Terazosin reborn

By Lauren Martz, Staff Writer

In searching for compounds to treat stroke or sepsis, a Chinese team has found a new activity—and possibly a new indication—for an old drug. Terazosin, a generic α -blocker used in hypertension, inhibited apoptosis and extended survival in rodent models of stroke and sepsis by activating phosphoglycerate kinase 1.¹ Although the enzyme could be a viable target for new compounds, repurposing terazosin might be a lower-risk route to draw companies into an area with a reputation for little innovation.

There are in fact several compounds in development for stroke or sepsis, but researchers who spoke with SciBX noted that companies are often tentative about entering either field because the preclinical models are poorly predictive of results in humans.

“The problem is that we’ve cured a lot of rodents with stroke, but we haven’t cured a lot of patients,” said David Lowe, a board member at **Amarantus BioScience Holdings Inc.** “For this reason, the stroke space is often referred to as the graveyard of drug development in CNS.”

He added, “If someone can prove that the drug and this new mechanism are effective in a proof-of-concept trial in stroke patients, it should stimulate a lemming effect across the industry. As soon as something is successful, there will be a lot more companies buzzing around the stroke indication again.”

Amarantus is developing mesencephalic astrocyte-derived neurotrophic factor (MANF; ARP; ARMET), a human growth factor that mediates apoptosis, for indications including shock/trauma and various neurological diseases. It is in preclinical development.

Stroke is treated clinically with recombinant tissue plasminogen activator (tPA), which dissolves the underlying blood clot and restores blood flow to damaged brain tissue. Sepsis is treated with antibiotics, which target the bacterial cause of the inflammatory response. However, neither approach—and few, if any, of the compounds in development—addresses the apoptosis underlying the tissue toxicity and organ damage in either condition.

The **Genentech Inc.** unit of **Roche** markets the tPA Actilyse alteplase to treat stroke.

Francesca Bosetti, program director of the extramural research program at the **National Institute of Neurological Disorders and Stroke**, told SciBX, “tPA is the only approved drug for ischemic stroke, and it can only be used in a small percentage of patients. Side effects and a narrow treatment window of about four hours limit the number of patients eligible for the treatment.”

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She added, “There are no therapeutics to specifically treat the apoptosis or inflammation in the tissue resulting from stroke.”

Similarly, Xiaoyu Li said, in sepsis treatment “a large quantity of antibiotics is used to kill the bacteria, but it does not prevent organs from being damaged by the toxins from dead bacteria.” Li is the lead author on the study and an associate professor of bio-organic chemistry and chemical biology at **Peking University**.

To explore mechanisms underlying the apoptosis-induced damage in stroke and sepsis, Li teamed up with Lei Liu and colleagues at the university, in addition to researchers at the **National Institute of Biological Sciences**. Liu is an assistant professor in the School of Life Sciences at Peking University.

The team unearthed the new activity of terazosin inadvertently when performing a screen in *Drosophila* for genes altered by apoptosis.

Terazosin is approved to treat hypertension and benign prostatic hyperplasia (BPH), and it was developed by **Abbott Laboratories** in the 1980s to control blood pressure based on its activity as an antagonist of adrenergic receptor α_1 (ADRA1).

Apoptotic surprise

First, the group designed a model of lethal apoptosis in *Drosophila* and used it to screen for compounds that protect against cell death using a global transcription analysis for genes altered by apoptosis. They then matched the identified genes to 25 compounds using the **Broad Institute of MIT and Harvard’s** Connectivity Map for screening. Terazosin emerged as the only compound that significantly protected the flies from apoptosis and increased survival.

Because terazosin was not known to be associated with apoptosis, the next steps were to confirm the results in mammalian cells and determine if the drug was acting by its recognized adrenergic pathway.

Terazosin protected mouse macrophages from apoptotic cell death, showing that its effect was not limited to *Drosophila*. In addition, a terazosin analog with 1,000-fold lower affinity for ADRA1 was also able to block apoptosis. That suggested the drug was most likely acting via a different target.

To identify the target, the group used an affinity-based pull-down assay that would reveal proteins physically associated with the molecule and identified phosphoglycerate kinase 1 (PGK1) as a binding partner.

Low doses of terazosin activated PGK1 in *in vitro* activity assays, whereas suppressing PGK1 in macrophages with a specific shRNA blocked the drug’s antiapoptotic effects. Together, the results suggested low doses of the drug might protect against apoptotic cell death.

Liu, Li and colleagues also found that PGK1 interacted with heat shock protein 90 (Hsp90), a chaperone protein with antiapoptotic activity. In addition, they showed that inhibiting Hsp90’s ATPase blocked the effects of terazosin on macrophages. That suggested terazosin’s antiapoptotic activity could be caused by activation of PGK1 and Hsp90.

Finally, to test whether the antiapoptotic activity translated to a therapeutic effect, the team tested terazosin in rodent models of both stroke and sepsis.

In a rat model of stroke, i.p. administration of a low dose of terazosin after the injury decreased infarct brain volume compared with vehicle treatment.

In a mouse model of lipopolysaccharide (LPS)-induced sepsis, i.p. administration of terazosin 1.5 hours after disease induction increased survival compared with vehicle treatment. In a cecal ligation and puncture model of the disease, subcutaneous delivery of a low dose of terazosin up to six hours after sepsis onset increased survival compared with vehicle delivery. In addition, a lentivirus expressing *Pgk1* also increased survival in the cecal ligation model compared with a control virus.

The data were published in *Nature Chemical Biology*.

Stroke selection

Liu told *SciBX* that the team plans to pursue stroke as a new indication for terazosin, but more preclinical testing will be required before the drug can be tested in stroke patients. He said that one factor for choosing that indication was the high incidence of stroke in China.

Although the data are very early, Lowe thinks that repurposing terazosin might offer a way in for companies that want to make headway in stroke. “The poor translation from animal models to human patients increases risks to drug developers and investors, but repurposing a therapeutic with a favorable safety profile that has already been dosed in thousands of patients may shift the risk perception for companies,” he said.

However, finding a predictive preclinical pathway might still be the main sticking point.

Paul Lapchak, director of translational research in the departments of neurology and neurosurgery at **Cedars-Sinai Medical Center**, suggested the team study the effects of terazosin in larger species such as rabbits and primates. “Currently, the rabbit embolic stroke model is the only model that has been used to gain FDA approval of a drug—tPA,” he said.

Bosetti added, “These researchers used a good model of stroke. The permanent middle cerebral artery occlusion model is the most severe, and reperfusion will not occur. But the truth is that no animal model fully recapitulates stroke in humans due to the range of comorbidities present in patients.”

To address the failure of preclinical models, the National Institute of Neurological Disorders and Stroke was involved in the release of updated Stroke Treatment Academic Industry Roundtable preclinical recommendations in 2009.² The goal of the recommendations was to promote preclinical research guidelines that resemble clinical trial standards as closely as possible.

Among the recommendations is a requirement for calculations to justify the number of animals studied in preclinical experiments, said Bosetti. She noted that the Peking researchers used a very small sample size—only five animals per group—which would not meet the requirement. In addition, she wanted to see blinding and results from sham-operated control rats, which the group did not report.

Bosetti also noted that the experiment tested the effects of administering terazosin prior to stroke in the rats. “The large majority

of stroke patients don’t know when they are going to have a stroke,” she said. “There may be some application for a pretreatment, such as in patients having surgery with a risk of stroke, but the clinical application of a pretreatment would be very limited.”

Although the activity of terazosin suggests it could be repurposed, the researchers also think that designing a new therapeutic candidate might produce a more potent and specific agent. Lowe noted that a new compound would have greater potential for patent protection than a repurposed terazosin.

“PGK1 may be used as a target to design or screen for more potent and specific small molecule probes,” Li told *SciBX*. Because PGK1 is a kinase, it should be highly druggable, and discovery of a new ligand should be feasible, he added.

Li said that his team plans to design a new type of molecule based on a PGK1-activating mechanism different from that of terazosin. He said that an allosteric activator, for example, should avoid the differential effects on PGK1 at high and low doses that were seen with terazosin.

The team also plans to conduct additional preclinical studies for both stroke and sepsis prior to clinical testing, he said.

The authors raised one concern with activating an antiapoptotic pathway: the risk for cancer. They noted that PGK1 activation has been linked to multidrug resistance in animal models of cancer. “However, sepsis or stroke treatments usually only last for a couple of weeks, and therefore the risk for the patient to develop cancer is extremely low,” said Li.

Linx Pharmaceutical Co., which has a technology transfer agreement with Peking University, has filed patent applications in China and the U.S. for the use of terazosin and PGK1 activators in stroke and sepsis. Although the company has rights to the IP, Linx does not plan to pursue clinical development of a stroke or sepsis therapeutic based on this research, and the IP is available for licensing.

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