A chemical killer unmasked

Analytical techniques identify a contaminant in heparin.

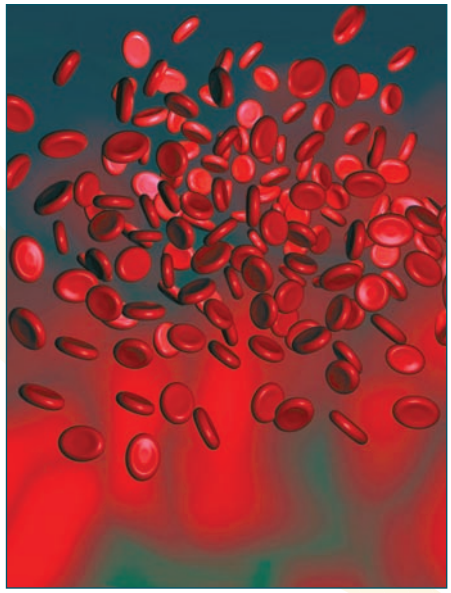
On February 29 of this year, Ram Sasisekharan at the Massachusetts Institute of Technology (MIT) received a call from Moheb Nasr of the Center for Drug Evaluation and Research, part of the U.S. Food and Drug Administration (FDA). Nasr was trying to sort out the nature of a contaminant his team had just discovered in heparin, an anticoagulant commonly administered to dialysis and cardiac patients. Using CE and heparinases (enzymes that degrade heparin), Nasr's group could plainly see that several batches of heparin were contaminated. He wanted Sasisekharan to figure out what that contaminant was and where it came from.

The story had been building for weeks. On February 1, the U.S. Centers for Disease Control and Prevention announced an outbreak of "acute allergic-type reactions" among individuals receiving blood dialysis (*MMWR* **2008**, *57*, 124–125). A total of 53 hemodialysis patients experienced 65 "confirmed or probable" cases from November 19, 2007, to January 21, 2008, at 19 dialysis centers in 12 states; 36 more "possible" cases were under investigation.

Of the 65 episodes, 61 occurred within minutes of injection with heparin from Baxter Healthcare. On January 17, Baxter issued a voluntary recall of nine lots of heparin, all of which were manufactured at the same plant in China. In late February, FDA inspected that facility and found a host of violations, which they enumerated in a February 28 report.

Identifying the contaminant

Heparin is a pig-derived, complex polysaccharide—"a soup," as Sasisekharan puts it, of molecules with different chain lengths and distributions. Fundamentally, heparin is a polymer of disaccharides, each unit of which contains uronic acid and glucosamine. In practice, the product is highly heterogeneous; different stereoisomers, sugars, and sulfation patterns come together to yield 32 possible disaccharide units.



Heparin is commonly administered to dialysis and cardiac patients to prevent blood clots.

After Nasr's call, and realizing "the enormity of the public-health issue surrounding these events, it became important to me to throw everything we had at it," Sasisekharan says. "Time was of the essence because people were dying." Indeed, from November 2007 to February 2008, FDA had received reports of 62 heparin-related deaths associated with allergic or hypotensive symptoms, compared with 3 in all of 2006.

Sasisekharan's strategy was to mobilize a multi-institutional, multidisciplinary team of researchers who could do the work as quickly as possible and also provide independent validation of one another's data. In addition to his own group, he requested help from researchers at the Giuliana Ronzoni Institute for Chemical and Biochemical Research (Italy) who had worked with heparin for decades. He subsequently solicited aid from Momenta Pharmaceuticals, where scientists had the high-throughput capability to run several analyses on all the samples in parallel.

"We did not want to do just one sample," Sasisekharan explains. "We comprehensively analyzed all the lots FDA provided, so we knew what was happening in all the lots in the clinic."

FDA sent the team 10 samples—6 contaminated lots of heparin and 4 controls. Sasisekharan coded all the materials, so the researchers would not know which samples were contaminated. "We wanted the data to tell us what they were," he says.

Simple ^{1}H and ^{13}C 1D NMR provided the first clues to the contaminant's identity. A signal at 2.16 ppm in the ^{1}H-NMR spectrum revealed the presence of an acetyl group distinct from that of heparin (2.04 ppm) or dermatan sulfate (2.08 ppm), a common contaminant. Signals in the ^{13}C spectrum at 25.6 ppm and 53.5 ppm revealed the presence of a novel O-substituted *N*-acetylgalactosamine, whereas signals in the range 103–105 ppm suggested a β-glycosidic linkage between monosaccharides.

But ^{1}H–^{13}C 2D spectra provided the first clue that something truly strange was occurring, says Sasisekharan: both sugars in the disaccharide unit contained two sulfate groups—a highly unusual configuration never previously seen in nature as a polymer.

The teams attempted to purify the contaminant by using three parallel approaches: one group enriched for the contaminant by degrading the heparin, another used an alcohol-based selective precipitation, and the third tried chromatography based on charge differences. These purified samples were then subjected to an alphabet soup of NMR protocols: HSQC, COSY, TOCSY, ROSEY, and HMBC. This combination of techniques "gives you literally a map of each atom in the monosaccharide and how that links to the other monosac-

charide," Sasisekharan explains. "It essentially gives you the complete chemical picture of the disaccharide repeat unit of this material."

In aggregate, the analyses pointed to a heparin-like compound composed of 2,3-*O*-sulfoglucuronic acid and 4,6-*O*-sulfo-*N*-acetylgalactosamine, with a β-1,3-linkage between the two sugars in the disaccharide and a β-1,4-linkage between adjacent disaccharide units. The team named the contaminant "oversulfated chondroitin sulfate" (OSCS), and to confirm that they were right, they synthesized OSCS by using standard chemistry and compared its behavior with that of their test samples via NMR. The two were essentially indistinguishable.

"OSCS is not a natural product," Sasisekharan says. "I don't know where it came from—I wish we did. But the only way to explain scientifically the large amounts of it was that it was made synthetically and introduced" into the samples.

All of this activity occurred over a period of ~2 weeks, Sasisekharan says. By March 5, 1 week after Nasr's call, and with the preliminary NMR data in hand, FDA announced that the contaminant was a heparin-like compound. The next day, the agency released a protocol for using NMR to screen heparin batches for the contaminant; since then, the number of reported cases has dropped off sharply. On March 16, the final data were presented to FDA, and 2 days later the agency announced that the contaminant was OSCS. Sasisekharan invited Rensselaer Polytechnic Institute's Robert Linhardt, who was collaborating with scientists at Baxter Healthcare, to join the team so that they could publish their results together; the paper detailing these findings was recently released (*Nat. Biotechnol.* **2008**, DOI 10.1038/nbt1407).

Proving causation

But that's only half the story. The question was: did OSCS actually cause the adverse reactions first noted in late November 2007? It looked like it might have. Scattered throughout the medical literature were reports linking sulfated heparin-like molecules with adverse effects. Indeed, Arteparon, an antiarthritis medication marketed in Germany that was structurally quite similar to the contaminant, was pulled from the market shortly after its release. The drug had been linked to both allergic reactions and death.

Sasisekharan assembled a new team of researchers from Harvard, MIT, Virginia Polytechnic Institute and State University (Virginia Tech), and FDA. First, in experiments with human plasma, the team demonstrated that both contaminated heparin and synthetic OSCS could activate two different biological pathways, the contact system and the complement system. These systems together induce an allergic reaction and a drop in blood pressure, two of the hallmark symptoms in affected patients.

Next, the team tested its samples with blood plasma from a battery of animal models, beginning with small animals such as mice, rats, and rabbits. None of these animals responded to OSCS as humans did. Undeterred, Sasisekharan called the pig-research facility at Virginia Tech, asking them to fast-track an experiment for him. The data came back positive 2 days later: when OSCS was administered to pigs, they exhibited the same symptoms found in humans (*N. Engl. J. Med.* **2008**, DOI 10.1056/NEJMoa0803200).

"That is a coincidence," says Sasisekharan. "We isolated heparin from pigs, and it was pigs that came to the rescue. And it also explains why initially the contaminant seemed to have no observed biological effect—because in mice and rats, it has none."

The papers detailing these chemical and biological findings were published April 23—just 5 months since the first report of an adverse reaction to heparin and a mere 8 weeks after FDA's call to Sasisekharan. Two days earlier, on April 21, Chinese authorities had held a press conference stating that OSCS was not the cause of the adverse reactions; FDA's response was that it had data suggesting OSCS was. With the release of these two papers, the world can now judge for itself. ◥

—Jeffrey M. Perkel