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Boswellic acids from frankincense inhibit lipopolysaccharide functionality through direct molecular interference

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ABSTRACT

Lipophilic extracts of gum resins of *Boswellia* species (BSE) are used in folk medicine to treat various inflammatory disorders and infections. The molecular background of the beneficial pharmacological effects of such extracts is still unclear. Various boswellic acids (BAs) have been identified as abundant bioactive ingredients of BSE. Here we report the identification of defined BAs as direct inhibitors of lipopolysaccharide (LPS) functionality and LPS-induced cellular responses. In pull-down experiments, LPS could be precipitated using an immobilized BA, implying direct molecular interactions. Binding of BAs to LPS leads to an inhibition of LPS activity which was observed *in vitro* using a modified *limulus* amoebocyte lysate assay. Analysis of different BAs revealed clear structure-activity relationships with the classical β-BA as most potent derivative (IC_{50} = 1.8 μM). In RAW264.7 cells, LPS-induced expression of inducible nitric oxide synthase (iNOS, EC 1.14.13.39) was selectively inhibited by those BAs that interfered with LPS activity. In contrast, interferon-γ-induced iNOS induction was not affected by BAs. We conclude that structurally defined BAs are LPS inhibiting agents and we suggest that β-BA may contribute to the observed anti-inflammatory effects of BSE during infections by suppressing LPS activity.

1. Introduction

Lipophilic extracts of the gum resin of *Boswellia* species (=*Boswellia* spec. extracts, BSE) are traditionally used in folk medicine to treat infections and various sterile inflammatory disorders [1]. Analysis of the composition of these extracts revealed a group of abundant pentacyclic triterpenes, termed boswellic acids (BAs), which are unique to *Boswellia* spec. [2]. There are four major β-configurated derivatives of BAs that vary at the C3 (OH– or acetoxy group) and C11 (oxo moiety present or absent) position, yielding 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA), 3-*O*-acetyl-β-boswellic acid (Aβ-BA), 11-keto-β-boswellic acid (KBA) and β-boswellic acid (β-BA) (Table 1). Attempts to reveal the biochemical basis of the pharmacological actions of BAs led to the identification of several molecular targets, including 5- and 12-lipoxygenase [1], cyclooxygenase-1 [3], cathepsin G [4], microsomal prostaglandin E_2 synthase-1 (mPGES-1) [5], human leukocyte elastase [6], IκB-kinases [7] and topoisomerases [8]. For many of these targets, AKBA was the most potent compound, but rather high IC_{50} values (1–50 μM) implicate rather low affinity. On the other hand, investigations of blood plasma levels of BAs reached after oral application of standard doses of frankincense extracts indicate a poor bioavailability of AKBA (<0.1 μM) [4,9], and targets for other BAs (in particular for those without 11-keto moiety, such as β-BA or Aβ-BA) with much better bioavailability have been less addressed. Together, additional targets for the various BAs may exist and contribute to the bioactivities of frankincense extracts.

Bacterial lipopolysaccharides (LPS) are potent activators of the mammalian innate immune system [10]. They induce strong inflammatory responses in many types of eukaryotic cells and

Abbreviations: Aβ-BA, 3-*O*-acetyl-β-boswellic acid; AKBA, 3-*O*-acetyl-keto-β-boswellic acid; β-BA, β-boswellic acid; BA-seph, β-BA-sepharose; cm-BA, 3-carboxymethylenoxy-β-boswellic acid; cm-KBA, 3-carboxymethylenoxy-11-keto-β-boswellic acid; glut-BA, EU, endotoxin units, 3-glutaroyl-β-boswellic acid; glut-KBA, FCS, fetal calf serum, 3-glutaroyl-11-keto-β-boswellic acid; IFN-γ, interferon-γ; KBA, 11-keto-β-boswellic acid; LAL, *limulus* amoebocyte lysate; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ox-BA, 3-oxaloyl-β-boswellic acid; ox-KBA, 3-oxaloyl-11-keto-β-boswellic acid; seph, EAH sepharose 6B; succ-BA, 3-succinoyl-β-boswellic acid; succ-KBA, 3-succinoyl-11-keto-β-boswellic acid; TLR4, toll-like receptor 4.

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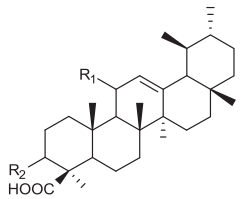
Table 1
Molecular structures of natural and synthetic BAs.



R_2	R_1	comp.	R_1	comp.
HO	H	β-BA	O	KBA
H_3C O O	H	Aβ-BA	O	AKBA
HO O O	H	ox-BA	O	ox-KBA
HO O O	H	succ-BA	O	succ-KBA
HO O O	H	glut-BA	O	glut-KBA
HO O	H	cm-BA	O	cm-KBA

several components of the respective signaling pathways have been identified so far. In macrophages, LPS-binding protein catalyzes the monomerization and transfer of LPS from LPS aggregates to membrane-bound CD14 proteins [10]. This complex activates the toll-like receptor 4 (TLR4)/MD2-receptor complex, followed by the induction of intracellular signaling cascades. These include the activation of p38 MAPK [11], JNK [12], and NF-κB [13], leading to the induction of inducible nitric oxide synthase (iNOS, EC 1.14.13.39) expression [14] and the release of various cytokines [10] that culminates in several critical disease states like sepsis or septic shock [15]. Such disorders are the leading cause of death in critically diseased patients [16]. Accordingly, agents that inhibit the LPS pathway may have beneficial effects in the course of these diseases.

We have recently constructed BA-affinity matrices composed of an insoluble resin part (i.e., sepharose) that is covalently linked via a glutaroyl residue to the C(3)-OH group of β-BA or KBA [4,17]. Using these constructs we identified molecular interaction partners of BAs including platelet-type 12-LO, COX-1, mPGES-1 and cathepsin G from lysates of human platelets or leukocytes, respectively [3–5,17]. By means of such protein fishing strategy using a BA-affinity matrix we report here the identification of LPS as a target of BAs and we demonstrate that structurally defined BAs can effectively suppress LPS activities and LPS signaling. Interestingly, suppression of LPS-induced responses by BAs or by BSE *in vitro* and *in vivo* were reported in previous studies but the molecular targets remained unresolved [7,18–21]. Our results strengthen the pharmacological potential of BAs and BSE in the treatment of infections and inflammatory disorders and provide novel insights into the molecular mechanistic of BAs in biological systems.

2. Materials and methods

2.1. Reagents

BAs were isolated from gum resins of *Boswellia* spec. extracts as previously described [22]. For the synthesis of the half-esters glut-KBA, glut-BA, succ-KBA and succ-BA, the KBA or β-BA were first treated with glutaric anhydride or succinic anhydride, respectively, and 4-pyrolidinopyridine in pyridin as described [23] (Table 1). Treatment of KBA or β-BA with oxalyl chloride in tetrahydrofurane (30 min, 20 °C) led to ox-KBA and ox-BA, respectively. The cm-KBA and cm-BA were synthesized from KBA and β-BA by addition of chloroacetic acid and sodium hydride (60%, m/v) in tetrahydrofurane. All chemical reagents were obtained by Merck KGaA (Darmstadt, Germany). The BA derivatives were analyzed by ^1H and ^{13}C NMR as well as by mass spectrometry. The LPS detection kit (*limulus* amoebocyte lysate (LAL), Pyrogene®) was obtained from Lonza (Basel, Switzerland). LPS from *Escherichia coli* O55:B5, interferon (INF)-γ, polymyxin B and all other fine chemicals were obtained by Sigma–Aldrich (Deisenhofen, Germany) unless stated otherwise.

2.2. Cells

RAW264.7 cells (provided by Dr. Lidia Sautebin, Univ. Naples, Italy) were cultured in DMEM/High Glucose (4.5 g/l) medium supplemented with heat-inactivated fetal calf serum (FCS, 10%, v/v), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 incubator. After 3 days, confluent cells were detached using a cell scraper and reseeded at 3×10^4 cells/cm^2 in cell culture flasks.

2.3. Determination of LPS activities

LPS activities were quantified using a cell-free, modified LAL assay kit, in which the LPS-dependent activation of recombinant factor C was coupled to a fluorimetric detection method [24]. In brief, LPS (from *E. coli* O55:B5, routinely used at 10 endotoxin units (EU)/ml) was incubated with testing compounds, fluorescent substrate and recombinant factor C according to manufacturer's manual. Fluorescence (excitation: 355 nm, emission: 460 nm) was measured in a fluorescent plate reader at the beginning of the incubation and after 1 h at 37 °C (Victor^3 plate reader, PerkinElmer, Rodgau-Juegesheim, Germany).

2.4. Immobilization of boswellic acids and protein pull-down assays

β-BA was treated with glutaric anhydride to form the half-ester glut-BA, and analyzed by ^1H and ^{13}C nuclear magnetic resonance as well as by mass spectrometry, as previously described [4]. Glut-BA was linked to EAH sepharose 4B (GE Healthcare Bio-Sciences, Freiburg, Germany) by standard amide coupling procedures. The carboxylic acid of the β-BA core was unlikely to react due to steric crowding. The success of the coupling reaction was determined by two methods. First, glut-BA was used in defined excess (2 μmol of the glut-BA per 1 μmol of NH_2 groups of the EAH Sepharose 4B). After the coupling reaction, the hypothetical excess of glut-BA (1 μmol) could be recovered. Second, treatment of glut-BA with KOH in isopropanol under reflux for ~3 h cleaved the ester bond and gave β-BA, detected by thin layer chromatography.

For pull-down experiments, LPS (1 μg) was solubilized in 375 μl buffer (50 mM HEPES (pH 7.4), 200 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.02% BSA) and incubated over night with the sepharose beads (BA-seph and seph). Then, the supernatants were collected and the sepharose beads were intensively washed. Precipitated molecules were finally eluted by addition of urea (4 M) and LPS was monitored via LAL assay as described above.

2.5. Determination of iNOS expression

RAW264.7 cells (2.5×10^6/ml, 100 μl, in DMEM + 2% FCS) were preincubated with BAs for 15 min and then LPS (1 μg/ml) or IFN-γ (10 ng/ml) was added for 21 h at 37 °C and 6% CO_2. Then, the medium was removed and cells were lysed on ice for 10 min in 75 μl lysis buffer (20 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Triton-X-100 (v/v), 0.5% Nonidet-P40 (v/v)). Lysates were centrifuged (10 min, 10,000 × *g*, 4 °C) and the supernatants (75 μl) were mixed with SDS-loading buffer (35 μl, 20 mM Tris (pH 8.0), 2 mM EDTA, 5% SDS (w/v), 10% β-mercaptoethanol (v/v), 2.5 mg/ml bromophenol blue, 10% glycerol) and heated for 5 min at 95 °C. Samples were analyzed by SDS-PAGE using a Mini Protean System (Bio-Rad, Munich, Germany) on an 8% gel. After electroblot to nitrocellulose (Hybond C, GE Healthcare Bio-Sciences, Freiburg, Germany), membranes were blocked by 5% nonfat dry milk in TBS (50 mM Tris–HCl, pH 7.4, 100 mM NaCl) for 1 h. After washing with TBS, membranes were probed with iNOS-specific antibodies (Cell Signaling Technologies, Danvers, USA) over night at 4 °C. Then, membranes were washed with TBS and incubated with alkaline phosphatase conjugated secondary antibodies. Immunoreactive bands were visualized with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

2.6. Cell viability tests

Cell viability was assessed by MTT assay as previously described [25]. Briefly, RAW264.7 cells (2.5×10^6/ml, 100 μl) were preincubated with BAs for 15 min and then LPS (1 μg/ml) or INF-γ (10 ng/ml) at 37 °C and 6% CO_2 were added for 21 h. DMSO was used as solvent never exceeding 0.5% (v/v). Afterwards, MTT reagent (5 mg/ml) was added for 30 min. Then, 100 μl SDS solution (20% SDS in a 1:1 (v/v) dimethylformamide/water solution) was added for 16 h. Plates were read on a multiwell spectrophotometer (Victor^3 plate reader, PerkinElmer, Rodgau-Juegesheim, Germany) at a wavelength of 620 nm and a reference wavelength of 690 nm.

2.7. Statistics

Data are expressed as means ± S.E. IC_{50} values are approximations determined by graphical analysis (linear interpolation between the points between 50% activity). The program Graphpad Instat (Graphpad Software Inc., San Diego, CA) was used for statistical comparisons. Statistical evaluation of the data was performed by one-way ANOVAs for independent or correlated samples followed by Tukey HSD post hoc tests. A p value of <0.05 (*) and <0.001 (***) was considered significant.

3. Results

3.1. LPS binds to BAs

The binding of LPS to β-BA was determined in pull-down experiments using immobilized β-BA (BA-seph). After incubation of LPS with BA-seph or seph, the beads were separated from the supernatants by centrifugation, intensively washed and bound LPS was eluted by 4 M urea. The occurrence of LPS in the respective fractions (i.e., in the eluates of precipitated beads or in the supernatants) was monitored using the modified LAL assay. LPS was clearly detectable in the eluates of BA-seph, whereas the eluates of seph (lacking β-BA) did not contain detectable LPS levels (Fig. 1). Accordingly, the amount of remaining LPS in the supernatant of the pull-down using BA-seph was diminished, compared to the control approach with seph that contained higher LPS levels in the supernatant. Attempts to conduct competition experiments with excess of soluble BAs (>1 mM) failed due to poor aqueous solubility of the BAs and neutralization of LPS by the excess of soluble BAs.

3.2. Inhibition of LPS activity by BAs

In order to analyze whether or not the direct interaction of BAs with LPS can also influence LPS functionality, BAs were tested for their ability to affect LPS activity in the modified LAL assay. The well-characterized LPS-neutralizing protein polymyxin B [27] was used a reference compound that completely inhibited LPS activity at a concentration of 100 nM (not shown). Among the major β-configurated BAs, only β-BA significantly modulated LPS activity that was reduced by approx. 70% at a final concentration of 10 μM, whereas KBA, Aβ-BA and AKBA failed in this respect (Fig. 2A). This indicates that the C(3)-acetoxy group as well as the C(11)-keto moiety may hamper the bioactivity. To gain deeper insights into the structure-activity relationships (SAR), further BA derivatives with structurally modified C3 position (Table 1) were synthesized and analyzed for their ability to inhibit LPS activity. As shown in Fig. 2B, all BA derivatives (i.e., cm-BA, ox-BA, succ-BA, glut-BA) lacking the 11-keto moiety efficiently inhibited LPS activity but the respective 11-keto analogs were inactive. Note that Aβ-BA was ineffective despite the lack of the 11-keto group. Interestingly, in contrast to Aβ-BA, the esterification of the C(3)-OH group with bifunctional acids or acid chlorides (e.g., succinic or glutaric acid, oxalyl chloride) retaining one free terminal carboxylate as well as etherification with a carboxymethyl moiety (in cm-BA) led to potent inhibitors of LPS. More detailed concentration response studies revealed IC_{50} values of 1.8 μM for β-BA, 1.9 μM for cm-BA and for glut-BA, each, and 2.5 μM for

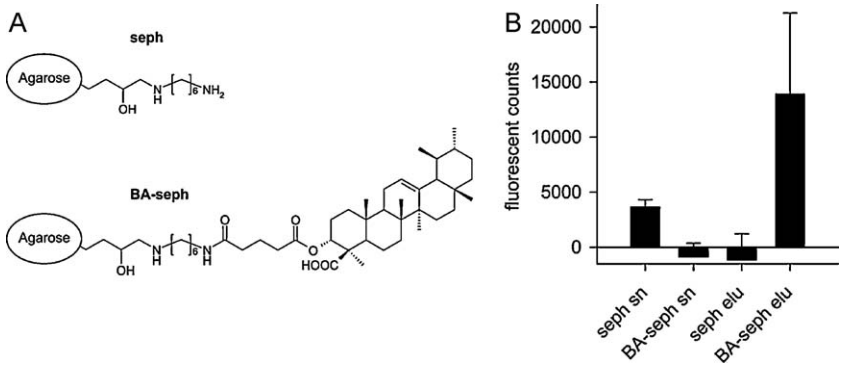


Fig. 1. LPS binds to a boswellic acid-affinity matrix. (A) Structure of seph and BA-seph. (B) LPS pull-down. Seph and BA-seph were incubated with LPS (1 μg/ml) and eluted with 4 M urea. Precipitated molecules were eluted and LPS levels of the eluates (elu) and supernatants (sn) were analyzed by a modified LAL assay. Data are given as mean + S.E., $n = 4$.

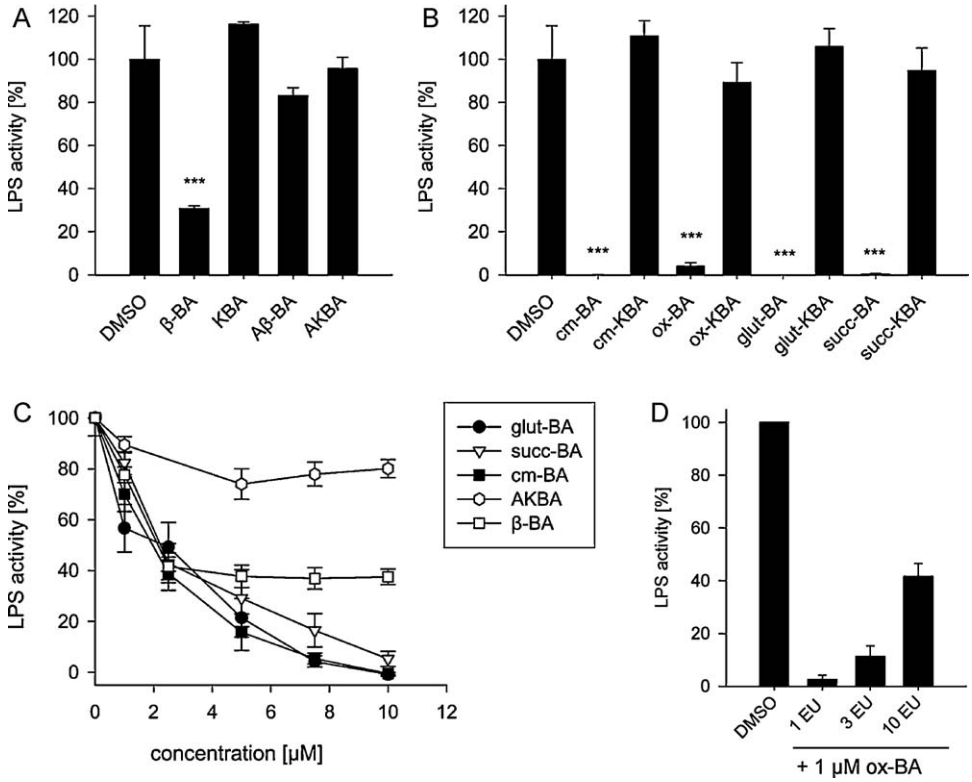


Fig. 2. BAs inhibit LPS activity in a cell-free LAL assay. LPS (10 endotoxin units (EU)/ml) was incubated with (A) natural occurring BAs (10 μM), (B) synthetic BAs (10 μM) and (C) both natural occurring and synthetic BAs at different concentrations; (D) LPS at various amounts (i.e., 1, 3, and 10 EU/ml) was incubated with or without 1 μM ox-BA. After 10 min, LPS activities were determined using a modified LAL assay. Data are given as mean ± S.E., $n = 3$, $^{***}p < 0.001$, *vs.* vehicle (DMSO).

succ-BA (Fig. 2C). To exclude unspecific interference of BAs with the LAL assay, such as inhibition of factor C or the fluorescent substrate, we varied the LPS amounts at a fixed BA concentration. As shown in Fig. 2D, at decreasing LPS amounts the efficiency of ox-BA was continuously enhanced, excluding interference with the LAL assay reaction. Together, our data show that β-BA (as naturally occurring BA) is a potent inhibitor of LPS, and apparently polar residues in C(3) position and the absence of the 11-keto group are structural determinants required for inhibition of LPS activity.

3.3. Inhibition of LPS-induced iNOS expression by BAs

Next, we wanted to investigate whether or not BAs may also interfere with LPS activity in a cell-based assay. LPS is a well-recognized activator of iNOS expression in rodent macrophages

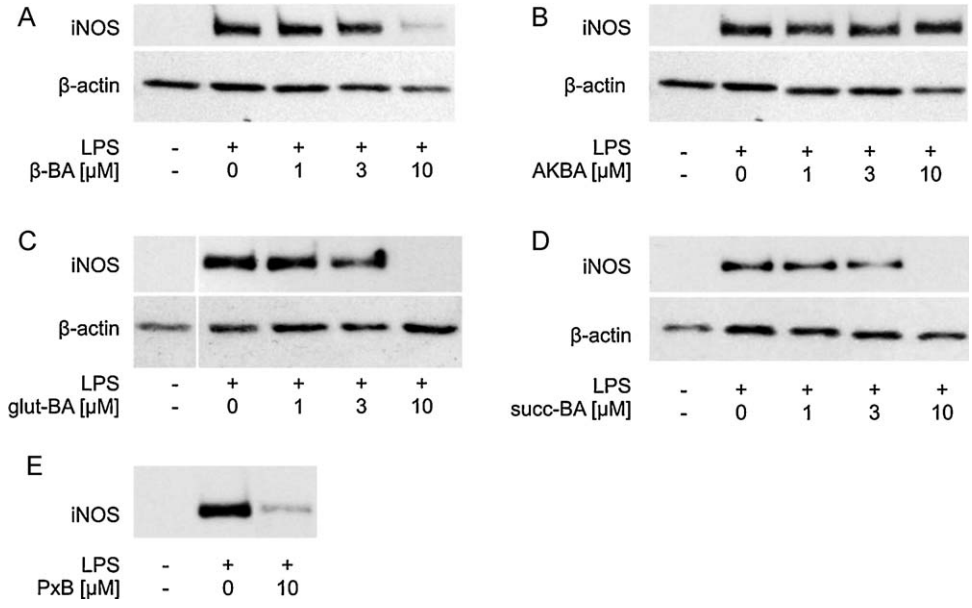


Fig. 3. LPS-induced iNOS expression is inhibited by BAs. RAW264.7 cells (2.5×10^6/ml) were preincubated with BAs for 15 min prior to LPS (1 μg/ml). After 21 h, cells were harvested and iNOS and β-actin were analyzed by SDS-PAGE and Western blot. (A) β-BA, (B) AKBA, (C) glut-BA, (D) succ-BA and (E) polymyxin B (PxB). Results shown are representative for three independent experiments.

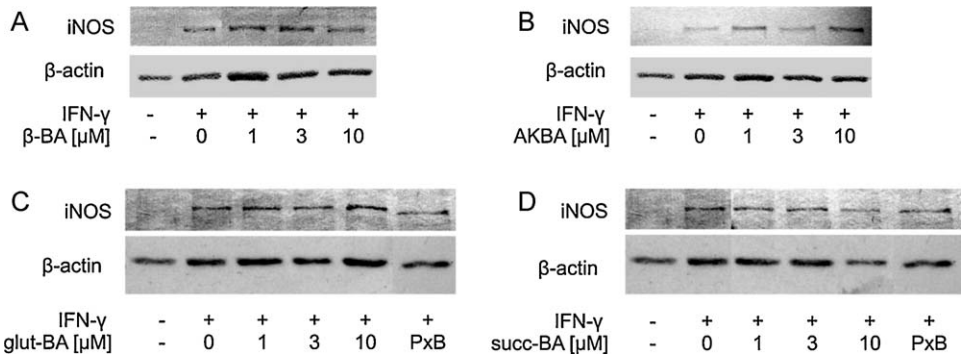


Fig. 4. IFN-γ-induced iNOS expression is not influenced by BAs. RAW264.7 cells (2.5×10^6/ml) were incubated with BAs for 15 min prior to IFN-γ (10 ng/ml). After 21 h, cells were lysed and both iNOS and β-actin levels were analyzed by SDS-PAGE and Western blot. (A) β-BA, (B) AKBA, (C) glut-BA and (D) succ-BA. PxB, polymyxin B. Results shown are representative for three independent experiments.

which eventually leads to an increased generation and release of nitric oxide [26]. When RAW264.7 cells were stimulated with LPS for 21 h, a clear induction of iNOS protein levels was observed (Fig. 3). The LPS-neutralizing polymyxin B, used as reference compound, blocked iNOS expression as expected. Of interest, the LPS-induced iNOS induction was antagonized by those BAs that inhibited LPS activity (i.e., β-BA, glut-BA, and succ-BA, 10 μM each) but not by AKBA that also failed to modulate LPS activity (Fig. 3) or by other inactive synthetic 11-keto-BAs (i.e., succ-KBA, glut-KBA, not shown). In order to further exclude unspecific interference of the active BAs on iNOS expression, RAW264.7 cells were stimulated with IFN-γ that also leads to an enhanced iNOS expression [28] similar as LPS. However, as shown in Fig. 4, BAs as well as polymyxin B did not influence IFN-γ-induced iNOS levels, suggesting a direct interrelation between LPS inhibition and suppression of LPS-induced iNOS expression.

Since BAs were reported to induce apoptotic cell death in various eukaryotic cell lines [1], it appeared reasonable that reduced iNOS expression in LPS-treated RAW264.7 cells could be due to cytotoxic effects of BAs. Thus, cell viability was analyzed after incubation of with LPS with and without BAs for 21 h by MTT assay. β-BA, AKBA and succ-BA did not significantly affect cell viability up to 10 μM, whereas glut-BA reduced cell viability at 10 μM by approx. 45% (Fig. 5).

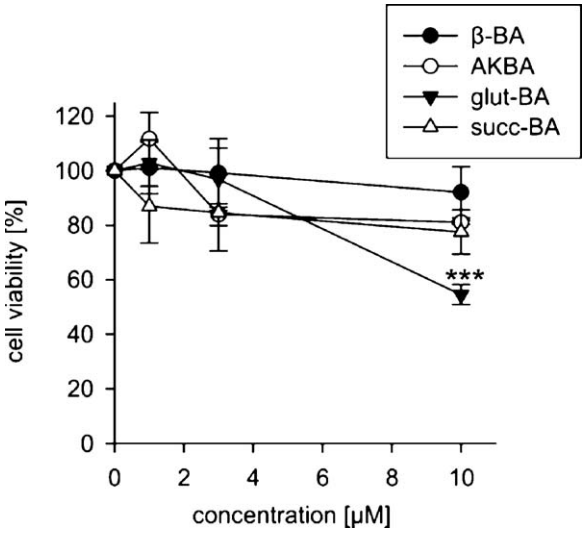


Fig. 5. Influence of BAs on cell viability. RAW264.7 cells were incubated with LPS (1 μg/ml) and BAs for 21 h. Viable cells were stained by MTT (5 mg/ml) and lysed. Absorption at 620/690 nm was measured as a marker of viable cells. Data are given as mean + S.E., $n = 3$. $^{***}p < 0.001$, *vs.* vehicle (DMSO).

4. Discussion

Here we showed that BAs with defined structural determinants directly interact with LPS leading to an inhibition of its biological activity. Thus, by means of a fishing approach for BAs [3,4,17], we demonstrate that LPS is as a direct binding partner of immobilized β-BA as LPS was specifically precipitated by BA-seph but not by the matrix (seph) alone. β-BA as well as select synthetic BAs inhibit the activity of LPS in a cell-free modified LAL assay that directly and specifically detects LPS [24], suggesting a direct functional interaction between the BAs and LPS. Importantly, this interference strongly depended on the structure of the BAs where in particular the presence of the 11-keto moiety essentially abolished the inhibitory BA effect. Finally, the biological relevance of LPS neutralization is demonstrated by the blockade of LPS-induced iNOS expression in RAW264.7 macrophages selectively by those BAs that inhibited the LPS activity in the LAL-assay. Since INF-γ-induced iNOS expression was unaffected under the same conditions, we conclude that BAs directly target LPS rather than unspecifically interacting with other molecules in downstream LPS signaling or in transcriptional or translational events of iNOS induction. Because BSE are commonly used in folk medicine to treat infection and inflammation [2], our data may provide a molecular basis for the beneficial effects of such extracts. Moreover, our findings may also explain the results by others that described the suppression of various LPS-induced cellular responses by BAs without concretely defining the BA target in the respective experimental set up [7,20,21].

The interference of BAs with LPS was demonstrated by the pull-down assay which was indeed a key experiment in this study. However, this result simply reflects a physical binding of the two molecules without any information about the functional consequence of such interaction. The inhibition of LPS activity by BAs in the modified LAL assay on the other hand clearly proves for the functionality. Note that under the experimental settings in this cell-free assay, only the direct interaction with LPS may suppress the monitored assay reaction. Interference of BAs with the activation of factor C or the fluorescent substrate is unlikely, since the efficiency of BAs in the LAL assay clearly depended on the amount of LPS. We observed that BAs lacking the 11-keto moiety displayed a very potent inhibition of LPS activity in this test system with IC_{50} values between 1.8 and 2.5 μM, whereas their corresponding 11-keto analogs were virtually ineffective. This suggests defined structural features of the respective pharmacophore and excludes unspecific (lipophilic) interactions between the BAs and LPS. The presence or absence of this 11-keto function also determines other bioactivities of BAs including inhibition of 5-LO [29], COX-1 [3], as well as Ca^{2+} mobilization and MAPK

activation in different cell types [30–32], for all of which the 11-keto moiety is a determinant. Note that Aβ-BA was essentially ineffective despite of the lack of the 11-keto moiety. It appears that a quite hydrophilic moiety at C(3) may be another determinant for LPS-inhibitory properties which is missing in Aβ-BA (where the C(3)-OH is masked by the esterification with a less polar acetyl residue). In fact, all LPS-inhibitory BAs contain a hydrophilic or even charged moiety connected to C3 such as the hydroxyl group in β-BA or the ω-carboxylic acid residue in cm-BA, ox-BA, succ-BA or glut-BA that are coupled via an ester or ether function to the C(3)-oxygen.

It should be emphasized that those BAs that neutralized LPS activity in the LAL assay (e.g., β-BA, ox-BA, succ-BA and glut-BA) also suppressed the LPS response in the cell-based assay, whereas AKBA (or cm-KBA, succ-KBA, glut-KBA) that failed in the LAL assay was inactive in the cell-based model as well. Interestingly, the suppression of iNOS expression by BAs in the cell-based assay depended on the stimulus. Activation of the transcription factors NF-κB and STAT-1α, and thereby activation of the iNOS promoter, seems to be an essential step for iNOS induction in most cells [33]. Both LPS as well as INF-γ can induce iNOS in macrophages and monocytes via convergent pathways such as NF-κB and MAPK signaling [34–37]. However, only the LPS-induced but not the INF-γ-induced iNOS expression in RAW264.7 cells was blocked by BAs, suggesting that the point of attack of BAs is upstream of the convergent signaling molecules and might be related to a direct interaction with LPS. Several previous reports by different groups have shown that BAs, BSE or other ingredients from BSE antagonize the actions of LPS *in vitro* [7,18,20,38–41] and *in vivo* [19,21], and some of these studies [18,19,38,39,41] even showed that BSE blocks LPS-induced iNOS expression or iNOS activity. Unfortunately, the defined mechanisms of how these LPS-induced responses were blocked are unknown. Consequently, the fact that BAs can directly inhibit LPS add to the understanding of the biological activities of BAs and may provide the underlying molecular basis of these reported observations.

Neutralization of LPS may be beneficial for the treatment of a number of diseases, and there are indications that several disorders, where BSE were actually beneficial, might be mediated via LPS-induced signal transduction cascades. For example, inhalation of LPS leads to bronchoconstriction [42–44], a change in non-specific airway responsiveness [42,43,45] and to a reduction of alveolar capillary diffusion [46]. A participation of LPS in chronic airway diseases as asthma has been suggested [47]. Also, high levels of iNOS and nitric oxide are associated with asthma [48,49]. Interestingly, a clinical trial demonstrated the effectiveness of BSE application during asthma [50]. Thus, in a double-blind placebo controlled study, forty asthma patients were treated with a BSE (3 × 300 mg/day) for 6 weeks. 70% of the BSE-treated patients showed an improvement in several disease parameters, compared to 27% of the control group. Besides asthma, BSE have been used in folk medicine to treat arthritic diseases [2], and in several animal studies a beneficial effect on arthritis could be demonstrated [51–53]. Of interest, elevated iNOS levels have been found also in rheumatoid arthritis and osteoarthritis [54]. A connection of high iNOS levels and LPS during the course of these diseases has still to be elucidated, but LPS was at least shown to be involved in reactive arthritis [55]. In addition, the use of BSE might be beneficial in the treatment of Crohn's disease [56]. While the exact molecular mechanism involved in Crohn's disease is still to be elucidated, there are indications that bacteria might be involved [57], and nitric oxide seems to contribute to the disease [58]. Possibly, LPS from prokaryotic membranes may be involved in the disease as well.

Among the naturally occurring BAs in BSE, β-BA might be the most relevant BA derivative that inhibits LPS-induced responses. The concentration of β-BA required for efficient inhibition of LPS activity is clearly in the range of blood plasma levels reached (6.4–10.1 μM) after oral application of standard doses of BSE [4,9]. As discussed above, BSE have been shown to be effective in diseases connected to elevated LPS levels and neutralization of LPS by β-BA may be a part of the molecular actions responsible for the beneficial BSE effects. Finally, our data may encourage for expanded studies on the influence of β-BA and BSE in LPS-mediated diseases such as sepsis and septic shock or certain other bacterial infections where LPS plays crucial roles. Whether BAs are beneficial in these diseases remains to be elucidated, but since sepsis is one of the major causes of death in critically diseased patients [16], future investigations of the therapeutic benefit of BSE or BAs deserves particular attention.

Conflict of interest statement

None declared.

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