and serum prepared. After that, mice were sacrificed and livers were immediately excised and used either for homogenates or for histology.

Biochemical Markers

The enzymatic activities of AST, ALT and ALP were assayed spectrophotometrically by Antech Diagnostics (Louisville, KY, USA). Liver homogenate were prepared by the following technique: livers were excised and rinsed in saline. A small section from each liver was placed in 10% PBS-formalin solution to be used in histological slides. The rest was frozen in liquid nitrogen and stored at -80°C for later analysis. Frozen liver was grounded to a fine powder and 20-25 mg of powder was solubilized. Protein concentrations were assayed using the bicinchroninic acid kit (Pierce, Rockford, IL, USA). The GSH levels were measured by the GSH test kit (Dojindo Labs, Kumamoto, Japan), SOD as described by Prasanna and Purnima [10] and malondialdehyde (MDA) as shown in Yadav et al. [11].

Cell Culture

RAW 264.7 cells were cultivated in 96-well plates at $2.5x10^5$/ml concentration for 24 hrs. at 37°C. The next day, the media were removed and the cells were treated with new complete media

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supplemented with 500 ng/ml LPS and tested samples at appropriate concentrations and cultivated for a further 24 hrs. Medium was collected, stored at -80° C and used for subsequent evaluation of TNF-α.

Ear Inflammation

Thirty minutes after dosing with test substances, 0.03 ml of xylene was topically applied to the anterior surface of the right ear. The left ear was used as a control. Two hours after xylene application, animals were sacrificed and both ears were removed and weighted. To reduce the errors arising from individual body weight, the relative weights of the ears were calculated along with the differences between the intact ear and induced ear [12] as follows:

Relative weight (%) = (absolute ear weight/body weight) x 100

Results

First, we evaluated the effects of individual samples on proliferation of human breast cancer cell line ZR-75-1. We found no effects on proliferation in serum-free conditions (Figure 1). In addition, when used in serum conditions no effects were found either (data not shown).

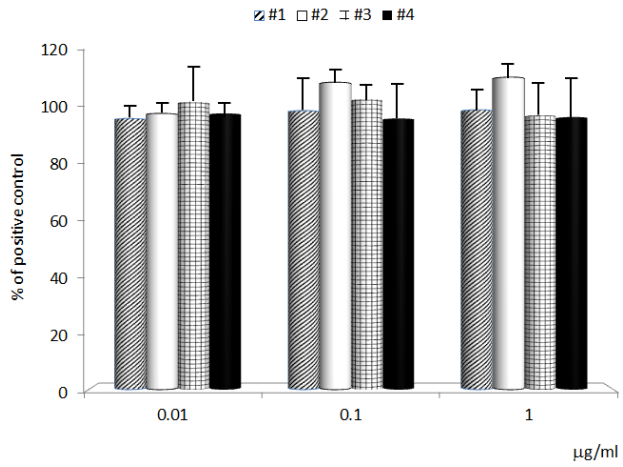

Figure 1: Effects of individual samples on proliferation of ZR-75-1 cells. Each value represents the mean ± SD.

Next, we focused on LPS-induced production of TNF-α by RAW264.7 cells *in vitro*. As shown in Figure 2, a concentration-dependent inhibition of TNF-α secretion was observed after adding different doses of samples into the culture medium. Control (LPS) groups showed 14,315 ± 2,311 pg/ml of TNF-α.

The xylene-induced acute inflammation mouse ear model has been used as a classic model of inflammation for decades. In this model, we tested the direct anti-inflammatory effects of feeding with boswellin by observation of ear weight. Table 1 summarized the effects of samples with boswellin and shows whereas all samples significantly decreased the relative weight of induced ears, only sample #2, and in case of relative weight sample #1, decreased also the relative weight.

In the last part of the study, we focused our attention on LPS-induced hepatotoxicity. After LPS treatment, individual groups were fed with tested samples for 14 days. Use of LPS caused significant stimulation of serum levels of AST, ALT and ALP. Supplementation with boswellin showed that all samples decreased the levels of these enzymes, but only significant effects were observed in the case of sample #1 and sample #2 (Table 2). Similar data were observed when we focused our attention on hepatic enzymes (Table 3). Treatment with LPS reduced levels of GSH and SOD, but stimulated levels of MDA. Individual curcumin samples helped to improve the liver damage tested by enzymatic levels. The most active samples were samples #1, #2 and to a lesser extend sample #4.

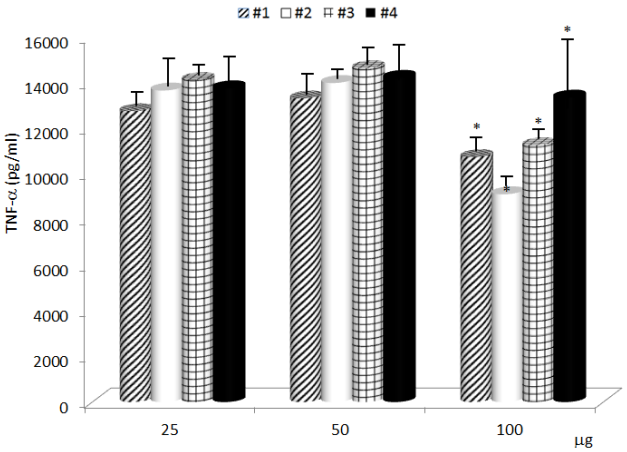

Figure 2: Effects of different doses of curcumin on LPS-mediated TNF-α secretion by RAW 264.7 cells. Values represent a mean of three independent experiments. *Significant difference between control and experimental group at P ≤ 0.05 level.

Discussion

Extracts from *Boswellia* species are traditionally used in folk medicine to treat various diseases. Boswellic acid has strong effects on colorectal cancer growth, manifested via suppression of NF-kB activation and downregulation of cyclooxygenase-2, bcl-2 and matrix metalloproteinase-9 [13]. In some cases, boswellic acids were combined with other molecules. A hybrid boswellic acids-NSAID showed synergistic effects with non-steroidal anti-inflammatory drugs, probably via inhibition of COX-2 [14].
RAW264.7 cell experiments confirmed the anti-inflammatory effects of Boswellin and are in agreement with the previously published data [15]. Compared to LPS-stimulated controls, boswellin led to a reduction in the formation of pro-inflammatory cytokine, but a rather high dose of 100 µg/ml was necessary to obtain significant changes.

Xylene-induced inflammation represents a well-established animal model of acute inflammation. As the result of pretreatment of mice with boswellin samples, the changes associated with acute inflammation such as the marked increase of induced ear weight and increase of the thickness of ear tissue were significantly suppressed. These results represent direct evidence that food supplementation with boswellin might help to lower the induction of the acute inflammation.

Boswellin has been found to have palliative effects on of experimentally-induced colitis (for review see [16]). An acute ulcerative colitis induced by application of acetic acid was improved by both pretreatment and treatment with Boswellia extract, most probably via inhibition of inflammatory mediators [17]. Inflammatory bowel disease is a family of health problems defined as a form of autoimmune disease manifested by persistent bowel inflammation. Boswellin has both anti-inflammatory and

Table 1. Effects of tested samples on changes in ear weight

Group	Absolute weight (g)		Relative weight (%)	
	Intact ear	Induced ear	Intact ear	Induced ear
Control	0.130 ± 0.011	0.182 ± 0.010	0.499 ± 0.063	0.627± 0.058
Sample #1	0.125 ± 0.018	0.171 ± 0.015*	0.454 ± 0.039	0.551 ± 0.033*
Sample #2	0.126 ± 0.022	0.143 ± 0.020*,**	0.421 ± 0.044	0.522 ± 0.051*,**
Sample #3	0.131 ± 0.011	0.173 ± 0.035*	0.474 ± 0.055	0.585 ± 0.109
Sample #4	0.129 ± 0.015	0.185 ± 0.036*	0.482 ± 0.049	0.603 ± 0.123

Mean ± SD (n=9). *Significant differences between intact and induced ear at $P \leq 0.05$ level, ** Significant differences between control and experimental induced ear at $P \leq 0.05$ level.

Table 2. Effects of tested samples on serum ALT, AST, and ALP.

Sample	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**
PBS	74.5 ± 4.4	20.1 ± 2.1	25.3 ± 2.2
LPS	152.7 ± 8.2	157.1 ± 9.2	178.3 ± 10.7
Sample # 1	133.9 ± 8.7	132.2 ± 8.1	125.5 ± 10.6*
Sample # 2	118.3 ± 9.1*	101.8 ± 8.5*	92.3 ± 7.8*
Sample # 3	131.6 ± 8.6	142.3 ± 8.9	144.3 ± 15.3
Sample # 4	141.5 ± 9.1	156.6 ± 14.2	161.4 ± 9.8

*Significant difference between tested and LPS group at $P \leq 0.05$ level.

Table 3. Effects of tested samples on level of hepatic enzymes GSH, MDA and SOD.

Sample	**GSH** (mol/mg protein)	**MDA** (nmol/mg protein)	**SOD** (U/mg protein)
PBS	18.6 ± 1.3	8.9 ± 0.5	55.9 ± 5.4
LPS	10.7 ± 1.0	65.4 ± 7.8	20.7 ± 1.3
Sample # 1	15.8 ± 1.9*	45.4 ± 5.1*	27.4 ± 3.6*
Sample # 2	16.2 ± 1.9*	39.4 ± 6.3*	39.0 ± 2.8*
Sample # 3	12.9 ± 1.3	54.3 ± 8.2	24.2 ± 2.0
Sample # 4	12.3 ± 1.5	55.4 ± 3.8*	23.4 ± 4.6

*Significant differences between tested groups and LPS group at $P \leq 0.05$ level.

anti-oxidant effects as demonstrated in the treatment of knee arthritis [18], having potential for innovative treatment of inflammatory bowel disease. Sample #2 is in fact a mixture of boswellin, curcumin, ginger extract and black pepper extract. The low activity of this sample observed in our experiments can be explained by the possibility that individual components do not act in synergy, but act against each other.

The mechanisms of action of boswellin are not clear, most probably due to the abundant group of constituents in such extracts. Some studies suggested that the direct target of boswellic acids the antimicrobial peptide LL-37, which subsequently modulates its LPS-inhibitory activity [19]. However, more studies are necessary to fully establish the action of boswellic acid.

Conclusion

Our study confirmed the anti-inflammatory effects of boswellin. However, as only one of the four samples tested in our study had significant effects, it seems that boswellin extracts used routinely in literature represent precisely isolated molecule, most of all isolated and well-characterized boswellic acid, and not the crude boswellin offered commercially.

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