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Inhibition of Respiratory Burst Activity in Alveolar Macrophages by Bisbenzylisoquinoline Alkaloids: Characterization of Drug–Cell Interaction

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ABSTRACT: *The objective of this study was to investigate the effects of various bisbenzylisoquinoline (BBIQ) alkaloids on respiratory burst activity of alveolar macrophages and to characterize the interaction of these drugs with alveolar phagocytes. BBIQ alkaloids were chosen for study because they exhibit a wide range of antifibrotic potencies in a rat model, with tetrandrine being very effective and tubocurarine being ineffective. These drugs inhibited zymosan-stimulated oxygen consumption with a potency sequence of tetrandrine (TT) ≈ fangchinoline (FA) > berbamine (BE) ≈ cepharanthine (CE) ≈ cycleanine (CY) ≫ tubocurarine (TU). This inhibition of respiratory burst activity could not be attributed to a drug-induced decline in the ATP content of these pneumocytes. Drug binding to alveolar macrophages was directly dependent on temperature and drug concentration. The sequence for binding capacity was FA > TT ≈ BE ≈ CY > CE ≫ TU. Therefore, there was no simple relationship between binding capacity and inhibitory potency. Binding capacity was not related to lipophilicity of these alkaloids. In addition, tetrandrine failed to bind to metabolically dead cells or sonicated macrophage preparations. These data suggest that the interaction of BBIQ alkaloids with phagocytes is not simply nonspecific binding to membrane lipids. Alteration of the cytoskeletal system with vinblastine, taxol, or cytochalasin B decreased tetrandrine binding by approximately 33% when added separately and by 93% when added jointly. Pre-exposure of alveolar macrophages to stimulants increased the ability of BBIQ alkaloids to inhibit both oxygen consumption and superoxide release. These data suggest that the mechanism by which BBIQ alkaloids inhibit activation of phagocytes involves microtubules and*

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bules and microfilaments. Pre-exposure of macrophages to stimulants would change the conformation of cytoskeletal components and may make these structures more susceptible to drug interaction.

INTRODUCTION

Tetrandrine is a bisbenzylisoquinoline alkaloid that is isolated from the root of the traditional Chinese medicinal herb *Stephania tetrandra.* The molecular weight of tetrandrine is 622.73 and its empirical formula is $C_{38}H_{42}O_6N_2$. Its structure is characterized by two 17-carbon ring members connected by a double oxygen bridge.

Tetrandrine has been reported to exhibit antitumor, antihypertensive, and antifibrotic properties [1–3]. It is currently used in China as a treatment for silicosis and has been reported to improve diffusion capacity and decrease the size of lesions visualized on chest radiographs of patients suffering from pulmonary fibrosis [4]. However, information is lacking thus far as to the mechanism(s) by which tetrandrine expresses its antifibrotic action.

Tetrandrine has been shown to inhibit collagen synthesis by pulmonary fibroblasts and to reduce the formation of silicotic nodules in silica-exposed rats [3, 5, 6]. It is possible that tetrandrine acts on alveolar macrophages to prevent the silica-induced release of fibrogenic factors from these phagocytes. Indeed, we have shown that tetrandrine inhibits silica-induced secretion of interleukin-1 from pulmonary macrophages [7]. The antifibrotic potential of tetrandrine may also result from its ability to depress silica-induced activation of pulmonary phagocytes and thus decrease oxidant-induced lung damage. Data from our laboratory indicate that in vitro treatment of alveolar macrophages with tetrandrine significantly inhibits zymosan-stimulated superoxide release, hydrogen peroxide secretion, and oxygen consumption [8]. Similar effects were also seen after in vivo treatment of silica-exposed rats with tetrandrine [8]. This drug is also effective in preventing the activation of polymorphonuclear leukocytes; i.e., tetrandrine decreases chemiluminescence, degranulation, superoxide anion release, and bacterial killing by neutrophils [8–10].

Mo et al. [11] have measured the antifibrotic potency of several bisbenzylisoquinoline alkaloids in the silicotic rat model and compared the effects of these analogues with tetrandrine. Tetrandrine (50 mg/kg) was found to decrease collagen formation by 52%. In addition, only small macules were observed in tetrandrine-treated rat lungs 4 weeks after intratracheal installation of silica (50 mg) compared with the presence of nodules and large macules in the silica-exposed controls. Cycleanine was also found to be potent; fangchinoline and cepharanthine exhibited slightly lower potency; berbamine was less effective; while tubocurine and tubocurarine were ineffective in decreasing collagen formation or preventing the appearance of silicotic nodules. Our laboratory has shown that for tetrandrine and tubocurine there was a rela-

tionship among antifibrotic potency, macrophage deactivation, and binding to alveolar macrophages [12]. The antifibrotic drug tetrandrine bound strongly to alveolar macrophages and inhibited particle-induced activation of these phagocytes, while tubocurine bound weakly and had little effect on stimulant-induced activation.

The objectives of the present investigation were to quantitate the effects of various alkaloids, i.e., analogues of tetrandrine that exhibit various levels of effectiveness in preventing fibrosis [11], on the activation of alveolar macrophages and to determine if a relationship exists between the ability to inhibit respiratory burst activity and the level of drug binding to these phagocytes. In addition, we characterized parameters affecting drug–cell interaction such as dose and temperature. Finally, attempts were made to determine if binding of drugs to these pneumocytes was nonspecific or related to specific target sites that may play crucial roles in the stimulus–secretion coupling system in these phagocytes.

METHODS

Isolation of Alveolar Macrophages

Male Sprague–Dawley rats (175–225 g) obtained from Charles River Laboratories (Wilmington, MA) were anesthetized with sodium pentobarbital (0.2 g/kg) and exsanguinated by cutting the renal artery. Alveolar macrophages were obtained by pulmonary lavage with a Ca^{2+}, Mg^{2+}-free phosphate-buffered medium (145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5.5 mM glucose; pH 7.4). Cells were centrifuged at 500*g* for 5 min, washed, and resuspended in a Hepes-buffered medium (145 mM NaCl, 5 mM KCl, 10 mM Hepes, 5.5 mM glucose, and 1.0 mM $CaCl_2$; pH 7.4). Cell counts and purity were measured using an electronic cell counter equipped with a cell sizing attachment [13]. Membrane integrity was determined microscopically by monitoring the exclusion of trypan blue dye [14].

To obtain dead alveolar macrophages, cells (1×10^6/mL) were incubated with 5 mM NaCN and 5 mM iodoacetic acid at 37°C for 45 min. The cell pellet was obtained by centrifugation (500*g* for 5 min), resuspended in Hepes-buffered medium (2×10^6 cells/mL), and incubated at 37°C for 75 min. Membrane integrity was monitored by trypan blue exclusion and these cells were used as dead alveolar macrophages when viability was less than 20%. To lyse the cells, alveolar macrophages (2×10^6 cells/mL) were sonicated for 20 s on ice.

Source of Bisbenzylisoquinoline (BBIQ) Alkaloids

The following BBIQ alkaloids were kindly supplied by the Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, People's Republic of China: tetrandrine (TT), fangchinoline (FA), cepharanthine

(CE), berbamine (BE), and cycleanine (CY). Tubocurarine (TU) was obtained from Sigma Chemical Company (St. Louis, MO). Since these drugs exhibit a broad range of antifibrotic potency, i.e., ranging from very active to ineffective [11], they were chosen to probe the relationship among macrophage binding, inhibition of phagocyte activity, and antifibrotic potential. Stock solutions of alkaloids were prepared by mixing the drug with a few drops of water, adding HCl until dissolved, adjusting pH to 6.5 with NaOH, and then bringing up to volume with water.

The structure of tetrandrine is given in Fig. 1. Important structural characteristics of tetrandrine include (1) methoxy groups at C_7 and C_{12}; (2) uncharged nitrogens at N_2 and $N_{2'}$; and (3) a double oxygen-bridged ring (C_8–$C_{7'}$ and C_{11}–$C_{12'}$) of 18 bond lengths. Fangchinoline has a hydroxyl substitution at C_7, while berbamine has a hydroxyl substitution at C_{12}. Cepharanthine has a condensed methylenedioxy ring at C_6 and C_7 and the 18-bond-length double oxygen ring between C_8–$C_{7'}$ and C_{12}–$C_{11'}$. Cycleanine exhibits an 18-membered oxygen-bridged ring at C_8–$C_{12'}$ and C_{12}–$C_{8'}$ with methoxy substitution at C_7 and $C_{7'}$. Tubocurarine is characterized by hydroxyl substitutions at both C_7 and C_{12}, a 20-bond-length oxygen-bridged ring between C_8–$C_{12'}$ and C_{13}–$C_{7'}$, and positively charged ammonium nitrogens at positions N_2 and $N_{2'}$ bound to two methyl groups instead of one.

Binding of Alkaloids to Alveolar Macrophages

Binding of BBIQ alkaloids to alveolar macrophages was carried out in Hepes-buffered medium at 2×10^6 cells/mL. Drugs were added to cell suspensions and incubated at the desired temperature. A 0.3-mL aliquot of sample mixture was withdrawn at each time point and cells were separated by centrifugation for 20 s in an Eppendorf 5412 centrifuge (Brinkmann, Westbury, NY). The supernatant was saved for drug analysis and the cell pellet was used to determine cell viability. The concentration of drug in the supernatant was monitored by reverse-phase high-performance liquid chromatography (HPLC) us-

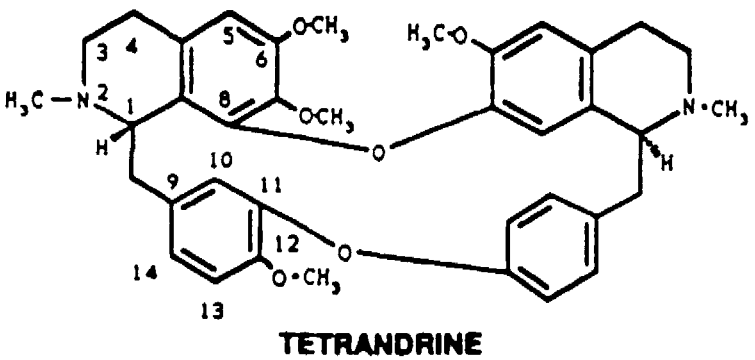

Figure 1 Structure of tetrandrine showing the characteristic double oxygen-bridged ring.

ing a Waters HPLC system equipped with a Model 440UV detector (Waters Associates, Milford, MA). BBIQ alkaloids were separated from other substances using a μBondapak C_{18} column and a mobile phase of CH_3CN/Hepes buffer (pH 4.5)/*n*-butanol (37 : 60 : 3) with a flow rate of 1 mL/min. The samples were detected by UV at 254 nm. The drug level of a test sample was determined by integrating the absorbance peak and comparing this value to a standard, i.e., drug in medium alone. Note that drug remaining in the supernate declined as binding to the cells rose. In the absence of cell binding, recovery approached 100%.

Oxygen Consumption Measurement

Oxygen consumption was measured with a Gilson K-IC oxygraph equipped with a Clark electrode (Gilson Medical Instruments, Middletown, WI). Cells (4×10^6 cells/mL) were preincubated at 37 °C for 10 min in 1.8 mL of Hepes-buffered medium. After preincubation, the cell suspension was transferred to a temperature-controlled chamber (37 °C) and resting oxygen consumption was monitored. Stimulant-induced oxygen consumption was determined after the addition of 2 mg/mL zymosan or 3 μM PMA (phorbol-12-myristate-13-acetate) to the cell suspension. To investigate the effects of BBIQ alkaloids on oxygen consumption by alveolar macrophages, drugs (50 μM) were added either before or after the addition of the stimulant and oxygen consumption was monitored for 5 min. The oxygraph was calibrated using media equilibrated with gases of known oxygen content.

Measurement of ATP Concentration of Alveolar Macrophages

Alveolar macrophages (2×10^6 cells/mL) were incubated at 37 °C for 20 min in Hepes-buffered medium in the absence or presence of 25 μM BBIQ alkaloids. Cells were centrifuged at 1980*g* for 1 min, washed once, and resuspended in 0.135 mL of 0.5 M tris-acetate buffer, pH 7.4. A 10-μL aliquot for the cell suspension was used for cell counting. Triton X-100 (1 : 200 dilution) was added to cell suspension at equal volume to disrupt the cell membrane, the sample was thoroughly mixed by vortexing for 10 s, and 50 μL was immediately analyzed for ATP content with a Lumi-Aggregometer Model 400 (Chrono-Log, Haverstown, PA) using the firefly luciferase method as described by Wolff and Doppen [15]. The concentration of ATP was determined by measuring the emission of light resulting from mixing the sample with 50 μL of firefly lantern extract (Sigma Chemical, St. Louis, MO) in a total reaction mixture of 0.5 mL. The ATP concentration was calculated based on ATP standard solutions.

Statistical Analysis

Data were presented as means ± standard errors of 4 to 6 experiments. Data were compared using a Student's t test with significance set at $p < .05$.

RESULTS

Previously, we have shown that tetrandrine was a potent inhibitor of particle-stimulated superoxide release, hydrogen peroxide secretion, and oxygen consumption by alveolar macrophages [8, 12]. In the present study, the effects of several other BBIQ alkaloids on zymosan-stimulated oxygen consumption by alveolar macrophages were compared with that of tetrandrine (Fig. 2). The results indicate that several BBIQ alkaloids exhibited significant inhibition of these phagocytes. The potency of these drugs in inhibiting particle-stimulated respiratory burst activity was as follows: TT ≈ FA > BE ≈ CE ≈ CY ≫ TU. Similarly, PMA-stimulated oxygen consumption by alveolar macrophages was also inhibited by these BBIQ alkaloids (data not shown). Although these alkaloids inhibited activation of phagocytes with stimulants, they did not affect resting oxygen consumption or trypan blue exclusion under similar conditions, indicating that the inhibition noted above was not due to decreased cellular viability.

To characterize the mechanism in which these alkaloids inhibited stimulant-induced activation of alveolar macrophages, their binding to alveo-

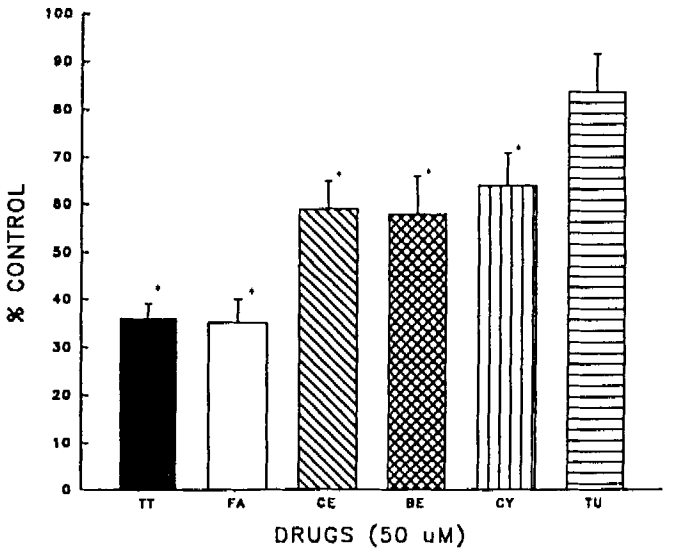

Figure 2 The effect of BBIQ alkaloids on zymosan-stimulated oxygen consumption by alveolar macrophages. Drug (50 μM) was added to the cells before addition of zymosan (2 mg/mL). After each addition, oxygen consumption was measured. Values represent means ± standard errors of four experiments. The control value for zymosan-stimulated oxygen consumption by alveolar macrophages was 4.63 ± 0.33 nmol/10^6 cells min^{-1}. *Significant decrease from control.

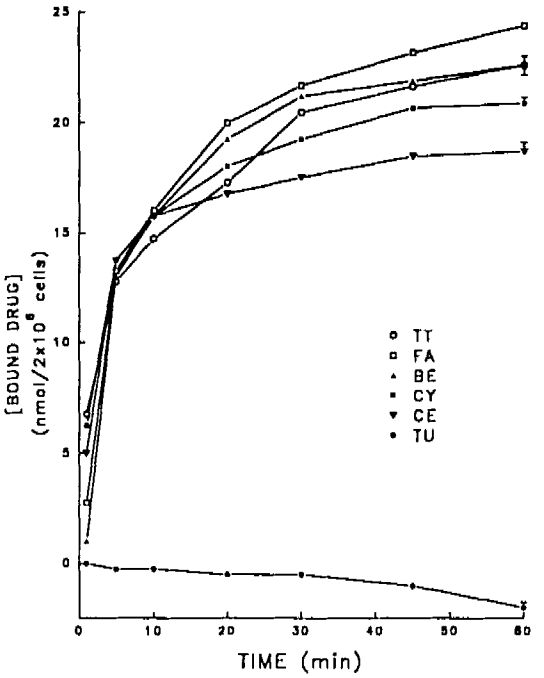

Figure 3 Time course for the binding of BBIQ alkaloids to alveolar macrophages. Alveolar macrophages (2×10^6/mL) were treated at 37 °C with BBIQ alkaloids (25 μM) for various times. The concentration of the drug in the supernatant was measured by reverse phase HPLC, monitored by UV at 254 nm. The cell pellet was used for determining cell viability. Values represent means ± standard errors of four experiments.

lar macrophages was determined. With the exception of tubocurarine, all of these BBIQ alkaloids bound to alveolar macrophages in a time-dependent manner with the rate of binding being greatest during the first 5 min of incubation (Fig. 3). The sequence for maximal binding of these alkaloids to alveolar macrophages was as follows: FA > TT ≈ BE ≈ CY > CE ≫ TU. Figure 4 shows that the interaction between tetrandrine and macrophages was concentration dependent, with half maximal binding being achieved with 11–12 μM tetrandrine. Figure 5 shows that the interaction of tetrandrine, fangchinoline, and cepharanthine with alveolar macrophages was also temperature dependent. At room temperature, all of these alkaloids exhibited approximately the same binding strength toward cells. At 37 °C, fangchinoline and tetrandrine exhibited greater binding to cells than cepharanthine. At low temperatures, tetrandrine bound to alveolar macrophages more avidly than either cepharanthine or fangchinoline. The activation energies (ΔE_a) for the drug–cell interactions were 12.0, 13.0, and 9.7 kcal/mol for tetrandrine, cepharanthine, and fangchinoline, respectively.

To further characterize the nature of the interaction between tetrandrine and alveolar macrophages, the binding of tetrandrine to cells killed with cya-

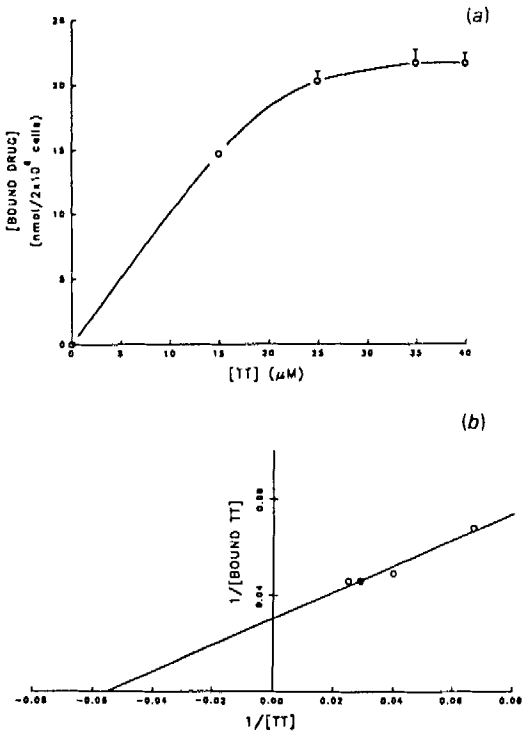

Figure 4 Concentration dependence of tetrandrine binding to alveolar macrophages. Data are plotted as TT binding vs. TT concentration (A) and as 1/bound TT vs. 1/TT concentration (B). Alveolar macrophages (2×10^6/mL) were treated at 37 °C with various doses of tetrandrine and the concentration of unbound drug was determined in the supernate by HPLC. Cell viability was monitored using the cell pellet. Values represent means ± standard errors of four experiments.

nide and iodoacetic acid or lysed by sonication was studied. The results shown in Fig. 6 indicate that tetrandrine did not bind to either dead or lysed cells. This absence of binding could not be explained by the release of factors from leaky cells that might have inhibited binding, since supernate obtained from sonicated phagocytes did not alter tetrandrine binding. Therefore, these results suggest that cell integrity is essential to alkaloid binding and that the interaction of alveolar macrophages with tetrandrine is not simply nonspecific binding of the drug to the cell membrane.

The effect of vinblastine and taxol (microtubular modifiers) and/or cytochalasin B (a microfilament inhibitor) on the binding of tetrandrine to the alveolar macrophages is shown in Table 1. These cytoskeletal modifiers blocked the binding of tetrandrine to alveolar phagocytes by approximately 33% when added separately and by 93% when all three inhibitors were added simultaneously. These results suggest that the integrity of the cytoskeletal structure plays an important role in the interaction of tetrandrine with alveolar macrophages.

Activation of phagocytes has been associated with changes in the cytoskele-

tal system such as actin polymerization [16]. Therefore, we investigated the effect of prior phagocyte activation on the ability of BBIQ alkaloids to inhibit respiratory burst activity of alveolar macrophages. Stimulation of macrophages with zymosan substantially enhanced the ability of BBIQ alkaloids to inhibit respiratory burst activity; i.e., drugs were more effective in inhibiting oxygen consumption when added after the addition of stimulant than when added prior to stimulation (Table 2). Similarly, tetrandrine was a more potent inhibitor of superoxide anion release when added after, rather than prior to, stimulation. The inhibitory potency of tetrandrine was 90 ± 4% when added after PMA compared to a 62 ± 3% inhibition of superoxide anion release when added prior to PMA. Similar results were seen with zymosan-stimulated superoxide release.

Since ATP synthesis is associated with cellular respiration, we investigated the effects of BBIQ alkaloids on ATP content of alveolar macrophages. There was no relationship between the ability of drugs to inhibit the respiratory burst (Fig. 2) and any effect on ATP content (Table 3). Therefore, the ability of BBIQ alkaloids to inhibit phagocytotic activity was not the result of depletion of cellular ATP.

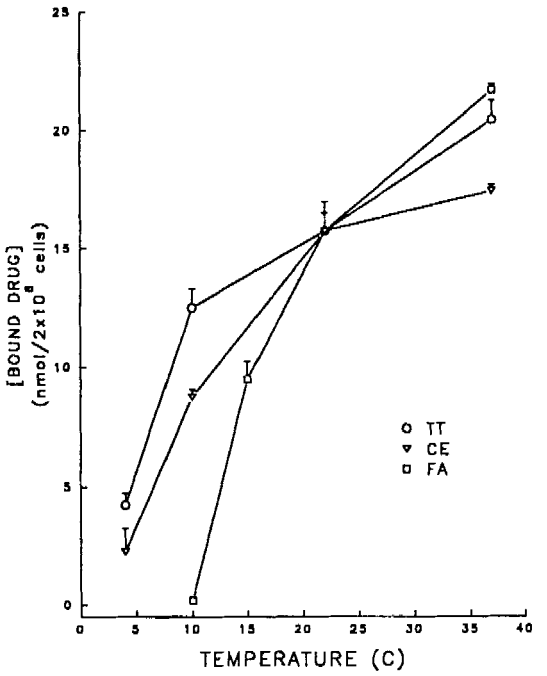

Figure 5 Temperature dependence of BBIQ binding to alveolar macrophages. After preincubation of alveolar macrophages (2×10^6/mL) at the desired temperature for 10 min, 25 μM of alkaloid was added to the reaction mixture. Cells were incubated with drugs for 30 min and then separated by centrifugation. The supernatant was saved for drug analysis by HPLC and the cell pellet was used for determining cell viability. Values are means ± standard errors of four experiments.

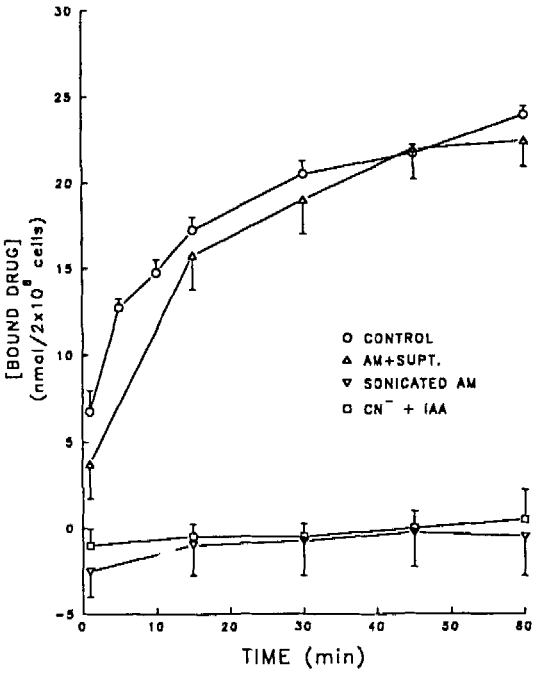

Figure 6 The effect of cell viability on tetrandrine binding. Viable, lysed, or dead cells were incubated with 25 μM tetrandrine. At desired time point, aliquots of the sample were removed and separated by centrifugation, and the concentration of drug in the supernatant was determined by HPLC. Values represent means ± standard errors of four experiments.

Table 1 Effects of Cytoskeletal Modifiers on the Binding of Tetrandrine to Alveolar Macrophages

Treatment	Binding of tetrandrine (% bound)
Tetrandrine	82 ± 3
Vinblastine + tetrandrine	55 ± 5*
Cytochalasin B + tetrandrine	56 ± 4*
Taxol + tetrandrine	23 ± 4*
Vinblastine + cytochalasin B + tetrandrine	26 ± 7*$^{+}$
Taxol + cytochalasin B + tetrandrine	28 ± 2*$^{+}$
Taxol + vinblastine + cytochalasin B + tetrandrine	6 ± 9*$^{+}$

Note. Alveolar macrophages (2×10^6/mL) were incubated with 25 μM tetrandrine in the absence or presence of vinblastine (10^{-4} M), cytochalasin B (20 μg/mL), and/or taxol (20 μM) at 37°C for 30 min. After the incubation period, an aliquot of the sample was removed and separated by centrifugation. The concentration of the drug in the supernatant was determined by HPLC and the cell pellet was used for determination of viability. Values are means ± standard errors of four experiments. *Significant decrease from tetrandrine alone; $^{+}$significant decrease from one modifier.

Table 2 Effects of Alkaloids on Zymosan-Stimulated Oxygen Consumption by Alveolar Macrophages

	Zymosan-stimulated oxygen consumption (% inhibition)	
Drug (50 μM)	Pretreated with drug	Post-treated with drug
Tetrandrine	65 ± 3	119 ± 7
Fangchinoline	63 ± 8	102 ± 11
Berbamine	33 ± 11	105 ± 10
Cycleanine	35 ± 2	112 ± 2
Cepharanthine	40 ± 8	105 ± 15
Tubocurarine	16 ± 4	88 ± 14

Note. Resting oxygen consumption was 2.39 ± 0.04 nmol/10^6 cells min^{-1}. Zymosan (2 mg/mL) increased oxygen consumption to 6.90 ± 0.30 nmol/10^6 cells min^{-1}. Drugs did not affect resting oxygen consumption. Values are means ± standard errors of four experiments. Pretreated with drug indicates that cells were exposed to drug 5 min prior to addition of zymosan. Post-treated with drug indicates that cells were stimulated with zymosan for 5 min prior to addition of drug.

DISCUSSION

Data presented in this investigation indicate that a number of bisbenzylisoquinoline alkaloids bound to alveolar macrophages and inhibited the ability of these phagocytes to respond to zymosan particles. At a dose of 50 μM drug, the inhibitory potency of these alkaloids varied from a 65% depression of the respiratory burst in the presence of tetrandrine and fangchinoline to approximately 40% inhibition with cepharanthine, berbamine, and cycleanine to only 10% block with tubocurarine. The sequence of maximal binding capacity toward alveolar macrophages at 37°C was FA > TT ≈

Table 3 Effect of Alkaloids on ATP Content in Alveolar Macrophages

Drugs (25 μM)	% of control
Tetrandrine	76 ± 6
Fangchinoline	105 ± 5
Berbamine	95 ± 8
Cycleanine	66 ± 4
Tubocurarine	106 ± 6

Note. Values are means ± standard errors for four experiments. The ATP content of the drug-treated cells is expressed as a percentage of the control. The control value of ATP content in alveolar macrophages was 1.17 ± 0.15 nmol/10^6 cells.

BE ≈ CY > CE ≫ TU. Mo et al. [11] reported that antifibrotic activity followed a sequence of TT ≈ CY > FA ≈ CE > BE ≫ TU. At the extremes, i.e., comparing tetrandrine and tubocurarine, there was a relationship among binding, phagocyte inhibition, and antifibrotic activity as had been suggested previously [12]. However, when all of the alkaloids were considered, no strong relationship between total binding and pharmacological effect was apparent. The correlation coefficient between binding and inhibition of oxygen consumption was 0.70, that between inhibition of oxygen consumption and antifibrotic potency was 0.59, while that between binding and antifibrotic potency was 0.62.

Ma et al. [17] reported that the lipophilicity of these BBIQ alkaloids exhibited the following sequence: TT > CY > CE > BE ≈ FA > TU. Hydroxyl substitution found in berbamine, fangchinoline, and tubocurarine resulted in significant declines in lipophilicity. The positively charged ammonium nitrogens further increased the hydrophilic nature of tubocurarine. Our data indicate that the correlation between lipophilicity and binding or inhibitory potency was poor. The correlation coefficient between lipophilicity and inhibition of the respiratory burst was 0.26, that for lipophilicity and antifibrotic potency was 0.58, while that between binding and lipophilicity was 0.26. These results argue against nonspecific interaction of BBIQ alkaloids with membrane lipids and favor interaction with specific sites.

Binding of BBIQ alkaloids to alveolar macrophages was extensive. For example, when 25 μM tetrandrine was added to 6×10^6 macrophages (2×10^6 cells/mL), the supernatant drug concentration was decreased to 2–3 μM in 1 h at 37 °C. Therefore, approximately 88–92% of the total drug added was associated with macrophages. The cytoplasmic volume of water in alveolar macrophages is 1.94 μL/(2×10^6) cells [18]. If cell-associated drug was the result of membrane transport or endocytosis, the intracellular concentration would approach 11,900 μM, i.e., a 476-fold accumulation from the supernate. This does not seem possible. Therefore, drug–cell interaction reported in the present study must represent true binding.

Drug binding to macrophages was rapid with a half-time of < 5 min. Binding was dose dependent. For tetrandrine, half-maximal binding was reached between 11 and 12 μM drug. Binding of BBIQ alkaloids also increased as temperature was raised. Activation energies for drug–cell interaction ranged from 10 to 13 kcal/mol. The rapid half-times and relatively low activation energies for these interactions argue against transport or endocytotic processes accounting for drug accumulation. Rather, the data suggest true drug binding.

As stated above, nonspecific interaction between drugs and membrane lipids of the macrophages does not seem to explain alkaloid binding. Further evidence against simple interaction with membrane lipids is that drug–cell interaction depended strongly on cell viability; i.e., drugs did not bind to metabolically dead or sonicated cells. However, BBIQ alkaloids interact with

cytoskeletal structures, since binding was inhibited by microtubule perturbants vinblastine or taxol, and by the microfilament modifier cytochalasin B. These results support those reported by Liu et al. [19] and Chen et al. [20], which demonstrated direct interaction between tetrandrine and isolated preparations of microtubules.

Activation of phagocytes has been associated with actin polymerization and structural alterations of the cytoskeleton [16]. It is of interest that activated macrophages were more susceptible to the inhibitory action of alkaloids. Pre-exposure to zymosan or PMA enhanced the ability of drugs to block the oxygen consumption or superoxide anion release. These data suggest that stimulants would change the conformation of cytoskeletal components and may make these structures more susceptible to drug interaction. Note that data from our laboratory indicate that taxol, vinblastine, and cytochalasin B inhibit oxygen consumption and superoxide release from alveolar macrophages, implicating cytoskeletal changes in the stimulus–secretion coupling scheme [21].

The data above indicate that the cytoskeletal network plays an important role in alkaloid–cell interaction. However, the mechanism(s) by which BBIQ alkaloids act are still not completely understood. Seow et al. [22] have shown that tetrandrine did not block concanavalin A receptors in lymphocytes. Castranova et al. [8] also suggested that tetrandrine did not block the interaction between zymosan and membrane receptors in alveolar macrophages. Furthermore, they reported that tetrandrine had no effect on stimulant-induced membrane depolarization. In contrast, inhibition of phosphoinositide metabolism due to tetrandrine has been reported [22]. Since pathways for stimulus–secretion coupling in phagocytes are complex, further investigation is required to resolve the site(s) of action of tetrandrine and its analogues.

The fact that structurally similar BBIQ alkaloids exhibited widely different binding and inhibitory potency toward alveolar macrophages, as well as different antifibrotic potential, suggests that these drugs may represent useful probes to elucidate the role of pulmonary phagocytes and their secretory products in the fibrogenic process. Indeed, our laboratory is currently investigating the action of tetrandrine on fibroblast proliferation and the ability of alveolar macrophages to secrete fibrogenic cytokines in response to silica.

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REFERENCES

1. Kupchan SM, Atland HW: Requirements for tumor-inhibitory activity among benzylisoquinoline alkaloids and related synthetic compounds. J Med Chem 16:913–917, 1973.

2. Zeng FD, Shaw DH, Jr, Ogilvie RI: Kinetic disposition and hemodynamic effect of tetrandrine in anesthetized dogs. J Cardiovasc Pharmacol 7:1034–1039, 1985.
3. Yu XF, Zou CQ, Lim MB: Observation of the effect of tetrandrine on experimental silicosis of rats. Ecotoxicol Environ Safety 7:306–312, 1983.
4. Li Q, Xu Y, Zhow Z, et al: The therapeutic effect of tetrandrine on silicosis. Chin J Tuber Respir Dis 4:321–328, 1981.
5. Huang T, Liu Y, Zhao S, Li Y: Changes of acid-soluble collagen from lungs of silicotic rats and tetrandrine-treated silicotic rats. Acta Biochem Biophys Sintca 13:61–68, 1981.
6. Liu B, Zou C, Li Y: Studies on the contents of glycosaminoglycans from lungs of silicotic rats and tetrandrine-treated silicotic rats. Ecotoxicol Environ Safety 7:323–329, 1983.
7. Kang JH, Lewis DM, Castranova V, Rojanasakul Y, Banks DE, Ma JKH: Inhibitory action of tetrandrine on macrophage production of interleukin-1 (IL-1)-like activity and thymocyte proliferation. Exp Lung Res 18:719–733, 1992.
8. Castranova V, Kang JH, Moore MD, Pailes WH, Frazer DG, Schwegler-Berry D: Inhibition of stimulant-induced activation of phagocytic cells with tetrandrine. J Leukocyte Biol 50:412–422, 1991.
9. Seow WK, Ferrante A, Li S, Thong YH: Antiphagocytic and antioxidant properties of plant alkaloid tetrandrine. Int Arch Allergy Appl Immunol 85:404–409, 1988.
10. Matsuno T, Onta K, Sato E, Nobori K, Inoue B, Utsumi K: Inhibition of metabolic response of polymorphonuclear leukocyte by biscoclaurine alkaloids. Biochem Pharmacol 36:1613–1618, 1987.
11. Mo CG, Yu XF, Zou CQ: Studies on the relationship between structure and bioactivity of bisbenzylisoquinoline alkaloids. Wei Sheng Yan Jiu 4:118–125, 1982.
12. Castranova V, Kang JH, Ma JKH, et al: Effects of bisbenzylisoquinoline alkaloids on alveolar macrophages: correlation between binding affinity, inhibitory potency, and antifibrotic potential. Toxicol Appl Pharmacol 108:242–252, 1991.
13. Lane FC, Mehta JR: In vitro human tumor sensitivity assay using cell counting and sizing. Am Biotech Lab 8:12–27, 1990.
14. Phillips HJ: Dye exclusion tests for cell viability. In Kruse PR, Patterson MK (eds), Tissue Culture Methods and Applications. New York, Academic Press, pp 406–408, 1973.
15. Wolff K, Doppen W: Luminometric method. In Bergmeyer HU (ed), Methods of Enzymatic Analysis, 3rd ed. Deerfield Beach, FL, Verlag-Chemie, Vol 7, pp 357–364, 1985.
16. Rao KMK, Castranova V: Phenylmethylsulfonyl fluoride inhibits chemotactic peptide-induced actin polymerization and oxidative burst activity in human neutrophils by an effect unrelated to its antiproteinase activity. Biochim Biophys Acta 969:131–138, 1988.
17. Ma JKH, Mo CG, Malanga CJ, Ma JYC, Castranova V: Binding of bisbenzylisoquinoline alkaloids to phosphatidylcholine vesicles and alveolar macrophages: relationship between binding affinity and antifibrogenic potential of these drugs. Exp Lung Res 17:1061–1077, 1991.

18. Castranova V, Bowman L, Miles PR: Transmembrane potential and ionic content of rat alveolar macrophages. J Cell Physiol 101:471–480, 1979.
19. Liu L, Chen N, Cai G, Li Z, Yang J, Li Y: Studies on the effect of tetrandrine on microtubules, I: biochemical observation and electron microscopy. Ecotoxicol Environ Safety 15:142–148, 1988.
20. Chen N, Liu L, Cai G, Yang J, Li Y: Studies on the effects of tetrandrine on microtubules, II: observation with immunofluorescence techniques. Ecotoxicol Environ Safety 15:149–152, 1988.
21. Ma JYC, Barger MW, Ma JKH, Castranova V: Effect of tetrandrine on the cytoskeletal control of alveolar macrophage respiratory burst. FASEB J 6:A1911, 1992.
22. Seow WK, Ferrante A, Goh H, Chalmers AH, Li S, Thong YH: In vitro immunosuppressive properties of the plant alkaloid tetrandrine. Int Arch Allergy Appl Immunol 85:410–415, 1988.