Overexpression of the Multidrug Resistance Protein-4 Transporter in Patients Undergoing Coronary Artery Bypass Graft Surgery
A Cause of Aspirin Resistance?*

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Millions of people take aspirin every day for the prevention of atherothrombotic cardiovascular events. Aspirin inhibits thrombus formation by irreversibly inactivating the cyclo-oxygenase (COX)-1 enzyme in platelets, thereby blocking platelet production of thromboxane A₂, a potent vasoconstrictor and platelet agonist (1). Multiple studies have reported that in some individuals the effectiveness of aspirin for cardiovascular prevention is limited by “resistance” to its antiplatelet effects (2). Although the validity of aspirin resistance, as an entity, continues to be debated, observational studies consistently show that as many as one-third of individuals prescribed aspirin have less-than-expected inhibition of platelet function, which is independently associated with an increased risk of cardiovascular events (3–5).

So-called aspirin resistance can often be overcome simply by improving patient adherence or increasing the dose or frequency of aspirin treatment (6–9), but there are also unequivocal causes of aspirin resistance, such as drug–drug interactions in which nonsteroidal anti-inflammatory drugs prevent access of aspirin to platelet COX-1 (2). Multiple studies have reported that in some individuals the effectiveness of aspirin for cardiovascular prevention is limited by “resistance” to its antiplatelet effects (2). Although the validity of aspirin resistance, as an entity, continues to be debated, observational studies consistently show that as many as one-third of individuals prescribed aspirin have less-than-expected inhibition of platelet function, which is independently associated with an increased risk of cardiovascular events (3–5).

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In a second set of experiments, Mattiello et al. (12) confirmed the role of MRP4 in aspirin transport with platelets obtained from an in vitro cell culture system and megakaryocytes transfected with a small interfering ribonucleic acid that interferes with expression of MRP4. Compared with platelets obtained from untransfected megakaryocytes, platelets obtained from MRP4 small interfering ribonucleic acid–transfected megakaryocytes demonstrated increased cytosolic concentrations of aspirin and salicylic acid and reduced concentrations of thromboxane, findings that are consistent with the role of MRP4 in aspirin transport.

The third set of experiments involved the use of immunoblot, immunoelectron microscopy, and immunofluorescence studies to demonstrate that—compared with platelets collected from healthy volunteers—platelets collected 5 and 10 days after procedure from patients undergoing CABG surgery had increased MRP4 expression. The MRP4 expression was preferentially localized at the plasma membrane on day 5 compared with day 10 after surgery.

Multidrug resistance proteins are adenosine triphosphate–dependent efflux pumps localized in cellular plasma membranes that have broad substrate specificity for transport of endogenous anionic substances and play a key role in hepatobiliary, renal, and intestinal drug elimination (13). One of 9 distinct multidrug resistance proteins, MRP4 is expressed in the membrane of platelet dense granules in platelets and, to a lesser extent, in the plasma membrane of platelets (14) as well as in red cells, the kidney, prostate, and liver (15). Drug substrates for MRP4 include nucleoside analogues used in the treatment of human immunodeficiency virus as well as antibiotic and antineoplastic agents. Inhibition of MRP4 by dipyridamole could explain the enhanced thromboxane suppression that has previously been reported when dipyridamole is added to aspirin (16). Genes encoding MRPs are highly polymorphic (17), and variants leading to lack of functional protein account for hereditary conditions such as Dubin–Johnson syndrome (MRP2 deficiency) and pseudoxanthoma elasticum (MRP6 deficiency). To date, however, no disease has been directly linked to altered MRP4 activity.

Mattiello et al. (12) are the first to demonstrate that aspirin is a substrate for MRP4, and their data provide a plausible mechanism for post–CABG aspirin resistance and the attendant increase in risk of cardiovascular events (18). As with so many new discoveries, these findings raise important new questions. What is the mechanism of upregulation of MRP4 after CABG surgery? Does MRP4 upregulation also occur in other high platelet production states (e.g., acute coronary syndromes, bleeding, sepsis) and contribute to the aspirin resistance that has been reported in patients with acute coronary syndrome and undergoing percutaneous coronary intervention and in patients with diabetes? From a therapeutic perspective, the most important question is whether MRP4–induced aspirin resistance can be overcome by more frequent doses of aspirin or by the use of an MRP4 inhibitor such as dipyridamole and, if so, whether this leads to an improvement in clinical outcomes? If the favorable effect of adding dipyridamole to aspirin in patients with previous ischemic stroke of arterial origin is mediated by inhibition of MRP4–mediated transport (19), are there other, more potent inhibitors of MRP4–mediated transport—such as cilostazol (20)—that might further enhance the effectiveness of aspirin?

Further studies are clearly required to validate and extend the provocative findings by Mattiello et al. (12) concerning the role of MRP4 in post–CABG aspirin resistance. Molecular studies should ideally be performed in parallel with functional and biochemical studies to examine the potential role of MRP4 gene polymorphisms that alter the expression and function of the transporter as determinants of the occurrence and severity of aspirin resistance (17). Confirmation of the causal role of MRP4 in aspirin resistance could give rise to new more effective antiplatelet strategies for patients undergoing CABG surgery, thereby potentially reducing the very high rates of graft failure and major
cardiovascular events in these patients. If MRP4 is also shown to contribute to aspirin resistance in other clinical settings, the future of aspirin alone as the foundation antiplatelet therapy in high-risk populations (21) could be challenged by alternative treatments that are less vulnerable to this mechanism. In the meantime, however, aspirin has been proven to be effective for the prevention of graft failure (22,23) and is likely also effective for the prevention of major cardiovascular events in patients undergoing CABC surgery (24) and thus should continue to be used according to guideline recommendations (25,26).

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