THE ANGEL’S WING MYSTERY

An UNSTABLE AMINO ACID may have something to do with deadly mushroom poisoning

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WITH STALKLESS WHITE FANS spreading like scattered petals on decaying wood, angel’s wing mushrooms must appear heavenly to mushroom hunters. But in 2004, the ethereal-looking fungus was linked to 17 deaths in Japan from acute encephalopathy, a type of degenerative neurological condition characterized by lesions in the brain.

Japan’s Ministry of Health, Labour & Welfare traced the source of the poisoning to the angel’s wing mushroom—also known as Pleurocybella porrigens—but the cause of the encephalopathy has yet to be identified. Vitamin D analogs, fatty acids, and saccharides have all been fingered in chemical investigations. Recently two groups of chemists at different schools in the southern city of Shizuoka, working in collaboration with and spearheaded by Shizuoka University’s Hirokazu Kawagishi and University of Shizuoka’s Toshiyuki Kan, reported evidence that an unstable aziridine amino acid may be the chemical culprit (Angew. Chem. Int. Ed., DOI: 10.1002/anie.201004540).

The angel’s wing poisoning is unusual for several reasons. “It is noteworthy that there has only been one large poisoning event from P. porrigens—the 2004 cases in Japan,” says Michael W. Beug, a retired environmental chemistry professor and mycologist at Evergreen State College in Olympia, Wash. Even with all deadly mushroom species combined, there is, on average, about one death per year in all of North America, Beug notes. Since the 2004 outbreak, there has been only one other report of angel’s wing poisoning. It occurred in 2009 in Japan.

This is the first time that a mycotoxin has been associated with encephalopathy, Beug says. And the time from consumption to poisoning was unusually long for a mycotoxin, taking anywhere from 13 to 29 days. Another mystery: Most of the poisoning victims were undergoing dialysis treatment for kidney failure prior to consuming the angel’s wing.

“In Japan, especially in the northern area in the country, this mushroom is very popular,” says Kawagishi, who notes that the mushroom is usually boiled in water for a minute or so before it is consumed. It is very delicious, he adds. “To tell the truth, I had eaten this mushroom every year before the food-poisoning incident,” Kawagishi says. Last year, while studying compounds from the angel’s wing mushroom, Kawagishi’s group identified a group of six new cytotoxic amino acids (Tetrahedron, DOI: 10.1016/j.tet.2009.11.044). They all feature a β-hydroxyvaline unit attached to endogenous molecules, such as alcohols and sugars. This finding prompted the researchers to speculate that these amino acids must have come from a common precursor: an aziridine ring with a carboxylic acid on one end and two methyl groups on the other.

The researchers suspected that a nucleophilic attack of such a compound by endogenous molecules could result in ring opening of the aziridine. But no aziridine amino acid had ever been isolated from the angel’s wing before, indicating that if the compound did exist, it is very labile.

Setting out to confirm the existence of the putative aziridine amino acid, which they dubbed pleurocybelleziridine, Kawagishi and Kan’s team esterified extracts from the mushroom, hoping to create both the methyl and diphenylmethyl esters of the compound. These derivatives, they reasoned, would be less reactive than the carboxylic esters of the compound. They were right. The chemists isolated both esters of pleurocybelleziridine from the esterified mushroom extracts. And the compounds were identical to synthetic versions the group had prepared, confirming the presence of the aziridine amino acid.

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They also found that the amount of pleurocybellastrinidine in the mushroom is strikingly high—accounting for about 0.025% of the total weight.

**JAPAN’S WEATHER**  In the autumn of 2004 was ideal for the angel’s wing fungi. Kegy says, “Early typhoons led to perfect conditions for the mushroom, so it started fruiting about a month earlier than usual. It reached three to four times its normal size.” Because of the abundance of angel’s wing, its consumption was up, and that could account for the poisoning outbreak.

“It is possible that under the unusual conditions of 2004, unusually large concentrations of pleurocybellastrinidine could have been present,” Kegy adds. “In examination of other toxic natural products in mushrooms, I have found wide variations in the concentration from one collection to another collection. Even when grown under controlled conditions, I have observed up to 10-fold variations in concentration from one strain to another and even from mushrooms of the same strain collected at various stages in the fruiting cycle.”

The brain tissue of the poisoning victims showed symptoms characteristic of eroded myelin, the protective barrier that surrounds critical brain cells. To see whether pleurocybellastrinidine could damage the myelin sheath in brain cells, Kawagishi and Kan’s team examined the effect of both the aziridine amino acid and its corresponding methyl ester on rat oligodendrocyte cells. They found that pleurocybellastrinidine was indeed toxic to the cells. But they observed no such toxicity in cells treated with the corresponding methyl ester or with the previously identified β-hydroxyvaline units attached to endogenous molecules. From this, Kawagishi and Kan concluded that both the carboxylic acid and the aziridine are crucial for cytotoxicity.

Kegy believes that Kawagishi and Kan’s explanation that pleurocybellastrinidine is the cause of the angel’s wing’s toxicity is plausible. “The nagging question is whether or not the chemical is stable enough to have made it to the brain of the affected individuals,” he adds. “We may never know for sure what caused the 17 deaths in 2004. However, I think that we now have a promising candidate.”

For now, Kawagishi and Kan are working with a multidisciplinary team to figure out the mechanism of the poisoning and why it seemed to affect only those with advanced kidney disease.