Once a PON-1 an enzyme

By Tracey Baas, Senior Editor

In March 2010, the FDA added a boxed warning to the blockbuster drug Plavix clopidogrel, an antiplatelet agent, noting that carriers of variants of the liver enzyme cytochrome P450 2C19—present in up to 14% of the population—could experience diminished drug efficacy due to lower levels of drug metabolism. However, these variants only account for about a tenth of all cases in which clopidogrel exhibits low efficacy—the rest stem from an as of yet unknown mechanism.¹

Now, a European team has put a new enzyme on the map—paraoxonase-1 (PON-1)—and thinks genotyping the esterase could be a robust marker for identifying patients who are poor Plavix metabolizers.² According to researchers polled by SciBX, the results will have to be confirmed in independent, large-scale studies before the enzyme can be adopted as a clinical marker.

Plavix, a thienopyridine prodrug from **Bristol-Myers Squibb Co.** and **sanofi-aventis Group**, is converted *in vivo* by cytochrome P450 (CYP) enzymes, including cytochrome P450 2C19 (CYP2C19), to its bioactive thiol metabolite. In turn, this metabolite inhibits a key mediator of platelet aggregation called ADP P2RY12 (adenosine diphosphate purinergic receptor P2Y G protein-coupled 12).

Plavix posted more than \$9 billion in sales in 2010.

A team led by Dirk Taubert, senior lecturer of pharmacology at the **University Hospital of Cologne**, began to suspect that another factor aside from CYP2C19 had to be responsible for the diminished drug metabolism following some intriguing findings by his group: *In vitro*, combinations of clopidogrel with microsomes from CYP2C19-expressing human embryonic kidney 293 (HEK293) cells resulted in no conversion to the active thiol.³

“We knew there had to be another enzyme contributing to the activation of clopidogrel,” said Taubert, who is senior author of a *Nature Medicine* paper describing the findings.

In order to find the mystery enzyme, his group generated stable HEK293 cell lines that expressed enzymes involved in drug metabolism, such as CYP enzymes and esterases. Preparations from these cell lines showed that PON-1 played a critical role in activating Plavix (see **Figure 1**, “Activation of clopidogrel”). PON-1 is an esterase that is synthesized in the liver and is associated with high-density lipoproteins in blood.

The researchers determined that a polymorphism in the gene for the PON-1 enzyme resulted in more efficient clopidogrel activation. The polymorphism results in the substitution of the amino acid glu-

Figure 1. Activation of clopidogrel. The first metabolic step [a] takes place in the small intestine and liver, where clopidogrel is oxidized by cytochrome P450 (CYP) enzymes to 2-oxo clopidogrel, which is still not active. The second metabolic step [b] takes place mainly in the blood, where the 2-oxo clopidogrel intermediate is converted to the active thiol metabolite, which inhibits ADP P2RY12 (adenosine diphosphate purinergic receptor P2Y G protein-coupled 12).

In *Nature Medicine*, Bouman *et al.* show that this second step [b] relies on paraoxonase-1 (PON-1) to mediate hydrolytic cleavage of the 2-oxo clopidogrel ring structure to a thiol and yield the fully active metabolite. They postulate that there is no place for CYP enzymes in the second metabolic step because the conversion is not associated with oxidation or reduction but rather involves a hydrolytic cleavage.

tamine (Q) at position 192 with arginine (R).

In a case-cohort study in patients with coronary artery disease who had stent procedures, individuals heterozygous or homozygous for the Q amino acid were more likely to have lower plasma amounts of active PON-1 and active clopidogrel metabolite than individuals homozygous for the R amino acid.

In addition, QR heterozygotes had more than 4-fold risk and QQ homozygotes had more than 12-fold risk of stent thrombosis compared with RR homozygotes. Results were confirmed in another independent prospective cohort.

“The strength of the study is that it has demonstrated a conclusive mechanistic and clinical link between the presence of a genetic polymorphism, suboptimal clopidogrel active metabolite generation using a pharmacokinetic measurement, decreased clopidogrel responsiveness using a pharmacodynamic measurement and adverse clinical outcomes,” said Taubert. “Such a study was completely missing.”

The devil is in the detail

Researchers contacted by *SciBX* were surprised by the PON-1 findings for two reasons. First, the results in the *Nature Medicine* paper are in contrast with two previous papers that showed clopidogrel was activated only by CYP enzymes.^{4,5} Second, PON-1 was absent in a previous genomewide association (GWA) study of Plavix’s impact on platelet aggregation.¹

Miho Kazui, a drug metabolism and pharmacokinetics researcher at **Daiichi Sankyo Co. Ltd.**, noted that Taubert’s group biologically synthesized the drug’s active metabolite from 2-oxo clopidogrel. “Typically, studies use a chemically synthesized active metabolite to measure the active metabolite of clopidogrel in plasma or *in vitro* assays,” said Kazui, who was a lead author on one of the previous papers investigating clopidogrel.

“We would be interested in conducting the *in vitro* experiments ourselves to see if PON-1 could really hydrolyze 2-oxo clopidogrel to the active thiol,” Kazui told *SciBX*.

Patrick Dansette and Daniel Mansuy, directors of research emeriti at the **Centre National de la Recherche Scientifique (CNRS)**, **University Paris Descartes**, also were concerned about the paper’s discordant results with previous findings. They wanted to understand why the *in vitro* results of the two previous papers had not been reproduced using Taubert’s HEK293 cytochrome P450 system or human liver microsomes and to see confirmation of the PON-1 results by other investigators.

Guillaume Pare added that “the fact that the only GWA study of clopidogrel efficiency with respect to ADP-stimulated platelet aggregation did not report the association of PON-1 with platelet response to clopidogrel remains puzzling. More research will definitely be needed to explain this discrepancy.”

Pare is an assistant professor of pathology and molecular medicine and the director of the genetic and molecular epidemiology laboratory at **McMaster University**.

Eric Topol, professor of translational genomics at **The Scripps Research Institute** and director of the **Scripps Translational Science Institute**, agreed. “The *PON-1* gene variant or its locus doesn’t even show up as being associated with clopidogrel pharmacogenomic response,” he said. Topol wanted to see the PON-1 findings “independently replicated by an outside group. This would certainly strengthen the story.”

Taubert countered that the GWA study “investigated a healthy population of related Amish individuals—a genetically closed population—and the results cannot simply be extrapolated to other

populations of unrelated individuals with coronary artery disease. Moreover, they measured platelet inhibition as a surrogate parameter for active metabolite formation, so the study would likely miss the association of platelet inhibition with the PON-1 locus.”

Decisions, decisions

If the findings about PON-1 are confirmed, the results could provide a much broader biomarker of Plavix’s activity than CYP2C19.

“These findings suggest that genotyping patients for their CYP2C19 status is not of value as far as their clinical outcome is concerned. Genotyping for their PON-1 status may be of more clinical value, but that should await confirmatory studies of Taubert’s findings,” said Neville Ford, founder of **Woodfield Clinical Consulting LLC**.

“The PON-1 polymorphisms appear to be much more powerful predictors of risk than CYP2C19,” noted Deepak Bhatt, associate professor of medicine at **Harvard Medical School**, chief of cardiology of the **VA Boston Healthcare System** and director of the integrated interventional cardiovascular program at **Brigham and Women’s Hospital**.

“It seems logical that testing for this polymorphism should help guide the choice of a patient’s antiplatelet therapy,” added Bhatt. “Before this can happen, though, the study needs to be replicated in a few larger, more diverse populations and, importantly, outcome studies need to be performed to prove that altering therapy based on PON-1 status in fact improves clinical outcomes. If all that happens, we may indeed achieve the goal of tailored antiplatelet therapy.”

Taubert agreed. “Drawing definite clinical conclusions based on the evidence of observational studies or small clinical investigations is always a source of uncertainty and bias. Thus, we would like to next use results from large prospective randomized controlled trials

investigating clinical endpoints with respect to the *PON-1 Q192R* gene polymorphism and PON-1 enzymatic plasma activity. This would allow us to assess the predictive effectiveness of the PON-1 diagnostics and the potency for individualizing antiplatelet therapy.”

Pierre Fontana, head of the laboratory of haemostasis in the Department of Angiology and Haemostasis at **Geneva University Hospitals**, said two key issues need to be explored before doing PON-1 activity profiling in the clinic.

“First, the biological test to assess PON-1 activity should be standardized, and a cutoff value under which a patient is at significant risk should be determined and evaluated in several prospective studies,” said Fontana. “Secondly, what do we do with patients with low PON-1 activity? A large-scale study evaluating the clinical impact of a strategy aiming to tailor antiplatelet therapy in those patients is needed. The last step of the bioactivation of prasugrel is very similar to that of clopidogrel, and the possibility that PON-1 is involved in the metabolism of prasugrel was just discussed by Christian Gachet” at a cardiology conference.

Fontana said that “other classes of ADP inhibitors that are not thienopyridines, such as ticagrelor, might be investigated for those with low PON-1 activity.”

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**—Guillaume Pare,
McMaster University**

Effient prasugrel is marketed by Daiichi for acute coronary syndrome. Brilinta ticagrelor from **AstraZeneca plc** is approved for acute coronary syndrome (ACS). Cangrelor from **The Medicines Co.** and AstraZeneca is in Phase III testing for ACS and Phase II/III trials for thrombosis.

Clopidogrel and prasugrel are both prodrugs that need to be metabolized to their active metabolites to irreversibly bind to ADP P2RY12, the key mediator of platelet aggregation. Ticagrelor and cangrelor require no further transformation to directly and reversibly bind to ADP P2RY12.

“Prasugrel’s metabolism should be checked with respect to the role of PON-1,” said Gachet, director of an **Institut National de la Santé et de la Recherche Médicale** (INSERM) unit at the **University of Strasbourg**.

The PON-1 work published by Taubert’s team is not patented and not available for licensing.

Baas, T. *SciBX* 4(4); doi:10.1038/scibx.2011.93

Published online Jan. 27, 2011

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