

DECEMBER 07, 2000

Antiviral Drug Works by Causing "Genetic Meltdown"

The antiviral drug ribavirin works by creating such extreme mutation rates in viruses that it drives them into "genetic meltdown," according to researchers. Uncovering the mechanism by which ribavirin disables viruses offers researchers information that can be used to design more effective antiviral drugs.

Ribavirin's mutagenic mechanism was reported in the December 2000 issue of *Nature Medicine* by a research team that included lead author Shane Crotty, who is a Howard Hughes Medical Institute predoctoral fellow at the University of California, San Francisco (UCSF), Craig Cameron at Pennsylvania State University and scientists from Schering-Plough Research Institute.

"Ribavirin is one of the few drugs that shows activity against a variety of RNA viruses," said Crotty. These viruses use RNA rather than DNA as their genetic material. Ribavirin is used to treat severe cases of respiratory syncytial viruses in children, lassa fever and hepatitis C infections, where it is used in combination with interferon-alpha.

"Unfortunately, only about thirty to forty percent of hepatitis C patients respond to the treatment," said Crotty. "Once people are chronically infected, they don't appear to get rid of the virus and there is a high incidence of liver failure in these patients. Nevertheless, ribavirin plus interferon-alpha is the closest we've gotten to a cure right now," he said.

Thus, said Crotty, there is a great deal of interest among pharmaceutical companies in understanding ribavirin's mechanism of action. Researchers have suggested several theories about how ribavirin works, including speculation that ribavirin blocks viral protein synthesis or that it halts the transcription of RNA needed for viral replication.

To determine whether ribavirin does, indeed, interfere with RNA transcription, Cameron and his colleagues used a sensitive analytical assay to see whether ribavirin is incorporated into viral RNA. Those experiments revealed that a form of the drug called ribavirin triphosphate (RTP) is incorporated into viral RNA. RTP resembles the nucleotide building blocks that normally make up RNA.

"This finding was reasonable to expect because other antiviral drugs that resemble nucleotides are also incorporated into the viral genetic material, acting as chain terminators to block further RNA elongation," said Crotty. "However, what was confusing was that Cameron's experiments also showed that ribavirin was not a chain terminator because RNA translation continued," he said.

To understand how ribavirin affects viruses *in vivo*, Crotty, working in the laboratory of Raul Andino at UCSF, tested the drug's effect on live poliovirus using an assay he had developed to determine the level of mutation in poliovirus. Crotty's assay measured how frequently poliovirus in culture mutates to develop resistance to the molecule guanidine, both in the absence and in the presence of ribavirin. Crotty's measurements revealed that ribavirin increased the mutation rate of the poliovirus cultures by a large amount.

"Normally, a high rate of mutation is a survival trait used by viruses such as HIV, influenza and poliovirus to escape the immune system or to develop resistance to antiviral drugs," explained Crotty. "Importantly, data from other researchers studying poliovirus have indicated that the virus is mutating as much as it possibly can, and if it mutates any more, it starts dying. Its genetic information is degraded so much that it doesn't make sense.

"My experiments convinced me that ribavirin is pushing poliovirus into what's called an 'error catastrophe'—in other words, a genetic meltdown," said Crotty.

Evidence that ribavirin pushes viruses to mutate suggests that the apparent failure of clinical trials of ribavirin used alone against hepatitis C virus might not have been real failures, said Crotty. "Nobody knows how to grow hepatitis C in culture, so there is no assay to determine how much viable virus is left after treatment with ribavirin," said Crotty. "So, to indicate clinical effectiveness, those studies measured the blood levels of viral RNA. My data suggest that while viral RNA is present in the blood, it might be mutated to the point where it is useless."

Of broader importance, said Crotty, is that this discovery offers hope that pharmaceutical companies may be able to develop improved versions of ribavirin or completely new nucleotide mimics that can also be incorporated into the viral RNA to induce lethal genetic meltdown in RNA viruses.