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β-lactams are a broad class of antibiotics known to target penicillin-binding proteins (PBPs), which are enzymes involved in the assembly of the bacterial cell wall. These drugs halt cell wall synthesis, but the molecular mechanisms that ultimately lead to bacterial killing remain unclear. Now, Cho *et al.* show that β-lactams not only inhibit PBPs, but also induce the degradation of cell wall components, which depletes bacterial resources and enhances bacterial killing.

> “the inhibition of the transpeptidase activity of PBPs prevents peptidoglycan crosslinking and results in the accumulation of uncrosslinked glycan chains in the periplasm, which are degraded by Slt”

The main component of the bacterial cell wall is peptidoglycan, a polymer that consists of adjacent glycan chains that are crosslinked to each other by peptide chains. The assembly of peptidoglycan depends on the activity of different PBPs: class A PBPs (such as PBP1a and PBP1b) are bifunctional and have both glycosyltransferase activity (which polymerizes the glycan chains) and transpeptidase activity (which crosslinks neighbouring glycans), whereas class B PBPs (such as PBP2 and PBP3) have only transpeptidase activity. Importantly, β-lactams inactivate PBPs by binding to and modifying the active site of the transpeptidase domain, resulting in the inhibition of peptidoglycan crosslinking.

To investigate how PBP inhibition results in bacterial killing, the authors monitored peptidoglycan synthesis and decay in *Escherichia coli* cells treated with mecillinam, which is a β-lactam specific for PBP2. As expected, compared with untreated cells, mecillinam treatment reduced the insertion of newly synthesized glycan chains into the cell wall, as measured by the incorporation of radiolabelled meso-diaminopimelic acid (mDAP) — an amino acid that is only present in peptidoglycan. Surprisingly, treatment with mecillinam also resulted in the accumulation of a large pool of peptidoglycan turnover products, which suggests that rather than just inhibiting peptidoglycan crosslinking by the PBPs, mecillinam treatment also induced the degradation of the nascent peptidoglycan produced by the cell wall synthesis machinery. Furthermore, the β-lactam-induced degradation of peptidoglycan was not exclusive to mecillinam; it was also observed following treatment with cephalexin (which targets PBP3) and with cefsulodin (which targets PBP1a and PBP1b). Together, these data suggest that β-lactams not only block peptidoglycan crosslinking by modifying the transpeptidase activity of PBPs but also lead to peptidoglycan degradation, which results in the depletion of bacterial resources.

But how is peptidoglycan degraded following β-lactam treatment? To address this question, the authors screened a library of transposon mutants for resistance to mecillinam and identified a mutant carrying an insertion in the gene encoding a soluble lytic transglycosylase (Slt), which is a cell wall-degrading enzyme that cleaves uncrosslinked peptidoglycan strands in the periplasm. Notably, a mutant *E. coli* strain lacking Slt showed reduced peptidoglycan degradation following treatment with mecillinam compared with wild-type bacteria, demonstrating that Slt is responsible for peptidoglycan decay following β-lactam treatment.

Taken together, these data suggest a new model for the action of β-lactams in which the inhibition of the transpeptidase activity of PBPs prevents peptidoglycan crosslinking and results in the accumulation of uncrosslinked glycan chains in the periplasm, which are degraded by Slt; this initiates a futile cycle of peptidoglycan synthesis and decay that exhausts bacterial resources and contributes to bacterial killing.

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ORIGINAL RESEARCH PAPER Cho, H. *et al.* Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. *Cell* **159**, 1300–1311 (2014)

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