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COMMENTARY

On the link between antibiotic resistance, diabetes, and wastewater

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The study by Lucero et al. (https://doi.org/10.1085/jgp.202313464) sheds light on the remarkable capabilities of bacterial transporters to adapt to new selective pressures. Their findings provide insight into the mechanism of a subtype of SMR transporters.

Introduction

These seemingly unrelated issues of antibiotic resistance, diabetes, and wastewater are actually interconnected. Grasping this connection is essential in addressing the challenges we face today and preventing future ones.

A recent study conducted by Stockbridge's laboratory has found a link between small multidrug resistance (SMR) transporters and the metabolism of metformin, a common drug used to treat type II diabetes. Previous research shows that microbial communities play a crucial role in the breakdown of metformin in the environment, and SMR transporters, particularly SMRGdx, are believed to be necessary for this process. Metformin, a biguanide drug, is structurally similar to nitrogenous waste products such as guanidinium (Gdm^+) and guanylurea, which SMRGdx exports. The study conducted by Lucero et al. (2024) used various methods to investigate how SMRGdx and other SMR proteins interact with metformin metabolites. These proteins are linked to horizontal gene transfer in wastewater bacteria that degrade metformin.

The research provides a comprehensive analysis of the SMRGdx subtype, including biochemical, structural, and mechanistic attributes. These findings are relevant not only to other members of the SMR family but also to other multidrug transporters (MDTs), proteins best known for their role in microbial antibiotic resistance (this issue: Lucero et al., 2024).

Metformin in wastewater

Metformin is a widely prescribed drug for type II diabetes. It is taken in gram quantities daily by over 150 million people and excreted in an unchanged form, along with its degradation product guanylurea (Foretz et al., 2023). These two compounds are the most frequently found anthropogenic chemicals in wastewater worldwide, with concentrations measured up to the low μM range in sampled waste and surface waters. Unfortunately, typical wastewater treatment protocols do not remove these compounds, leading to environmental concerns about their accumulation in surface water (Briones et al., 2016).

Interestingly, metformin has been linked to changes in the composition of microbial communities in the gut and wastewater treatment plants. Some studies suggest that it may act as a co-selective agent, promoting the survival of antibiotic-resistant bacteria in the presence of antibiotics. On the other hand, recent research has identified bacteria that use metformin as a nitrogen and carbon source, indicating that biodegradation of metformin and guanylurea could be a viable strategy for remediating these compounds (Martinez-Vaz et al., 2022).

MDTs and antibiotic resistance

MDTs are membrane proteins that recognize a wide range of antibiotics. They remove the antibiotics from the cell in an energy-dependent process and are responsible for the resistance in some microorganisms. A prominent feature of these MDTs is their extremely broad specificity, which means that a single transporter can confer resistance against various drugs. Some MDTs have even been reported to confer resistance to dozens of toxic compounds with few common features. A hypothesis suggests that each MTD has evolved to remove a specific natural compound or a group of similar ones. This means that they are not substantially different from regular transporters. The ability of multidrug transporters to remove toxins is considered an opportunistic side effect that is only detected when bacteria are exposed to drugs in experimental or clinical situations. The concept that MDTs may have other natural functions was first suggested in *Bacillus subtilis*, where it was shown that the transporter Blt is part of an operon that detoxifies spermidine (Neyfakh, 1997). Many other MDTs have since been associated with various biological functions, including removing, besides antibiotics, heavy metals, organic pollutants, plant-produced

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compounds, quorum sensing signals, and bacterial metabolites such as breakdown products of metformin. Furthermore, mammalian homologs have evolved to fulfill various functions, including neurotransmitter transport, reabsorption of essential molecules, or secretion of toxic ones in the kidney (Henderson et al., 2021; Schuldiner et al., 1995).

The SMR family and metformin transport

The SMR family comprises small four-transmembrane helical proteins known for their role in antibiotic and antiseptic resistance (Schuldiner, 2009; Burata et al., 2022). These proteins are among the smallest membrane transport proteins, perfect candidates for detailed biochemical and biophysical analysis. EmrE has been the prototype for members of the SMR family and is the most extensively studied member of the SMRQac subtype. It is known to transport substrate across the cell membrane by exchanging protons. EmrE possesses a unique characteristic—it has only one membrane-embedded charged residue, Glu14, which is conserved in hundreds of homologous proteins in bacteria and archaea. Glu14 plays a crucial role in the coupling mechanism, as its deprotonation is necessary for substrate binding (Schuldiner, 2009).

There are two types of SMR, each with different abilities to transport substances. Stockbridge et al. previously showed that while SMRQac can transport various antimicrobial substances, including antiseptics like benzalkonium, which are commonly found in wastewater, SMRGdx is highly specific and does not provide robust resistance to classical antimicrobials. In its primary physiological context, SMRGdx exports the nitrogenous waste product Gdm^+ and its breakdown product guanylurea (Burata et al., 2022). The aforementioned evidence supports a role of SMRGdx in the metabolism and biodegradation of metformin. Therefore, the study in this issue embarks on a detailed analysis of the molecular and evolutionary implications of this finding.

In this study, the Stockbridge lab investigated whether several genomic and plasmid-associated SMRs transport metformin or other byproducts of microbial metformin metabolism. They examined four SMRGdx homologs, including Gdx-Clo, the structurally characterized genomic protein from *Clostridales* (Kermani et al., 2020).

The study revealed that genomic and plasmid-associated SMRGdx homologs efficiently transport guanylurea with transport kinetics similar to the physiological substrate Gdm^+. In an elegant part of the study, metformin metabolites were tested for transport using solid-supported membrane (SSM) electrophysiology. The experiment involved reconstituting proteoliposomes with purified proteins to monitor charge movement across the liposome membrane. The Gdx-Clo homolog, the best characterized SMRGdx homolog, was found to transport only Gdm^+ and guanylate. However, the other three SMRGdx homologs tested also transported singly substituted biguanides, including the metformin degradation product methyl biguanide and the related antidiabetic drug buformin. On the other hand, metformin, a doubly substituted biguanide, exhibited currents barely above the detectable limit by SMRGdx proteins. These findings are consistent with prior observations that SMRGdx transports guanidinium ions with single hydrophobic substitutions, while doubly substituted guanidiniums are not.

Previous research studies have shown that both Gdx-Eco and EmrE have a well-coupled 2 H^+: 1 Gdm^+ stoichiometry. However, it has been suggested that the transport stoichiometry may vary among some transported substances for EmrE. To determine the coupling stoichiometry of Gdx-Clo and plasmid-associated Gdx-pAmi, Stockbridge et al. conducted experiments using the SSM setup. In these experiments, a potassium gradient and the potassium ionophore valinomycin were used to establish several membrane potentials, and pyranine, a pH-sensitive fluorescent dye, was used to monitor substrate-coupled proton movement. When the equilibrium reversal potential was established, no substrate movement was detected. The equilibrium potential detected, which was −60 mV in this case, is in agreement with a 2 H^+: 1 solute coupling stoichiometry.

Lucero et al. (2024) solved a crystal structure of Gdx-Clo in the presence of guanylurea to investigate if it occupies the same binding site as guanidinium in Gdx-Clo. The crystal structure of Gdx-Clo with guanylurea was solved up to 2.1 Å, which showed that guanylurea occupies the same binding pocket as Gdm^+ in Gdx-Clo. The Gdm^+ group is positioned between the central glutamates.

Conclusion

This research holds significant implications for the scientific community. It not only offers a comprehensive examination of the structurally characterized SMRGdx homolog Gdx-Clo, laying a solid groundwork for future mechanistic studies of this model transport protein, but also shines a spotlight on the potential role of SMRGdx transporters in the microbial management of metformin and its microbial metabolic byproducts. These findings reveal how native transport physiologies adapt to new selective pressures, underlining the relevance of the reported findings to microbiology and drug resistance.

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