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"Superbugs Bunny" Outsmarts Our Immune Defense

Dieter Haebich* and Franz von Nussbaum*[a]

Introduction

For centuries, mothers have relied on the protective power of carrots and its ability to boost their baby's immune systems.[1] The toddler's golden yellow cheeks openly signal healthy development and the ability to withstand infectious adversity. Quite similarly, the human pathogen *Staphylococcus aureus* also counts on carotenoids to evade host immune defense, in particular being attacked and killed by human neutrophils. In fact, the characteristic golden color (Latin: *aureus*) conveyed by carotenoid surface pigmentation is the eponymous attribute of this bacterium.

Evolving resistance calls for novel therapeutic approaches

Mortality due to methicillin-resistant *S. aureus* (MRSA)[2] has exceeded the number of HIV-associated deaths in the US.[3] Bacteria rapidly mutate and outsmart antibiotic pressure by clever mechanisms that quickly spread through the microbial population and select for resistant organisms.[4] Multi-resistant and hyper-virulent microbes such as MRSA have become a physician's nightmare in hospitals and in the community (e.g. CA-MRSA USA300).[5] These "superbugs" demand "superdrugs"[6] that address novel therapeutic approaches without cross-resistance to antibiotics in clinical use.[7] Only a concerted effort to comprehend the fundamental biology of bacterial pathogens and the persistent commitment for antibacterial drug discovery will assure future therapy.[8] On the other hand, many analysts and managers cultivate a wary view of the antibacterial market.[9] As shareholder value interests frame the research strategies of pharmaceutical companies, innovative approaches with an especially high entrepreneurial risk have become more difficult to promote through R&D.[8]

[a] *Dr. D. Haebich, Dr. F. von Nussbaum*
Bayer HealthCare AG
Medicinal Chemistry Europe
42096 Wuppertal (Germany)
Fax: (+49) 202 368149
E-mail: dieter.haebich@bayerhealthcare.com
franz.nussbaum@bayerhealthcare.com

Color and virulence: anti-infective therapy based on virulence factor neutralization

In the March 7, 2008 issue of *Science*, the research teams of C.-I. Liu, G. Y. Liu, and Y. Song propose a new antibiotic approach that targets the bacterial pigment staphyloxanthin in *S. aureus*, an impressive example for anti-infective therapy based on bacterial virulence factor neutralization.[10] Their paper is an example of good science: a well-organized description of hypotheses, plus data and conclusions drawn from experimental tests of these hypotheses.

Staphyloxanthin (**5**) is an antioxidant that discriminates wild-type *S. aureus* from colorless (carotenoid-deficient) *S. aureus* mutants that are more vulnerable to oxidative stress, more susceptible to host neutrophil-based killing, and are less pathogenic in vivo in murine infection models (see Scheme 1 below).[11] To eradicate pathogens, the infected host's neutrophils and macrophages release reactive oxygen species (ROS) such as hydroxyl radicals ($HO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), peroxides (O_2^{2-}), singlet oxygen, and hypochlorites (OCl^-) during oxidative burst.[12] In systematic studies, the research teams of G. Y. Liu[11] and F. Götz[13] have demonstrated that the membrane-bound carotenoid staphyloxanthin (**5**) plays a crucial role in enhancing the virulence and fitness of *S. aureus* and its ability to cope with oxidative stress. The protective effect of this pigment on bacteria is a direct function of its antioxidant capacity. Hence, the authors suggest that blocking staphyloxanthin biosynthesis would render *S. aureus* more susceptible to innate immune system clearance and might be of value for treating *S. aureus* infections, in particular those which are resistant to established antibiotics.[10] Comprehending the fundamental biology behind these processes at the molecular level was a prerequisite for verifying this therapeutic concept.

The first steps in the biosyntheses of staphyloxanthin and cholesterol are alike

Staphyloxanthin (**5**) is a bacterial secondary metabolite that is not essential for the replication and growth of *S. aureus* in vitro, but furnishes the microbe with an evolutionary advantage for its survival in the infected host. F. Götz and co-workers[14] confirmed and extended the pioneering studies of J. H. Marshall and G. J. Wilmoth[15] on the biosynthesis of staphyloxanthin. The early steps in staphylococcal staphyloxanthin biosynthesis resemble those of the human cholesterol biosynthetic pathway. In contrast to Gram-negative bacteria,[16] both *S. aureus* and humans use the mevalonate pathway to produce the common precursor farnesyl diphosphate (**2**) (Scheme 1).[17] The first committed step in staphyloxanthin biosynthesis is catalyzed by the enzyme dehydrosqualene synthase (CrtM). CrtM promotes the head-to-head condensation of two C_{15} molecules of farnesyl diphosphate (**2**) to form presqualene diphosphate (**9**), which, after loss of diphosphate, rearranges to the C_{30} product dehydrosqualene (**3**) (Scheme 2). Stepwise oxidation of **3** by dehydrosqualene desaturase (CrtN) and diaponeurosporene oxidase (CrtP), and subsequent glucosylation and incorporation of a C_{15} acyl terminus, affords staphyloxanthin (**5**).[14] The similarity between the initial steps of bacterial staphyloxanthin biosynthesis and human cholesterol

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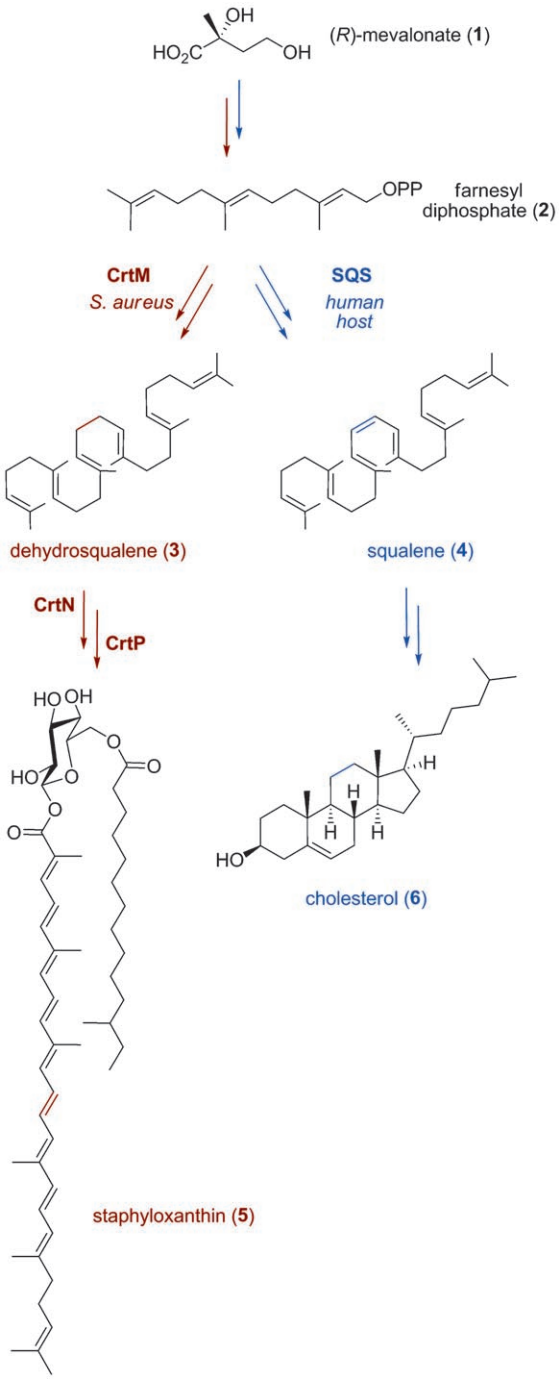

Scheme 1. Both *S. aureus* (red) and humans (blue) use the mevalonate pathway to biosynthesize staphyloxanthin and cholesterol.

biosynthesis stimulated the authors to presume that the enzymes involved, dehydrosqualene synthase (CrtM) and human squalene synthase (SQS), might be structurally related as well. They determined the X-ray crystal structure of *S. aureus* CrtM at 1.58 Å resolution and indeed found that despite moderate sequence identity, its overall fold showed structural similarity to human SQS (Figure 1). Bacterial CrtM contains a distinct hollow space, big enough to host dehydrosqualene (**3**) or squalene (**4**).[10]

Cholesterol biosynthesis inhibitors block *S. aureus* virulence

The authors consequently screened various known (racemic) inhibitors[18] of human SQS for activity against CrtM. They identified three phosphonosulfonate hit compounds and determined their X-ray crystal structures bound to CrtM. One of these squalene synthase inhibitors, BPH-652 (**12**), exhibited a K_i value of 1.5 nM against CrtM and indeed blocked staphyloxanthin pigment biosynthesis in vitro with an IC_{50} (median inhibitory concentration) value of 110 nM. The resulting non-pigmented bacteria were significantly more susceptible to killing by human blood and to innate immune clearance in a murine model of kidney infection. In this mouse model, a promising two-$\log_{10}$ decrease in colony forming units was observed after intraperitoneal treatment with 0.5 mg compound **12** for 4 days, twice daily (~12 mg kg^{-1} BID i.p.), starting one day prior to infection.[10]

Teaching an old dog new tricks?

In 1996, the squalene synthase inhibitor BMS-187745, the *S* enantiomer of **12** (= (*S*)-BPH-652, Figure 2), had been tested by Bristol–Myers Squibb in phase I clinical trials for the treatment of atherosclerosis and hyperlipidemia.[20] It exhibited low clearance ($C_L = 0.116$ mL h^{-1} kg^{-1}), an exceptionally long half-life ($t_{1/2} = 820$ h), but poor oral absorption ($F = 2.6\%$) in humans. Therefore, the bis-pivaloyloxymethyl ester BMS-188494 (**13**) was developed as a prodrug.[21] Compared with the polar parent drug (*S*)-**12** ($\log P = -1.3$), the corresponding lipophilic ester **13** ($\log P = 3.5$) exhibited higher bioavailability ($F = 26\%$). Up to a dose of ~3 mg kg^{-1}, ester **13** showed no safety or tolerability issues in an ascending multiple dose study.[22] The development of **13** was later discontinued, because no sufficient changes in total cholesterol, LDL cholesterol, or apoB levels could be demonstrated in treated subjects.

C.-I. Liu and G. Y. Liu's meticulous juxtaposition of the squalene biosyntheses in *S. aureus* and humans sparked a new idea for antibacterial research: clinically tested cholesterol-lowering drugs may be useful for the treatment of infectious diseases as mono- or combination therapy. However, some problems remain. In contrast to the chronic treatment of hyperlipidemia, a reliable anti-infective therapy will require much higher human

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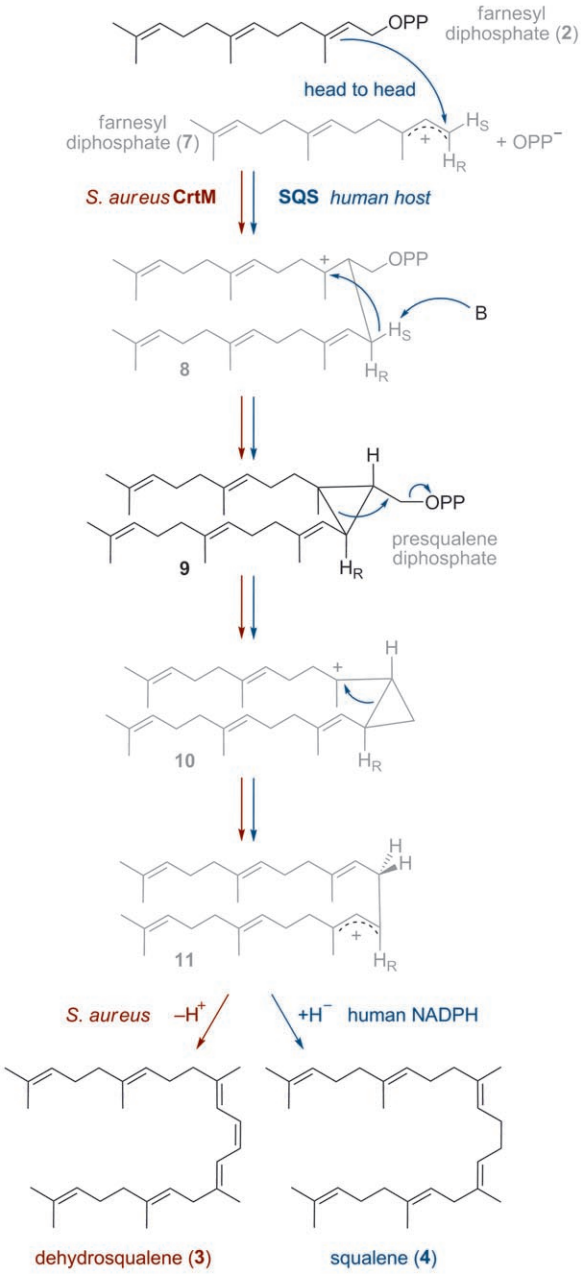

Scheme 2. Squalene scaffold biosynthesis in Gram-positive *S. aureus* (red) and its human host (blue); OPP = $P_2O_7^{3-}$.

doses (gram versus milligram quantities) to attain sufficient drug concentrations in the infected tissue to rapidly eradicate bacteria with the help of the host's immune defense. Whereas for a cholesterol-lowering drug the partial modulation of a single human target seems sufficient, for antibacterial therapy, essential bacterial biosynthetic pathways have to be blocked completely. Because **12** acts simultaneously as a potent inhibitor of bacterial CrtM and of human SQS, mechanism-based adverse effects in patients undergoing anti-infective therapy may represent a potential risk. The long half life of (*S*)-**12**, with mean residence times of ~1200 h, could be an issue for intensive care therapy.[21b]

Developmental and regulatory issues

Nontraditional ("secondary") anti-infective therapy based on virulence factor neutralization in place of clinically validated ("primary") bacterial targets, such as cell-wall biosynthesis, is innovative and encouraging but also enormously challenging from a developmental and regulatory point of view. In general, a virulence factor does not have a direct effect on bacterial cell growth. It does not exhibit in vitro potency (MIC), but rather manifests itself in the more complex in vivo setting. As MIC testing is the established standard procedure in hospitals to decide on a patient's therapy, virulence-factor-based antibiotics will have to find alternate ways to prove their suitability to the daily clinical situation. Moreover, it will be difficult to demonstrate antibacterial action and to satisfy the increasing regulatory requests of the US Food and Drug Administration (FDA).[23] While for more than a decade *non-inferiority* trials (the new agent must not be less effective than an established comparator drug) had been the standard in developing antibiotics, today the FDA increasingly requests *superiority* trials (the new agent must be superior to an established comparator drug), a decision possibly impelled by the FDA's experience with telithromycin and the public discussion on dispensable pseudo-innovations.[24] These changes to regulatory requirements for clinical trials could even further discourage the pharmaceutical industry from developing new antibiotics.[25] Nevertheless, the FDA has expressed interest and scientific flexibility to support innovative therapies for indications of high unmet medical need.

Conclusions

The clever discovery by C.-I. Liu, G. Y. Liu, Y. Song et al. illustrates how future anti-infective therapies could be boosted by bacterial virulence factor neutralization. At present, nontraditional therapy based on virulence factor neutralization appears to be enormously challenging from a developmental and regulatory point of view. On the other hand, the rising levels of clinical resistance among bacteria and the growing threat of a frightful public health crisis might eventually facilitate combination therapy and boost innovative principles to secure our future therapeutic options. For that prospect, the proficient work by C.-I. Liu, G. Y. Liu, and colleagues has provided a solid starting point. It could be rewarding to

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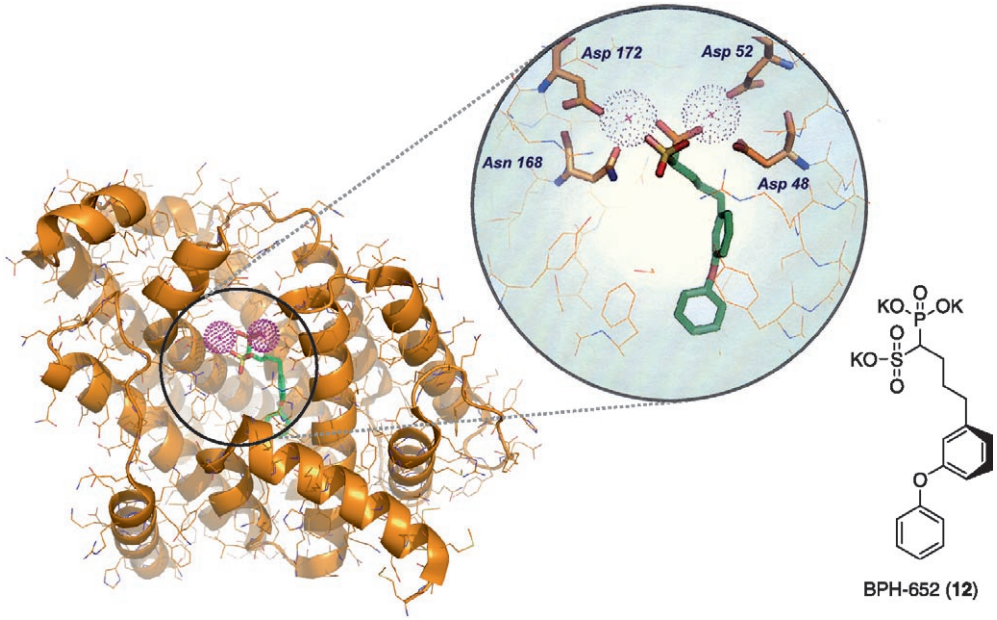

Figure 1. X-ray crystal structure of BPH-652 (**12**) bound to *S. aureus* CrtM.[10,19]

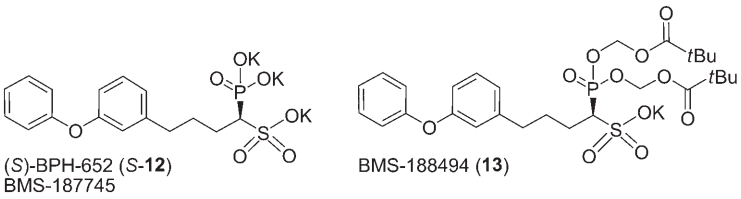

Figure 2. Phosphonosulfonate BMS-187745 [(*S*)-**12**] and its ester prodrug BMS-188494 (**13**).

explore the inherent synergistic potential of their concept in combination with established antibiotics and to perform studies in neutropenic infection models. This concept will probably be restricted to patients harboring a sufficient titer of neutrophils, which is not uniformly the case in severe bacterial infections. It will be crucial to assess the efficacy of a CrtM inhibitor in comparison with MRSA standard therapy and in a setup in which the animals are not treated prior to infection. It will require considerable commitment and effort to progress this valuable new therapeutic principle.

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Keywords: antibiotics · carotenoids · cholesterol · dehydrosqualene synthase · *Staphylococcus aureus* · staphyloxanthin

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