Chapter 6

SOS Response and *Staphylococcus aureus*: Implications for Drug Development

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Abstract

Damage in genetic material is induced through the action of several drugs (directly or indirectly). Specially, antimicrobials from quinolone class (such as ciprofloxacin) induce DNA damage that promotes the formation of the RecA filament leading to auto-cleavage of LexA and allows the expression of SOS genes, including the error-prone polymerase (like *umu*C). The SOS pathway plays a critical role in the acquisition of mutations that lead to the emergence of antibiotic-resistant bacteria and the spread of virulence factors. This chapter provides a comprehensive review about the SOS response of *Staphylococcus aureus* and the modulatory effects of new compounds (natural or synthetics) on this pathway. The effects of some SOS inhibitors are highlighted such as baicalein and aminocoumarins. Compounds able to prevent SOS response are extremely important to develop new combinatory approaches to inhibit *S. aureus* mutagenesis. The study of new SOS inductors could reveal new insights into the pathways used by *S. aureus* to acquire drug resistance; examples of these compounds are the lysine-peptoid hybrid LP5, cyclic peptide inhibitors, etc. These studies can impact the development of new drugs. In conclusion, we hope to provide essential information about the effects of compounds on SOS response from *S. aureus*.

Keywords: DNA damage, mutagenesis, virulence factors, small colony variants

1. Introduction

Drug resistance is a well-known problem involved in the treatment of bacterial infections and its incidence is escalating in an alarming rate [1–3]. As a result, microbial diseases are still among the most serious problems to public health system, especially in developing countries where infectious diseases still represent a major cause of human mortality. Especially alarming is the rapid global spread of multiresistant bacteria that cause common infections and that resist treatment with existing antimicrobial medicines [4, 5]. The classical paradigm suggests that antibiotic resistance emerges by selection of preexisting mutants in the bacterial population exposed to antibiotics [1]. In contrast, recent data suggested that mutations evolve after cells encounter antibiotic therapy. This kind of mutation is known as adaptive mutation, which is activated by the SOS DNA repair and mutagenesis pathways [6, 7].

The SOS system is the bacterial DNA damage response that is activated by DNA damage or stalled DNA replication caused by the exposure of bacteria to stressful conditions [8] such as antibiotic treatment [9], starvation [10], and oxidative stress [11]. Repair of damaged DNA is critical for bacterial survival, and during this process, some mutations may be introduced into the genome, which may result in bacterial drug resistance [12, 13]. Accumulation of single-stranded DNA (ss-DNA) is the signal that induces the SOS response by promoting the formation of the RecA filament, which in turn activates the auto-cleavage activity of LexA and allows expression of several genes [8]. The SOS response is a very orchestrated pathway by which the bacterial cell improves its capacity to inhibit cell division, repair DNA, and express error-prone DNA polymerases to replicate noninstructive DNA lesions [14]. This pathway has been widely studied in *Escherichia coli* where more than 40 genes are involved [15]. The first stage of SOS response is the expression of genes related to nucleotide excision repair mechanisms (*uvrA, uvrB, uvrD, polB, ruvA, ruvB*, and *dinI*). If the damaged is not repaired the genes responsible for recombination repair mechanisms (*recA* and *recN*) are expressed. Finally, if the SOS response is not successful, then the *sulA* and *umuDC* genes are expressed. SulA inhibits cell division and the *umuDC* operon encodes the error-prone DNA polV crucial in translesion error-prone DNA synthesis. When *sulA* is expressed in the late stage of the SOS gene expression, it arrests cell division by binding FtsZ and provides extra time for the mutagenic error-prone polymerases to acquire mutations that allow cells to escape from the metabolic and genomic stress [8, 16–18].

Some difference may be found in the SOS response for each species. For example, this process in *Bacillus subtilis* is mediated by a similar number of genes than in *E. coli*, however, only seven genes are common for these two bacteria [19]. In the case of *Staphylococcus aureus*, only sixteen genes have been identified under the control of LexA, between them one error-prone polymerase is designated here as *umuC* (SACOL1400) [9, 20]. The SOS pathway plays a critical role in several processes related to pathogenesis of *S. aureus*, such as emergence of antibiotic resistant strains [21], dissemination of virulence factors [22], and increase of the frequency of small colony variants (SCVs) [23]. In this sense, this chapter aims to provide a comprehensive review about the modulatory effects of compounds (natural or synthetics) on SOS response of *S. aureus*.

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2. Overview of SOS response in *Staphylococcus aureus*

Since DNA damage may occur as a result of environmental agents and drugs, the role of SOS pathway in different conditions has been studied in *S. aureus* [21–23]. Most of these studies have focused on the effects of clinical relevant antibiotics, especially those from fluoroquinolone class, such as ciprofloxacin. The induction of SOS response in this pathogen has been associated with mutagenesis, spread of virulence factors, and formation of small colony variants [22–27]. We discuss some of the papers related to these subjects in the following topics.

2.1. SOS response affects the expression of virulence factors in *Staphylococcus aureus*

To prove this concept, the effects of SOS response in the dissemination of pathogenicity island-encoded virulence factors in staphylococci was evaluated [22]. *S. aureus* pathogenicity island (SaPI) comprises a large family of highly mobile phage-related chromosomal islands, which carry a range of virulence genes, for example, TSST1 (toxic shock syndrome toxin), SEB (staphylococcal enterotoxin B), and other superantigens [28]. SaPI are widely distributed among Gram-positive bacteria and they are considered as prototypes for the understanding of the mobile mechanisms of pathogenicity islands, since horizontal gene transfer has an extremely important role in bacterial evolution [29]. It was demonstrated that SOS induction (by ciprofloxacin) induced SaPI excision and replication with participation of at least three different temperate phages (80, 11, and 147). SOS pathway also regulates the replication and high-frequency transfer of this element, as well as of SaPI1. Theses finds suggested that SOS activation by antibiotics may lead to the spread of staphylococcal virulence genes, an unintended consequence [22].

The influence of subinhibitory concentrations (Sub-MIC) of others antibiotics in the induction of SOS response and horizontal transfer of virulence factors in *S. aureus* was also evaluated [24]. The authors used antibiotics with different action mechanisms such as lactams (ampicillin, penicillin, ceftriaxone, and cloxacillin), macrolide-β lincosamide-streptogramin B antibiotics (erythromycin), aminoglycosides (kanamycin), chloramphenicol, and tetracycline. From these drugs, only β-lactams induced replication of SOS- inducible prophages 80α and 11ϕ, resulting in SaPIbov1 transfer. The effects of ciprofloxacin and trimethoprim (a folic acid inhibitor from sulfonamide class) on phage induction and expression of phage-encoded virulence factors were evaluated using *S. aureus* strains isolates from patients with cystic fibrosis [25]. This study analyzed the integration of phages into the chromosomal gene coding for β-hemolysin (*hlb*), these phages encode for accessory virulence determinants such as staphylokinase (*sak*; a plasminogen activator essential for bacteria dissemination from clots and abscesses and resistance against human defensins) and enterotoxins [30, 31]. Sub-MIC of both drugs resulted in delysogenization of strains and replication of *hlb*-converting phages in a dose-dependent manner. The involvement of SOS response in phage mobilization was demonstrated by increase of *recA* expression. In addition, induction of 13 was directly associated with phage-encoded virulence gene *sak* [25].

In another study, the expression of type 5 capsular polysaccharide (CP5) in *S. aureus* was shown to be linked to SOS response [32]. CP5 is one virulence factor that is important for protection

against phagocytes [33] and it is an attractive candidate for the development of immunotherapies [34]. The production of CP5 is influenced by various environmental agents (carbon dioxide, iron concentration, and specific nutrients) and controlled by a complex regulatory genetic network [35]. Using a transposon-mediated mutagenesis assay, seven genes were identified affecting the production of CP5, including *sbcD* and *sbcC* genes [35]. These genes are adjacent forming the *sbcDC* locus that negatively affects capsule production. Sub-MIC of SOS inducers (ciprofloxacin or mitomycin C) promoted the transcription of *sbcDC* locus and consequently repressed the CP5 production [32]. The authors suggested that this effect of SOS response in capsule expression could be related to (i) energy saving (the energy needed to capsule biosynthesis would be used for DNA reparation); (ii) improvement of adhesion capability (capsule absence would unmask the adhesion proteins present in cell membrane, promoting bacterial infection and thereby avoiding DNA-damaging agents). These results corroborate with previous study which showed that ciprofloxacin increased the expression of fibronectin-binding proteins (FnBPs) in fluoroquinolone-resistant *S. aureus* strains [36].

2.2. SOS response and mutagenesis in *Staphylococcus aureus*

Apart from its capacity to express virulence factors, *S. aureus* is extremely able to acquire resistance to virtually any antibiotic. For example, methicillin-resistant *S. aureus* (MRSA) strains are important etiologic agents of both nosocomial and community infections. It has been shown that Sub-MIC of fluoroquinolone drugs enhances methicillin resistance in community or nosocomial MRSA isolates [26, 37]. Community-associated MRSA isolates (CA-MRSA) grown in tryptic soy broth containing sub-MIC of fluoroquinolone (ciprofloxacin or levofloxacin) showed increased resistance in nafcillin agar, and this effect was dose-dependent. Through microarray analysis it was possible to conclude that alterations-induced fluoroquinolone drugs were mediated by SOS response [26]. In the same context, a later study evaluated the effects of Sub-MIC of ciprofloxacin in the development of rifampin resistance in methicillin-susceptible *S. aureus* (MSSA) and MRSA strains. Ciprofloxacin induced higher frequencies of rifampin-resistant mutants. A significant proportion of these mutants exhibited in-frame deletions or insertions in the *rpoB* gene at several positions, while those mutants from ciprofloxacin-free cultures essentially showed single-amino-acid substitutions [27].

2.3. Induction of SOS increases the frequency of small colony variants in *Staphylococcus aureus*

Recently, the activation of SOS response was linked with the enhanced incidence of small colony variants (SCVs) in *S. aureus* [11, 23]. *S. aureus* SCVs are marked by small colony with slow growth phenotype, which is associated with intracellular persistence and reduced antimicrobial susceptibility [38]. *S. aureus* switch to SCVs phenotype under the pressure of stress elicitors such as oxidative stress [11], cold stress [39], and drug treatment [23]. SCVs are frequently associated with latent or chronic infections, including device-associated infections, bone and tissue infections, and airway infections of cystic fibrosis patients [40].

S. aureus SCVs present mutations in one or few genes related to metabolic pathways resulting in atypical biochemical characteristics [41]. The nature of these mutations is directly

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related to the antibiotic resistance profile exhibited by the SCVs. Resistance to trimethoprim-sulfamethoxazole has been associated to alteration on *thyA* gene, which encodes thymidylate synthase [42, 43]. This enzyme is essential for DNA biosynthesis as it converts deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Diminished concentrations of intracellular dTMP lead to thymidine-dependent SCVs phenotypes (TD-SCVs), which is associated to trimethoprim-sulfamethoxazole resistance and hypermutability [44, 45].

On the other hand, mutations in genes related to menadione or hemin synthesis result in electron transport-defective strains as bacteria are unable to produce menaquinone and cytochromes, respectively [46, 47]. SCVs auxotrophic for menadione or hemin are resistance to aminoglycosides (such as gentamicin) due to a decrease in drug uptake [48]. Fluoroquinolones and mitomycin C Sub-MIC increased the generation of gentamicin-resistant SCVs with an increased mutation rate through activation of the SOS response [23]. The SOS response is also essential for the adaptation of *S. aureus* to oxidative stress, in this case by producing hydrogen peroxide–resistant SCVs [11].

3. New compounds able to modulate the SOS response in *Staphylococcus aureus*

Given the role of SOS response in *S. aureus* survival and pathogenesis, the effects of new antimicrobial candidates on SOS genes have become more frequently evaluated, especially those which target DNA structure or DNA replication machinery [49, 50]. Gottschalk et al. [49] reported an easy and inexpensive agar-based assay to detect the expression of *recA* induced by a compound. In this assay, a *S. aureus* 8325-4 derivative strain carrying the *recA* gene fused with the reporter gene *lacZ* (which encodes for β-galactosidase) is incorporated in agar plates containing X-Gal (5-bromo-4-β chloro-3-indolyl-D-galactopyranoside). X-gal is a chromogenic substrate for β-galactosidase that produces a rich blue color that can easily be detected visually. The compound to be tested should be added in wells in these agar plates and the expression of *recA* is monitored as a blue ring at the point of bacterial growth. Using this assay, the ability of some compound to active SOS response was revealed, such as the lysine-peptoid hybrid LP5 [49], some cyclic peptide inhibitors of the β-sliding clamp [50], and the amphibian peptide fallaxin analogue FL9 [51]. The induction of SOS response was also related to the anti–*S. aureus* action of new synthetic bis-indole antibiotics [52]. All these compounds inhibit the DNA replication of *S. aureus*.

Special attention has been given to the use of SOS inhibitors as therapeutic adjuvants in combating bacterial infections. These approaches involve inhibiting the SOS-mediated mutagenesis induced by drugs and thus improving their long-term viability. In these cases, LexA and RecA represent potential targets [53, 54]. In fact, the number of SOS inhibitors is still limited and most of the studies use *E. coli* as model [55, 56]. Regarding the suppression of SOS response in *S. aureus*, a study showed baicalein as a potential compound. Baicalein is the main component of the Chinese herb Scutellaria baicalensis Georgi (Labiatae), which shows anti–*S. aureus* and antioxidant activities [57, 58]. Baicalein inhibited the expression of some SOS genes (*recA*, *lexA*, and LexA-regulated DNA polymerase SACOL1400) and the

rifampin-resistant mutation ratio induced by Sub-MIC of ciprofloxacin. The authors correlated these effects in SOS response with a decrease in the formation of intracellular reactive oxygen species and ATP level after baicalein treatment [59].

In a later study, the effects of novobiocin in the SOS response induced by ciprofloxacin were evaluated. Novobiocin is an aminocoumarin, a class of antibiotics that interferes with ATPase activity of the gyrase subunit B and the topoisomerase IV subunit ParE without inducing double-strand breaks [60–62]. Differently from quinolones, aminocoumarin treatment does not activate SOS response. In fact, novobiocin inhibited the *recA* expression in a LexA-independent manner. Novobiocin was also able to suppress the SOS response induced by ciprofloxacin: it inhibited *recA* expression and partially reduced the induction of the error-prone polymerase *umuC* (regulated by LexA). These effects resulted in a reduction in the frequency of recombination, mutation, and the formation of nonhemolytic variants [20].

The concept that SOS response is a potential target was additionally explored using antimicrobial photoinactivation. Antimicrobial photodynamic therapy (aPDT) is a promising strategy for the treatment of localized infections, such as acne inflammation [63], periodontal, and peri-implant diseases [64]. aPDT consists in the use of three elements (photosensitizer agent, visible light, and oxygen), where the damage of different bacterial structures (cell envelopes, lipids, proteins, and DNA) would avoid the development of resistance [65]. The role of DNA damage and SOS response during photoinactivation was recently established. Different exogenous photosensitizers induced DNA damage and consequently the expression of *recA*. The repression of *recA* by novobiocin or gene deletion resulted in additional susceptibility of *S. aureus* toward photoinactivation through increase of DNA damage. These results suggested that the combination of *recA* inhibitors and photoinactivation could have a clinical relevance [66].

SOS response in *E. coli* has been shown to be regulated by ribonuclease E (RNase E), an enzyme involved in RNA metabolism (global mRNA degradation, maturation of rRNA, and small regulatory RNA) [67]. RNase E deficient strains exhibit a reduction in SOS activation, revealing that RNase E inhibitors could be possibly used as drug adjuvants [68]. Although RNase E orthologs have been identified in a range of other bacteria and in bacteria and chloroplasts [69, 70], RNA turnover is not regulated by an RNase E ortholog protein in *S. aureus* [70]. Instead, *S. aureus* has an mRNA degradosome complex formed by diverse proteins, including RNase enzymes (RNase J1, RNase J2, RNase Y, and RnpA), enolase, phosphofructokinase, polynucleotide phosphorylase (PNPase), and DEAD box RNA helicase. RnpA, a component of this complex, has been reported as a target to inhibit bacterial survival and pathogenesis [70, 71]. However, the role of mRNA degradosome complex in SOS response regulation remains to be elucidated in this pathogen.

4. Conclusion

The SOS response is an essential pathway for *S. aureus* survival and pathogenesis. This mechanism is activated by different stress situations (such as environmental alteration, drug, and toxins treatment), which lead to mutagenesis, phenotypical alterations, and spread of

virulence factors. All these consequences of SOS activation are important to pathogen dissemination and treatment failure. SOS proteins are potential target for therapies, especially those using quinolones and RecA/LexA inhibitors. These studies have shown that SOS inhibitors are able to decrease drug-induced mutagenesis in *S. aureus*. We hope that more researches will be performed in the future to identify more compounds that are able to modulate SOS response, as well as deeper *in vivo* studies to establish the clinical relevance of them.

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