rexresearch.com

Paul SAVAGE
CSA-54 vs AIDS

Ceragenins (Cationic Selective Antimicrobials, CSA) are novel bile cholic acid derivatives that mimic immune system T-cells & are very effective vs microbes & viruses including AIDS.

http://www.sltrib.com
The Salt Lake Tribune
February 7, 2006

Has BYU prof found AIDS cure?

by Bob Mims

Researchers, including a BYU scientist, believe they have found a new compound that could finally kill the HIV/AIDS virus, not just slow it down as current treatments do.

And, unlike the expensive, drug cocktails 25 years of research have produced for those with the deadly virus, the compound invented by Paul D. Savage of Brigham Young University appears to hunt down and kill HIV.

Although so far limited to early test tube studies, CSA-54, one of a family of compounds called Ceragenins (or CSAs), mimics the disease-fighting characteristics of anti-microbial and anti-viral agents produced naturally by a healthy human immune system.

Under a study sponsored by Ceragenix Pharmaceuticals, Savage and his colleagues developed and synthesized the compound for Vanderbilt University's School of Medicine. In his Nashville, Tenn., laboratories, Derya Unutmaz, an associate professor of Microbiology and Immunology, tested several CSAs for their ability to kill HIV.

While issuing a cautious caveat about his early results, Unutmaz acknowledged Monday that CSAs could be the breakthrough HIV/AIDS researchers have sought for so long.

"We received these agents [from BYU] in early October and our initial results began to culminate by November 2005. We have since reproduced all our results many times," he said. "We have some preliminary but very exciting results [but] we would like to formally show this before making any claims that would cause unwanted hype."

What studies to date show is a compound that attacks HIV at its molecular membrane level, disrupting the virus from interacting with their primary targets, the "T-helper" class white blood cells that comprise and direct the human immune system. Further, CSAs appear to be deadly to all known strains of HIV.

That would be a welcome development for the estimated 40.3 million people now living with HIV/AIDS globally, including nearly 5 million newly infected in the past year alone.

"We have devoted considerable resources to understand the mechanism of these compounds. We think this knowledge will enable us in collaboration with Dr. Savage to design even better compounds," Unutmaz said.

In addition to being a potential checkmate to HIV, the compounds show indications of being just as effective against other diseases plaguing humankind - among them influenza, possibly even the dread bird flu, along with smallpox and herpes.

Savage said he and his BYU research team had been studying CSAs for eight years, noting the compounds' value against microbial and bacteria infections. It was only a year ago they saw that CSAs killed viruses, too.

"They kill viruses very effectively and in a way paralleling our own, natural defenses," Savage said, noting that beyond the obvious use as a weapon against the AIDS pandemic, CSAs could help many others with non-HIV immune deficiencies.

Further, the compounds appear to have few limits on how they are delivered to patients. Although early indications are for application of CSAs with an ointment or cream, pills or injections may also be developed - if the compound gets to market.

BYU and Vanderbilt have jointly filed a patent on CSA technology, which has been licensed exclusively to Ceragenix.

Ceragenix CEO and Chairman Steven Porter said only further research will tell, but he was optimistic about the application of CSAs in the war on HIV/AIDS. There are indications that it could help battle antibiotic- and antiviral-resistance strains of disease as they manifest themselves.

"We are encouraged . . . that CSAs may provide a completely unique family of anti-infectives, potentially active against a wide range of viral, fungal and bacterial targets, including those resistant to current therapies," he said.

Assuming continued positive test results in animal and eventual human trials, Porter estimates it could be three to seven years before the compound is available by prescription. That transition could be accelerated, however, if the Food and Drug Administration should decide to fast-track the drug.

That day is still a long way off, though. First, researchers plan to publish their results in scientific journals, seeking peer review and independent confirmation of their findings. Assuming no flaws are found, several rounds of testing would follow.

Most of the nation's leading AIDS experts were attending the Conference on Retroviruses and Opportunistic Infections in Denver on Monday. The event's policies prohibits on-site news conferences or releases during the conference, and efforts to reach scientists there were not successful.

Of the few AIDS research luminaries reached, all said they preferred not to comment on the Vanderbilt tests until full results are published.

l Paul Savage and his Brigham Young University research team have invented CSA-54, a chemical compound that holds the promise of killing the HIV virus.

l CSA-54 is one of a family of compounds called Ceragenins that mimic the disease-fighting characteristics of a healthy human immune system.

l Tests at Vanderbilt University indicate the BYU compound also could be effective against

influenza, small pox and herpes.

l Assuming continued positive results, CSA-54 could be available in three to seven years.

---

http://en.wikipedia.org/wiki/Ceragenin

Ceragenin

Ceragenins, or cationic selective antimicrobials (CSAs), are synthetically produced small molecule chemical compounds consisting of a sterol backbone with amino acids and other chemical groups attached to them. These compounds have a net positive charge that is electrostatically attracted to the negatively charged cell membranes of certain viruses, fungi and bacteria. CSAs have a high binding affinity for such membranes (including Lipid A[1]) and are able to rapidly disrupt the target membranes leading to rapid cell death. While CSAs have a mechanism of action that is also seen in antimicrobial peptides, which form part of the body's innate immune system, they avoid many of the difficulties associated with their use as medicines.[2]

Ceragenins were invented by Dr. Paul B. Savage of Brigham Young University's Department of Chemistry and Biochemistry and exclusively licensed to Ceragenix.[2] In data previously presented by Dr. Savage and other researchers, CSAs have been shown to have broad spectrum antibacterial activity.[3] Dr. Derya Unutmaz, Associate Professor of Microbiology and Immunology at the Vanderbilt University School of Medicine, tested several CSAs in his laboratory for their ability to kill HIV directly. According to Unutmaz, "We have some preliminary but very exciting results. But we would like to formally show this before making any claims that would cause unwanted hype."[4]

On February 6, 2006, researchers (including Dr. Paul B. Savage) announced that a Ceragenin compound, CSA-54, appears to inactivate HIV. This conclusion seems to still be awaiting peer review.[5]

References

Ding B., Yin N., Liu Y., Cardenas-Garcia J., Evanson R., Orsak T., Fan M., Turin G., and Savage P.B.: Origins of Cell Selectivity of Cationic Steroid Antibiotics J. Am. Chem. Soc., 126(42), 13642 -13648, 2004.

"Vanderbilt University, Brigham Young University, and Ceragenix Pharmaceuticals Report Novel Drug Compound Kills Multiple HIV Strains; Synthetic Small Molecule Acts Through Unique Strain-Independent Virucidal Mechanism". Ceragenix Pharmaceuticals. 2006-02-06.

Savage PB, Li C, Taotafa U, Ding B, Guan Q (2002-11-19). "Antibacterial properties of cationic steroid antibiotics". FEMS microbiology letters.

"Chemical 'blocks HIV infection'". BBC News Online. 2006-02-09. x

Mims, Bob (2006-03-01). "Has BYU prof found AIDS cure?". The Salt Lake Tribune.

---

http://aac.asm.org/content/54/9/3708.fu

Depolarization, Bacterial Membrane Composition, and the Antimicrobial Action of Ceragenins ?

Raquel F. Epand1 // Jake E. Pollard2 // Jonathan O. Wright2, // Paul B. Savage2 and Richard M. Epand1,*

+ Author Affiliations
1 Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada
2 Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602

ABSTRACT

Ceragenins are cholic acid-derived antimicrobial agents that mimic the activity of endogenous antimicrobial peptides. Ceragenins target bacterial membranes, yet the consequences of these interactions have not been fully elucidated. The role of the outer membrane in allowing access of the ceragenins to the cytoplasmic membrane of Gram-negative bacteria was studied using the ML-35p mutant strain of Escherichia coli that has been engineered to allow independent monitoring of small-molecule flux across the inner and outer membranes. The ceragenins CSA-8, CSA-13, and CSA-54 permeabilize the outer membrane of this bacterium, suggesting that the outer membrane does not play a major role in preventing the access of these agents to the cytoplasmic membrane. However, only the most potent of these ceragenins, CSA-13, was able to permeabilize the inner membrane. Interestingly, neither CSA-8 nor CSA-54 caused inner membrane permeabilization over a 30-min period, even at concentrations well above those required for bacterial toxicity. To further assess the role of membrane interactions, we measured membrane depolarization in Gram-positive bacteria with different membrane lipid compositions, as well as in Gram-negative bacteria. We found greatly increased membrane depolarization at the minimal bactericidal concentration of the ceragenins for bacterial species containing a high concentration of phosphatidylethanolamine or uncharged lipids in their cytoplasmic membranes. Although membrane lipid composition affected bactericidal efficiency, membrane depolarization was sufficient to cause lethality, providing that agents could access the cytoplasmic membrane. Consequently, we propose that in targeting bacterial cytoplasmic membranes, focus be placed on membrane depolarization as an indicator of potency.

Ceragenins are a family of bile acid derivatives that have been modified to yield an amphiphilic morphology similar to that of endogenous antimicrobial peptides (Fig. 1) (9, 13). The majority of antimicrobial peptides adopt amphiphilic secondary structures in which cationic amino acid side chains (i.e., arginine, lysine, and histidine) are oriented on one face of the molecule while hydrophilic side chains are on the opposing face. This morphology has been termed “facially amphiphilic.” The ceragenins effectively reproduce this morphology owing to their bile acid scaffolding...

RESULTS

Permeabilization of the inner membranes and OMs of E. coli ML-35p.All three ceragenins, CSA-8, CSA-13, and CSA-54, rapidly permeabilized the OM of E. coli ML-35p in a concentration-dependent manner, as shown by the time-dependent increase in nitrocefin permeation (Fig. 2). The onset of permeabilization was more rapid with the ceragenins than with even the potent lytic peptide melittin, used as a positive control. However, the extent of nitrocefin permeation reached by higher concentrations of melittin was greater than that observed with the ceragenins. There were differences in the degrees of OM permeabilization among the ceragenins that correlate with their antimicrobial potencies. The most potent, CSA-13, at the lowest concentration exhibited an increase in permeabilization by nitrocefin while higher concentrations of CSA-8 and CSA-54 were required to achieve permeabilization. Interestingly, only ceragenin CSA-13 permeated the inner membrane of E. coli ML-35p to allow passage of ONPG (Fig. 2). For CSA-8 and CSA-54 no inner membrane permeabilization was observed even at high concentrations well above the MBC...

DISCUSSION

Structures of the ceragenins used in this study are presented in Fig. 1, and among these compounds CSA-8 is the least cationic, with three positive charges, and CSA-54 is the most positively charged with six. The primary distinguishing feature of CSA-13 is the lipid chain at C-24. Tables 1 and 2 give data on the composition of the major lipids in the bacteria tested as well as the MBCs of the ceragenins for this collection of Gram-positive and Gram-negative bacteria, respectively. As reported previously, CSA-13 is a broad-spectrum bactericidal agent, while CSA-8 and CSA-54 are somewhat less active against Gram-positive organisms and much less active against Gram-negative bacteria. We have proposed that the lipid chain in CSA-13 is necessary for effective traversal of the OM of Gram-negative bacteria and that this process is essential for bactericidal activity at low concentrations.

A key step in the activity of the ceragenins is selective association with bacterial membranes. All three ceragenins tested possess the structural elements required for association with the membrane lipid components, including lipid A in the OM of Gram-negative bacteria. We have shown previously that association of ceragenins with the OM disrupts the permeability barrier generated by the lipid bilayer. The ML-35p strain of E. coli has been engineered to monitor permeation of both cytoplasmic and outer membranes (15). Results from experiments with this strain and the ceragenins demonstrate that all three compounds effectively permeabilize the OM to nitrocefin at concentrations comparable to those of melittin (Fig. 2). However, only the most potent ceragenin, CSA-13, displayed the capacity to permeabilize the cytoplasmic membrane to ONPG, a property CSA-13 has in common with melittin. Even at concentrations well above the MBC, neither CSA-8 nor CSA-54 caused sufficient permeabilization of the cytoplasmic membrane to allow entry of the probe (Fig. 2). These results suggest that the bactericidal activity of CSA-13 toward E. coli may be a consequence of its ability to promote unregulated passage of small polar molecules through the cytoplasmic membrane. Nevertheless, the fact that CSA-8 and CSA-54 display bactericidal activity argues that they function by mechanisms that do not involve such cytoplasmic membrane disruptions that would allow passage of small molecules like ONPG.

There is a large electrical potential across the cytoplasmic membrane of bacteria. This electrical barrier can be dissipated by a perturbation of the membrane that would allow the flow of charge without necessarily allowing the passage of small organic molecules such as ONPG. Dissipation of this potential may be the mechanism by which CSA-8 and CSA-54 cause cell death without permeabilizing the cytoplasmic membrane to small molecules.

Measuring the depolarization of the cytoplasmic membrane of Gram-positive bacteria is straightforward using a cyanine dye because the probe has direct access to the cytoplasmic membrane. Using a fluorescent dye, we found that the ceragenins, in a time- and concentration-dependent manner, depolarized the lipid bilayer of the Gram-positive bacteria tested (as shown in Fig. 3, for example). A comparison of the percentage of membrane depolarization after 5.5 min with MBCs is given in Fig. 4. With all of the strains, a significant amount of depolarization was observed at the MBC for all the ceragenins tested.

Of particular interest is the observation that the extent of depolarization by these agents depended on the composition of the cytoplasmic membrane of the bacteria (Table 1). The genus Staphylococcus lacks phosphatidylethanolamine (PE) in the cytoplasmic membrane, similar to most other Gram-positive bacteria (with some exceptions such as in the genus Bacillus or Clostridium), presenting instead mostly phospholipids with anionic head groups (phosphatidylglycerol [PG] or cardiolipin [CL]). These Gram-positive bacteria showed the least depolarization at the MBC or at 0.5× MBC (Fig. 4). In contrast, B. cereus and B. polymyxa have a high PE content in the cytoplasmic membrane (Table 1), and with these organisms the ceragenins are comparably more efficient in their depolarization abilities (Fig. 4). This phenomenon has been observed with antimicrobial peptides (11), and the possibility exists that ceragenins and antimicrobial peptides have secondary, intracellular targets that influence antimicrobial activity. Nevertheless, the fact that significant levels of membrane depolarization occur at the MBC suggests that membrane depolarization may be sufficient to cause cell death and may be the primary mode of action of the ceragenins against the Gram-positive bacteria.

The lipid composition in bacteria varies greatly, and, in addition, bacteria are able to modify their lipid composition in response to environmental conditions and to satisfy requirements for outer wall biosynthesis. For these reasons, comparative antimicrobial activity assays for viability with antimicrobial agents are always carried out under the same conditions of growth in our studies. In the Gram-positive bacterium E. faecalis, the cytoplasmic membrane, besides PG and CL, consists of 20% lysyl-PG, a cationic lipid that would neutralize some of the membrane's negative charge (Table 1), and 26% phosphatidylkojibiosyl diglycerol, an uncharged lipid covalently linked to the polyglycerol moieties of lipoteichoic acid through a phosphodiester linkage (12). This lipid acts as an anchor between the membrane and the cell wall, with its fatty acids embedded in the membrane and the lipoteichoic acid extending from the surface of the membrane. With this lipid profile, E. faecalis proved to be similar to the Bacillus strains tested, which contain substantial amounts of PE, in its susceptibility to membrane depolarization by the ceragenins. This contrasts with S. aureus, which has a much larger residual amount of negative charge in its membrane. The finding that E. faecalis behaves similarly to other Gram-positive bacteria with high PE content suggests that the property of enhancing membrane depolarization is not a consequence of the specific structure of PE but, rather, of having zwitterionic or uncharged lipids in the cytoplasmic membrane, with biophysical properties different from those of the major anionic lipids, PG and CL. A similar situation was found with Streptococcus pyogenes in its susceptibility to the action of AMPs (8).

Membrane depolarization experiments with Gram-negative bacteria are complicated by the permeability barrier of the OM, which impedes access of the fluorescent probe to the cytoplasmic membrane. Access to the cytoplasmic membrane can be facilitated by EDTA, which disrupts the ion-mediated cross-linking of lipid A in the OM, making the bilayer more permeable. As described above, binding to lipid A by ceragenins also results in permeabilization of the OM. This activity of EDTA and the ceragenins can be observed in the loss of fluorescence of the probe after addition of these agents, which is likely caused by incorporation of the dye in the polarized cytoplasmic membrane (Fig. 5). Notably, the concentrations of ceragenins necessary to achieve permeabilization are about 1,000-fold lower than those required for EDTA. Because CSA-8 and CSA-54 display similar abilities to permeabilize the OM, only CSA-8 and CSA-13 were used in membrane depolarization studies. These studies were performed with three different strains of Gram-negative bacteria (Table 2), and results shown in Fig. 5 with K. pneumoniae and E. coli are representative.

With relatively low concentrations of CSA-8 (0.5 μg/ml), a drop in the fluorescence of the dye was observed, consistent with permeabilization of the OM and incorporation of the dye in the cytoplasmic membrane (Fig. 5B). At the MBC and above, the fluorescence of the dye increased, and this result suggests that incorporation of the dye was competitive with membrane depolarization. The MBC of CSA-8 is relatively high, and the OM-permeabilizing and bactericidal activities were separated by a substantial difference in concentrations (ca. 12 μg/ml). In contrast, with CSA-13 the difference in concentrations between these two activities is approximately 2 μg/ml (Fig. 5C). Nevertheless, with K. pneumoniae, permeabilization of the OM could be achieved without apparent cytoplasmic membrane depolarization at 0.5 μg/ml (Fig. 5C). Similarly, with E. coli, at concentrations of CSA-13 at or above the MBC, an initial decrease in fluorescence was followed by a large increase (Fig. 5D). At concentrations below the MBC, only a drop in fluorescence was observed.

Elucidation of the respective roles of membrane permeabilization and depolarization is of importance for understanding the mechanisms of action of antimicrobial agents and for providing evidence of the role of membrane integrity in bacterial viability. In addition, for Gram-negative bacteria it is important to understand the role of the OM in preventing access of the antimicrobial agents to the cytoplasmic membrane. As mimics of antimicrobial peptides, ceragenins display both OM permeabilization and cytoplasmic membrane depolarization activities. These activities can be separated to some extent with CSA-8 in Gram-negative bacteria. Only the most potent compound, CSA-13, can efficiently depolarize the cytoplasmic membrane of Gram-negative bacteria and induce solute leakage, causing a bactericidal effect. Nevertheless, from studies with both Gram-

negative and Gram-positive bacteria, it appears that membrane depolarization correlates to a large extent with the bactericidal activity of the ceragenins. Consequently, in continuing work to develop more potent and selective antimicrobials targeting bacterial membranes, focus should be placed on membrane depolarization as an indication of potency...

REFERENCES

Breukink, E., I. Wiedemann, C. van Kraaij, O. P. Kuipers, H. G. Sahl, and B. de Kruijff. 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. Science 286:2361-2364.

Bucki, R., A. G. Sostarecz, F. J. Byfield, P. B. Savage, and P. A. Janmey. 2007. Resistance of the antibacterial agent ceragenin CSA-13 to inactivation by DNA or F-actin and its activity in cystic fibrosis sputum. J. Antimicrob. Chemother. 60:535-545.

Chin, J. N., R. N. Jones, H. S. Sader, P. B. Savage, and M. J. Rybak. 2008. Potential synergy activity of the novel ceragenin, CSA-13, against clinical isolates of Pseudomonas aeruginosa, including multidrug-resistant P. aeruginosa. J. Antimicrob. Chemother. 61:365-370.

Chongsiriwatana, N. P., and A. E. Barron. 2010. Comparing bacterial membrane interactions of antimicrobial peptides and their mimics. Methods Mol. Biol. 618:171-182.

Ding, B., Q. Guan, J. P. Walsh, J. S. Boswell, T. W. Winter, E. S. Winter, S. S. Boyd, C. Li, and P. B. Savage. 2002. Correlation of the antibacterial activities of cationic peptide antibiotics and cationic steroid antibiotics. J. Med. Chem. 45:663-669.

El-Kosasy, A. M. 2006. Potentiometric assessment of Gram-negative bacterial permeabilization of tobramycin. J. Pharm. Biomed. Anal. 42:389-394.

Epand, R. F., C. M. Yip, L. V. Chernomordik, D. L. LeDuc, Y. K. Shin, and R. M. Epand. 2001. Self-assembly of influenza hemagglutinin: studies of ectodomain aggregation by in situ atomic force microscopy. Biochim. Biophys. Acta 1513:167-175.

Epand, R. M., R. F. Epand, C. J. Arnusch, B. Papahadjopoulos-Sternberg, G. Wang, and Y. Shai.2010. Lipid clustering by three homologous arginine-rich antimicrobial peptides is insensitive to amino acid arrangement and induced secondary structure. Biochim. Biophys. Acta 1798:1272-1280.

Epand, R. M., R. F. Epand, and P. B. Savage. 2008. Ceragenins (cationic steroid compounds), a novel class of antimicrobial agents. Drug News Perspect. 21:307-311.

Ericksen, B., Z. Wu, W. Lu, and R. I. Lehrer. 2005. Antibacterial activity and specificity of the six human a-defensins. Antimicrob. Agents Chemother. 49:269-275.

Friedrich, C. L., D. Moyles, T. J. Beveridge, and R. E. Hancock. 2000. Antibacterial action of structurally diverse cationic peptides on gram-positive bacteria. Antimicrob. Agents Chemother. 44:2086-2092.

Ganfield, M. C., and R. A. Pieringer. 1975. Phosphatidylkojibiosyl diglyceride. The covalently linked lipid constituent of the membrane lipoteichoic acid from Streptococcus faecalis (faecium) ATCC 9790. J. Biol. Chem. 250:702-709.

Lai, X. Z., Y. Feng, J. Pollard, J. N. Chin, M. J. Rybak, R. Bucki, R. F. Epand, R. M. Epand, and P. B. Savage. 2008. Ceragenins: cholic acid-based mimics of antimicrobial peptides. Acc. Chem. Res. 41:1233-1240.

Lehrer, R. I., A. Barton, K. A. Daher, S. S. Harwig, T. Ganz, and M. E. Selsted. 1989. Interaction

of human defensins with Escherichia coli. Mechanism of bactericidal activity. J. Clin. Invest. 84:553-561.

Lehrer, R. I., A. Barton, and T. Ganz. 1988. Concurrent assessment of inner and outer membrane permeabilization and bacteriolysis in E. coli by multiple-wavelength spectrophotometry. J. Immunol. Methods 108:153-158.

Li, C. H., L. P. Budge, C. D. Driscoll, B. M. Willardson, G. W. Allman, and P. B. Savage. 1999. Incremental conversion of outer-membrane permeabilizers into potent antibiotics for Gram-negative bacteria. J. Am. Chem. Soc. 121:931-940.

Ouhara, K., H. Komatsuzawa, S. Yamada, H. Shiba, T. Fujiwara, M. Ohara, K. Sayama, K. Hashimoto, H. Kurihara, and M. Sugai. 2005. Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, ß-defensins and LL37, produced by human epithelial cells. J. Antimicrob. Chemother. 55:888-896.

Papo, N., and Y. Shai. 2005. A molecular mechanism for lipopolysaccharide protection of gram-negative bacteria from antimicrobial peptides. J. Biol. Chem. 280:10378-10387.

Pollard, J., J. Wright, Y. Feng, D. Geng, C. Genberg, and P. B. Savage. 2009. Activities of ceragenin CSA-13 against established biofilms in an in vitro model of catheter decolonization. Anti. Infect. Agents Med. Chem. 8:290-294.

Tsubery, H., I. Ofek, S. Cohen, and M. Fridkin. 2000. Structure-function studies of polymyxin B nonapeptide: implications to sensitization of gram-negative bacteria. J. Med. Chem. 43:3085-3092.

Effect of Intracellular Expression of Antimicrobial Peptide LL-37 on Growth of Escherichia coli Strain TOP10 under Aerobic and Anaerobic Conditions Antimicrob. Agents Chemother. October 2013 57:10 4707-4716

Sensitization of gram-negative bacteria by targeting the membrane potential FASEB J. September 2013 27:9 3818

Antibacterial activity of the human host defence peptide LL-37 and selected synthetic cationic lipids against bacteria associated with oral and upper respiratory tract infections J Antimicrob Chemother March 2013 68:3 610-618

The Target of Daptomycin Is Absent from Escherichia coli and Other Gram-Negative Pathogens Antimicrob. Agents Chemother. January 2013 57:1 637-639

Antibacterial Mechanism of Action of Arylamide Foldamers Antimicrob. Agents Chemother. November 2011 55:11 5043-5053

---

http://www.ncbi.nlm.nih.gov/pubmed/19958044
J Parasitol. 2010 Jun;96(3):638-42.
doi: 10.1645/GE-2329.1.

Anti-trypanosomatid activity of ceragenins.

Lara D, Feng Y, Bader J, Savage PB, Maldonado RA.

Abstract

Cationic steroid antibiotics (CSAs), or ceragenins, are amphiphilic compounds consisting of a

cholic acid backbone that is attached to several cationic amines. In this study, we tested the hypothesis that CSAs possess antiparasitic activities with minimal to no effects on mammalian cells, and thus could be used as potential therapeutic agents against pathogenic trypanosomatids. To investigate this notion, we synthesized CSAs and determined their trypanocidal and leishmanicidal activities in vitro. The 3 ceragenins assayed, i.e., CSA-8, CSA-13, and CSA-54, showed several degrees of parasiticidal activity. CSA-13 was the most effective compound against Leishmania major promastigotes and Trypanosoma cruzi trypomastigotes, at LD(50) 4.9 and 9 microM, respectively. The trypanocidal activities of these ceragenins were also assessed by infectivity experiments. We found CSA-8 was more effective on T. cruzi intracellular amastigotes when the infected host cells were treated for 24 hr (LD(50), 6.7 microM). Macrophages and LLC-MK(2) (treated for 72 hr) showed relative low susceptibility to these compounds. Our results suggest that ceragenins are indeed promising chemotherapeutic agents against trypanosomatids, but they require further investigation.

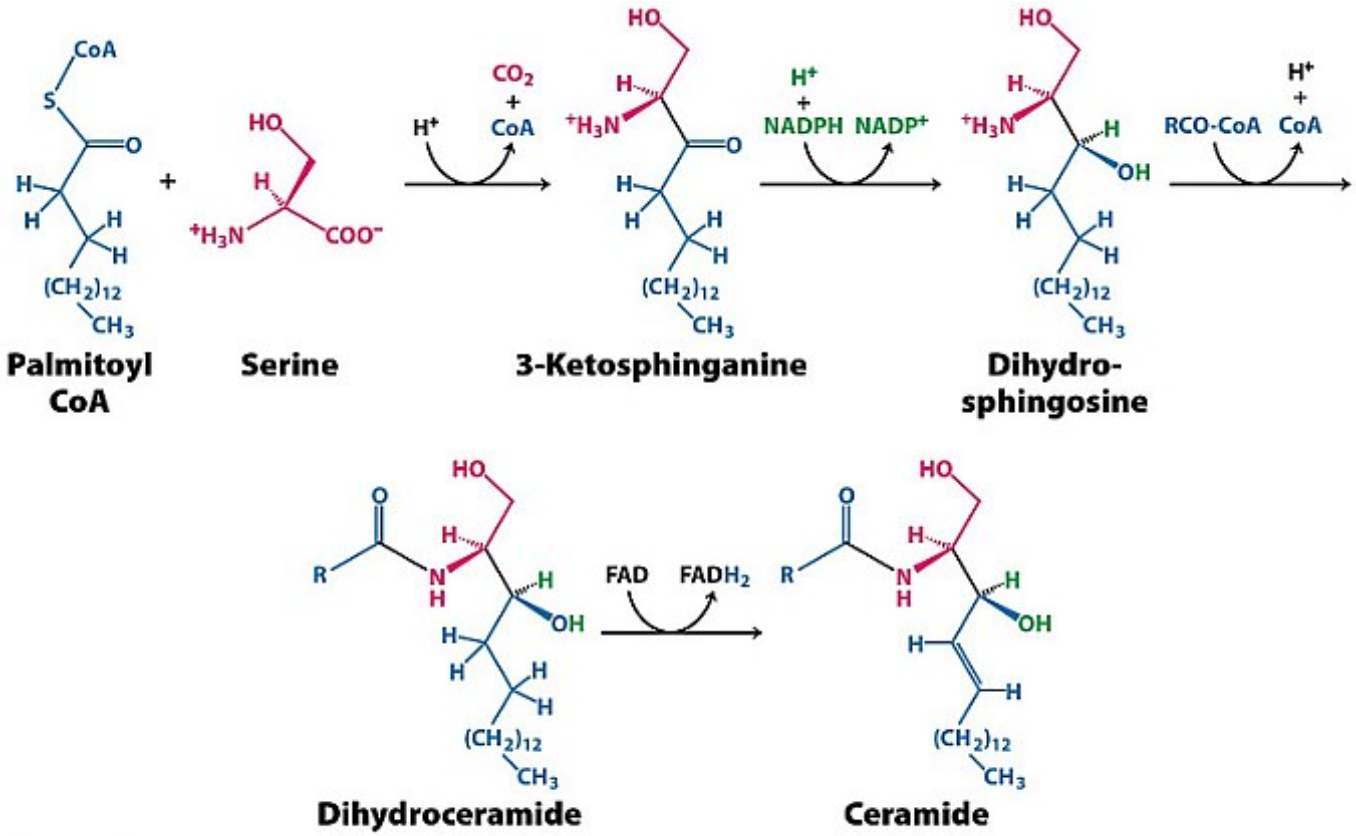

Figure 26.3
Biochemistry, Seventh Edition

Patents

BACTERIAL GLYCOLIPID ACTIVATION OF CD1D-RESTRICTED NKT CELLS US2008279894

[PDF]

[Excerpts]

Disclosed are methods for activating an NKT cell, methods of stimulating an immune response in a subject, methods of improving vaccine efficacy, and methods of treating an infection. Also

disclosed are methods of promoting tumor rejection, treating cancer, modulating autoimmunity and inhibiting allergen-induced hypersensitivity in subjects. The methods include contacting an NKT cell with a bacterial glycolipid complexed with a CD1 molecule to activate the NKT cell. The bacterial glycolipid may be derived from a member of the Class Alphaproteobacteria.

INTRODUCTION

[0003] The CD1d molecule is a member of the CD1 family of [beta]2 microglobulin-associated molecules. In contrast to class I and II major histocompatibility complex (MHC) molecules that present protein antigens to CD8+ and CD4+ T cells, respectively, CD1 molecules have evolved to capture and process both foreign and self lipid antigens for display to T cells. CD1a, -b, and -c molecules have been shown to present foreign microbial antigens to human TCR[alpha][beta] T cells. In contrast, CD1d-restricted T cells, or NKT cells, are a population of innate-like memory/effector cells expressing both NK receptors and a conserved, semi-invariant TCR (V[alpha]14-J[alpha]18/V[beta]8 in mice and V[alpha]24-J[alpha]18/V[beta]11 in humans). Like NK cells, NKT cells constitutively express mRNA but not protein for IFN-[gamma], evidencing their poised effector stage. NKT cells have been implicated in suppression of autoimmunity and graft rejection, promotion of resistance to pathogens, and promotion of tumor immunity.

[0004] While NKT cells are known to respond to [alpha]-GalactosylCeramide ([alpha]Gal-Cer), a surrogate ligand derived from a marine sponge, lack of knowledge of their natural antigens has previously precluded understanding of the mechanisms of their peripheral activation and recruitment, as well as their thymic development.

[0005] The inventors have previously identified a natural endogenous antigen, isoglobotrihexosylceramide (iGb3), which is presented to NKT cells by LPS-activated dendritic cells. This work suggests that iGb3 is a primary ligand for NKT cells. However, the partial diversity of the [beta]-chain of the TCR suggests that multiple natural antigen specificity may be possible.

SUMMARY

[0006] Described herein is the inventors' surprising discovery that glycolipids derived from members of the Class Alphaproteobacteria also act as natural ligands of CD1d molecules to activate NKT cells...

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

[0028] CD1-restricted T cells carry out both effector and helper functions and interact with a variety of cell types, including macrophages, dendritic cells, NK cells, T cells and B cells, thereby contributing to both innate and adaptive immune responses. A subset of these T cells, NKT cells, also known as CD1d-restricted T cells or CD1d tetramer<+> T cells, are characterized by invariant TCR[alpha] chains, self lipid reactivity and rapid effector responses. These cells play an important role in a number of immune functions, including antimicrobial responses, antitumor immunity and in regulating the balance between tolerance and autoimmunity.

[0029] In the absence of foreign antigens, NKT cells are stimulated by exposure to CD1<+> antigen presenting cells, such as monocytes, dendritic cells (DC) and macrophages. Classes of self-antigens that can be presented to and recognized by NKT cells include phospholipids, such as phosphatidylinositol, phosphatidylethanolamine and phophatidylglycerol, as well as sphingolipids. However, not all classes elicit a response in NKT cells in terms of cytokine release.

[0030] NKT cells also are known to recognize [alpha]-galactosylceramide ([alpha]Gal-Cer), a glycosphingolipid found in marine sponges. This molecule has no known immunological or other physiological function in mammals, but is widely used by investigators to study NKT activation. Prior to the present invention, activation of NKT by direct presentation of microbial glycolipids

was not known.

[0031] NKT cells are rapidly activated upon stimulation by CD1d presented polar lipid antigens. "Activation," as the term is used herein and in the art, refers to secretion by NKT cells of IFN-[gamma], IL-4, IL-2, IL-10, IL-13, GM-CSF or TNF-[alpha], or combinations of these cytokines, upon contact with CD1d presented stimulatory antigens. Alternatively, "activation" may refer to upregulated expression of cell-surface markers for activated T-cells, for example, CD69.

[0032] Activation of NKT cells in accordance with the invention comprises contacting an NKT cell, or more specifically, a T cell receptor (TCR) of the NKT cell, with a CD1d-complexed bacterial polar lipid. Glycolipids are suitable species of polar lipids. Thus, in some embodiments, activation of NKT cells comprises contacting an NKT cell with a bacterial glycolipid derived from a member of the Class Alphaproteobacteria. "A T cell receptor of an NKT cell," as the term is used herein, refers to the conserved, semi-invariant TCR of NKT cells comprising, e.g., V[alpha]14-J[alpha]18/V[beta]8 in mice and V[alpha]24-J[alpha]18/V[beta]11 in humans. "Contacting," as used herein, refers to the in vitro addition of bacterial glycolipid in solution to immobilized, soluble, or insoluble CD1d molecules, or to the in vivo administration of bacterial glycolipid to a subject having antigen presenting cells which express cell surface CD1d molecules.

[0033] Activation of NKT cells may be measured in vitro or ex vivo by any suitable method. An example of an in vitro test permitting evaluation of NKT cell activation is co-culturing NKT cells with antigen presenting cells (APC), such as dendritic cells (DC), in the presence of a bacterial glycolipid activator or putative activator, and subsequently assaying for IFN-[gamma] or other secreted cytokines in the supernatant. Alternatively, activation of NKT cells can be measured ex vivo by administering a bacterial glycolipid antigen to a subject or by administering CD1d<+> antigen presenting cells after ex vivo contact with bacterial glycolipids to a subject. The NKT cells from these subjects can be isolated by, e.g., CD1d-tetramer staining and gating via flow cytometry, and subsequently assayed for surface CD69 (early T-cell activation antigen) and/or intracellular IFN-[gamma] by suitable methods.

[0034] Alphaproteobacteria is a class in the phylum Proteobacteria comprised mostly of bacteria having two major phenotypes: purple non-sulfur bacteria and aerobic bacteriochlorophyll-containing bacteria. Bacterial members of the class of Alphaproteobacteria are primarily isolated from soil, lakes or ponds. Several members are known human pathogens.

[0035] The class Alphaproteobacteria includes six orders: Rhodospirillales, Rickettsiales, Rhodobacterales, Sphingomonadales, Caulobacterales and Rhizobiales (Garrity, G M et al., Taxonomic Outline of the Procaryotic Genera, BERGEY'S MANUAL of Systematic Bacteriology, 2<nd > Ed, April 2001, incorporated herein by reference). Bacterial glycolipids which may be useful in activating NKT cells may be derived from members of any of these orders. However, members of orders Rickettsiales, Sphingomonadales and Rhizobiales are contemplated to be particularly suitable.

[0036] The order Rickettsiales includes three families: Rickettsiaceae, Ehrlichiaceae and Holosporaceae. Polar lipids derived from members of Ehrlichiaceae in the genus Ehrlichia are contemplated to be suitably used in methods of the invention. For example, E. muris-derived glycolipids may be suitable.

[0037] The order Sphingomonadales includes the family Sphingomonadaceae. Glyclolipids derived from members of this family in the genus Sphingomonas, for example, from S. capsulata, are contemplated to be suitable.

[0038] The order Rhizobiales includes ten families: Rhizobiaceae, Bartonellaceae, Brucellaceae, Phyllobacteriaceae, Methylocystaceae, Beijerinckiaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Methylobacteriaceae and Rhodobiaceae.

[0039] Glycolipids derived from members of Brucellaceae in the genus Brucella are contemplated to be suitably used in methods of the invention.

[0040] Sphingomonas capsulata is a pathogen of the Alphaproteobacteria class which is a gram-negative, lipopolysaccharide (LPS)-negative bacteria whose cell wall lipids have been extensively characterized. Glycolipids derived from the cell walls of these bacteria may be used to activate NKT cells in accordance with the invention.

[0041] Similarly, members of the genus Ehrlichia are gram-negative, LPS-negative bacteria whose cell wall lipids may be used to activate NKT cells. Although the cell membrane lipids of Ehrlichia are not as well-characterized as those of Sphingomonas capsulata, it is contemplated that members of this genus will function to activate NKT cells in suitable activation assays, as well as in vivo.

[0042] Brucella is another genus in this class known to be pathogenic. The four species of this genus that can infect humans include B. abortus, B. suis, B. melitensis and B. canis. Brucellosis disease in humans is characterized as either an acute febrile disease or a persistent disease with a wide variety of symptoms. It is a true zoonosis in that virtually all human infections are acquired from animals. Subclinical infection is common. In contrast to Erlichia and Sphingomonas spp., the outer cell membrane comprises a dominant LPS component and three main groups of proteins. It is contemplated that particular fractions or components of these bacterial cell membranes may be used to directly activate NKT cells in accordance with the invention.

[0043] As noted, bacterial glycolipids are suitably derived from bacteria of the class Alphaproteobacteria. "Derived from," refers to isolation and/or purification from bacterial sources, and also refers to de novo synthesis of bacterial compounds, or compounds rationally designed based on bacterial compounds, using suitable synthetic processes known in the art. As will be appreciated by one of ordinary skill in the art, "bacterial glycolipids" may also include heat killed or attenuated bacteria in the context of the methods of the invention. For example, contacting a NKT cell with a bacterial glycolipid suitably includes contacting a NKT cell with a heat killed or attenuated bacteria, as well as isolated or synthetic bacterial glycolipids...

MODIFIED-GALACTOSYL CERAMIDES FOR STAINING AND STIMULATING NATURAL KILLER T CELLS
US8227581

Modified glycolipid compounds are provided. Also disclosed are methods for activating an NKT cell, methods of stimulating an immune response in a subject, and methods suitable for labeling NKT cells.

METHODS OF ACTIVATING NKT CELLS
US7998739

Provided are methods of activating an NKT cell which include a step of contacting the NKT cell with a sufficient amount of isoglobotrihexosylceramide (iGb3) to induce secretion of a cytokine from the NKT cell, stimulate proliferation of the NKT cell or upregulate expression of a cell surface marker on the NKT cell. Methods of activating an NKT cell population in a subject are also provided.

CATIONIC STEROID ANTIMICROBIAL COMPOSITIONS AND METHODS OF USE
US7754705

The invention provides methods for decreasing or inhibiting poxvirus infection or pathogenesis of a cell in vitro, ex vivo or in vivo, a symptom or pathology associated with poxvirus infection or pathogenesis in vitro, ex vivo or in vivo, or an adverse side effect of poxvirus infection or

pathogenesis in vitro, ex vivo or in vivo. In one embodiment, a method of the invention includes treating a subject with an invention compound (e.g., cationic steroid antimicrobial or CSA).

CATIONIC STEROID MICROBIAL COMPOSITIONS AND METHODS OF USE
WO2007089903
US2007191322

The invention relates to methods for decreasing or inhibiting influenza virus infection or pathogenesis of a cell in vitro, ex vivo or in vivo, a symptom or pathology associated with influenza infection or pathogenesis in vitro, ex vivo or in vivo, or an adverse side effect of influenza infection or pathogenesis in vitro, ex vivo or in vivo. In one embodiment, a method of the invention includes treating a subject with an invention compound (e.g., cationic steroid antimicrobial or CSA)

Biofouling-resistant ceragenin-modified materials and structures for water treatment
US 8529681

This invention relates to methods for chemically grafting and attaching ceragenin molecules to polymer substrates; methods for synthesizing ceragenin-containing copolymers; methods for making ceragenin-modified water treatment membranes and spacers; and methods of treating contaminated water using ceragenin-modified treatment membranes and spacers. Ceragenins are synthetically produced antimicrobial peptide mimics that display broad-spectrum bactericidal activity. Alkene-functionalized ceragenins (e.g., acrylamide-functionalized ceragenins) can be attached to polyamide reverse osmosis membranes using amine-linking, amide-linking, UV-grafting, or silane-coating methods. In addition, silane-functionalized ceragenins can be directly attached to polymer surfaces that have free hydroxyls.

CERAGENIN PARTICULATE MATERIALS AND METHODS FOR MAKING SAME
WO2013165574

Particulate ceragenin materials may be manufactured by (i) providing a ceragenin feed material comprised of ceragenin molecules, each having a sterol backbone and a plurality cationic groups attached thereto; (ii) fracturing the ceragenin feed material in a milling apparatus to produce a ceragenin particulate material having a particle size distribution with a median particle size in a range from 5 nm to 20 mum; and (iii) during fracturing, maintaining the ceragenin feed with a moisture content of less than or equal to 10% by weight.

ANTI MICROBIAL WASH COMPOSITIONS INCLUDING CERAGENIN COMPOUNDS AND METHODS OF USE FOR TREATING NON-MEAT FOOD PRODUCTS
WO2013163359

Particulate ceragenin materials may be manufactured by (i) providing a ceragenin feed material comprised of ceragenin molecules, each having a sterol backbone and a plurality cationic groups attached thereto; (ii) fracturing the ceragenin feed material in a milling apparatus to produce a ceragenin particulate material having a particle size distribution with a median particle size in a range from 5 nm to 20 mum; and (iii) during fracturing, maintaining the ceragenin feed with a moisture content of less than or equal to 10% by weight.

METHODS AND PRODUCTS FOR INCREASING THE RATE OF HEALING OF TISSUE WOUNDS
US2013243823

Disclosed are methods for increasing the rate of healing of a tissue wound by administering a composition including a therapeutically effective amount of at least one cationic steroid antimicrobial (CSA). Also disclosed herein are methods of promoting wound healing in a subject in need of such promotion, comprising administering a composition comprising a therapeutically

effective amount of at least one CSA. Additionally, disclosed herein are compounds and compositions comprising at least one CSA, or a pharmaceutically acceptable salt thereof, for use in the treatment of a tissue wound. Kits comprising such compositions and instructions on such methods are also contemplated herein.

COMPOSITIONS AND METHODS FOR TREATING BONE DISEASES AND BROKEN BONES
US2013243842

Disclosed herein are methods of promoting osteogenesis in a subject, comprising administering a composition comprising a therapeutically effective amount of at least one cationic steroid antimicrobial (CSA). Also disclosed herein are methods of promoting osteogenesis in a subject in need of such promotion, comprising administering a composition comprising a therapeutically effective amount of at least one CSA. Additionally, disclosed herein are compounds and compositions comprising at least one CSA, or a pharmaceutically acceptable salt thereof, for use in the treatment of bone disease or the treatment of broken bones. Kits comprising such compositions and instructions on such methods are also contemplated herein.

MEDICAL DEVICES INCORPORATING CERAGENIN-CONTAINING COMPOSITES
US2013245760

A medical device that includes a coating of a composite material that includes a polymeric material having a void structure and particulate ceragenin material (i.e., ceragenin particles) associated with the void structure. The average particle size of the ceragenin particles in the composite is in a range from 5 nm to 20 mum, 50 nm to 10 mum, 100 nm to 5 Xim, or 1 mum to 10 mum. The composite has a high loading of ceragenin particles (e.g., about 10% to about 25%, by weight). The composite has good polymer stability, the ability to release ceragenins from the ceragenin particles disposed in the composite over a sustained period of time at a characteristic elution rate, and the ability to kill large numbers of bacteria and other susceptible microbes over the sustained period of time.

Articles incorporating absorbert polymer and ceragenin compound
CN103313597

Also published as: US2012107382 // US2012108561 (A1) WO2012061651 // WO2012061648 // EP2635118

An absorbent article includes an absorbent polymer and a ceragenin compound. The ceragenin compound has a sterol group and a plurality of cationic groups that mimic naturally occurring antimicrobial peptides. The ceragenin compound is associated with the absorbent polymer such that upon absorption of a fluid, the ceragenin compound is incorporated or maintained in the absorbent article.

BACTERIAL GLYCOLIPID ACTIVATION OF CD1D-RESTRICTED NKT CELLS
US2008279894

Disclosed are methods for activating an NKT cell, methods of stimulating an immune response in a subject, methods of improving vaccine efficacy, and methods of treating an infection. Also disclosed are methods of promoting tumor rejection, treating cancer, modulating autoimmunity and inhibiting allergen-induced hypersensitivity in subjects. The methods include contacting an NKT cell with a bacterial glycolipid complexed with a CD1 molecule to activate the NKT cell. The bacterial glycolipid may be derived from a member of the Class Alphaproteobacteria.

ANTI-MICROBIAL FOOD PROCESSING COMPOSITIONS INCLUDING CERAGENIN COMPOUNDS AND METHODS OF USE
US2013236619

Disclosed herein are anti-microbial wash compositions and methods for using such compositions in

controlling microbe growth on a meat food product (e.g., a slaughtered meat carcass) by applying or contacting the anti-microbial wash composition with a surface of the food product to kill microbes (e.g., bacteria) on a surface of the food product. The anti-microbial wash compositions include a ceragenin compound dispersed in a fluid carrier. The ceragenin compound includes a sterol backbone and a number of cationic groups attached to the sterol backbone.

MEDICAL DEVICES INCORPORATING CERAGENIN-CONTAINING COMPOSITES
WO2013029059

A medical device that includes a coating of a composite material that includes a polymeric material having a void structure and particulate ceragenin material (i.e., ceragenin particles) associated with the void structure. The average particle size of the ceragenin particles in the composite is in a range from 5 nm to 20 [mu]??, 50 nm to 10 [mu]??, 100 nm to 5 [mu]??, or 1 [mu]?? to 10 [mu]??. The composite has a high loading of ceragenin particles (e.g., about 10% to about 25%, by weight). The composite has good polymer stability, the ability to release ceragenins from the ceragenin particles disposed in the composite over a sustained period of time at a characteristic elution rate, and the ability to kill large numbers of bacteria and other susceptible microbes over the sustained period of time.

INCORPORATION OF PARTICULATE CERAGENINS IN POLYMERS
WO2013029055

A composite that includes a polymeric material having a void structure and particulate ceragenin material (i.e., ceragenin particles) associated with the void structure. The average particle size of the ceragenin particles in the composite is in a range from 5 nm to 20 [mu]??, 50 nm to 10 [mu]??, 100 nm to 5 [mu]??, or 1 [mu]?? to 10 [mu]??. The composite has a high loading of ceragenin particles (e.g., about 10% to about 25%, by weight). The composite has good polymer stability, the ability to release ceragenins from the ceragenin particles disposed in the composite over a sustained period of time at a characteristic elution rate, and the ability to kill large numbers of bacteria and other susceptible microbes over the sustained period of time.

HYDROGEL MATERIALS INCORPORATING ELUTING CERAGENIN COMPOUND
WO2013013223

Also published as: US2013022651 // US2013053507 // WO2013013221

A hydrogel polymer includes a ceragenin compound. The ceragenin compound has a hydrophobicity/hydrophilicity that produces a release rate in a range of 0.1-100 [mu]g/ml for at least 3 days.