A conceptual illustration from Beiersdorf depicts how active compounds (blue and yellow spheres) might work to limit melanin production and prevent the formation of AGEs, within the skin.

THE TIRELESS QUEST TO ACHIEVE HEALTHIER SKIN

Human enzymes and proteins point the way to POTENT COMPOUNDS THAT PREVENT DETRIMENTAL REACTIONS IN THE SKIN triggered by sunlight and sugar.

The gap seemed insurmountable. Compound after compound tried by Ludger Kolbe, and his team at skincare company Beiersdorf in Hamburg, Germany, would succeed in petri dish tests, only to fail when they were finally tested on human skin.

The cause of their struggles was fairly obvious — the researchers, along with everyone else in the skincare industry, had been testing their compounds against an enzyme that was extracted from mushrooms.

Of course, there are some differences between mushrooms and humans — and one is in the molecular structure of the enzyme tyrosinase," says Kolbe, the chief scientist for photobiology at Beiersdorf. Tyrosinase is found in melanocytes, which are specialized skin cells that produce the ultraviolet absorbing pigment, melanin.

In the presence of sunlight, tyrosinase stimulates melanin production.

For decades, researchers in the skincare industry looking for solutions to tyrosinase hyperactivity have tested their compounds against the mushroom tyrosinase — the most widely studied tyrosinase — because it is much easier to obtain than human tyrosinase.

But by figuring out how to synthesize and work with human tyrosinase[1], Kolbe and his team at Beiersdorf have identified potent compounds that can counter tyrosinase hyperactivity.

OVERACTIVE IN SPOTS

Babies and children usually start out with an even distribution of the pigment, melanin. But as we get older, our skin cells begin producing different amounts of tyrosinase, in turn causing some cells to generate more melanin than others, explains Kolbe. This irregular pigmentation worsens with increased exposure to sunlight. In severe cases, hyperpigmented spots form large patches, a condition called melasma. This most commonly affects women of Asian and Latin descent who are of reproductive age[2].

"People consider it as a sign of ageing when they get irregular pigmentation like spots," says Kolbe, but the condition is not inevitable.

In his quest to inhibit tyrosinase where it is being overproduced, the initial challenge was to make enough human tyrosinase for testing. Unlike mushroom tyrosinase, extracting a sufficient amount of human tyrosinase from its source would require "square metres of human skin", which is impossible, Kolbe says.

Also, human tyrosinase is difficult to extract as it's bound to the membrane in skin cells — unlike mushroom tyrosinase, which is a free-floating protein and easy to isolate.

Working with collaborators at Marburg University in Germany, Kolbe's team first figured out how to modify the genetic code of the human tyrosinase, so that it could be produced as a free-floating protein. Then, they inserted this code into human kidney cells, coaxing them to become tiny factories of human tyrosinase.

With enough human tyrosinase for their experiments, Kolbe's team screened 50,000 compounds against the enzyme to see which one might inhibit its activity. Of these, a compound called amino-thiazolyl resorcinol showed the most promise. From there, the researchers spent a few more years synthesizing some 700 modified versions of amino-thiazolyl resorcinol and testing those.

630 ATTEMPTS

"Almost every single position in the molecule was changed," Kolbe recalls. "Most of the time, we made the molecule worse," he says. But the 630th molecule that they prepared turned out to be a winner.

Called isobutylamido thiazolyl resorcinol, in *in vitro* tests, it lowered the activity of human tyrosinase between 20 to 4,000 times more effectively than other known tyrosinase inhibitors[1].

SUN EXPOSURE ISN'T THE ONLY THREAT TO SKIN, EXCESS SUGAR ALSO POSES A RISK.

To further understand why the compound worked so well, Kolbe and his team used computational methods to model the structure of human tyrosinase. They found that the novel compound fits perfectly into the active site of human tyrosinase. This is where the chemical reaction of melanin production takes place, thereby effectively inhibiting the reaction, explains Kolbe. "It's like a cork in a bottle that's stuck. Nothing can get in, nothing can get out."

ROGUE SUGARS

Sun exposure isn't the only threat to skin, excess sugar and even normal cell metabolism over time can set off a chemical reaction called glycation that causes skin to deteriorate.

"It is a reaction between sugar and the proteins of your skin," explains Vasilisa Trotsenko, head of science communication at Beiersdorf. Simple sugars such as glucose or fructose that circulate in our bloodstream can react with the proteins in our body, including those found in our skin.

The glycation process and its final products, known as Advanced Glycation End-products (AGEs) will influence all layers of the skin, she adds. AGEs can cause inflammation and produce harmful reactive oxygen species. The glycation reactions also cause skin proteins to stiffen irreversibly, potentially leading to wrinkles, loss of elasticity and brightness, and changes to complexion.

▲ Kolbe and his team at Beiersdorf have discovered how to lower the activity of human tyrosinase in laboratory experiments.

SUGAR SCAVENGERS

To combat glycation, Julia Weise, head of the biological testing laboratory, and her team at Beiersdorf, set out to identify molecules that could prevent skin proteins from glycation.

They screened some 1,700 compounds for their ability to inhibit the formation of AGEs in protein solutions. In preliminary *in vitro* experiments, a candidate molecule that effectively and dose-dependently prevented sugar from reacting with the proteins to form AGEs, was the amino acid derivative N-acetyl-L-hydroxyproline (NAHP)[3,4].

Key to the assessment of NAHP's performance was a 3-dimensional model of the human skin, adapted by Weise and her team. They obtained fibroblasts, a type of skin cells, from human donors and incubated them in the lab with collagen to create a jelly-like hydrogel filled with skin cells. The ability of this hydrogel to contract and spring back served as a potential indicator of the extent of glycation after exposure to glucose.

To further validate their results, the researchers also used skin samples that were stressed *ex vivo* with sugar to induce glycation. The AGEs formed exhibit a green fluorescence when exposed to ultraviolet light, explains Trotsenko, which the scientists used to measure their levels in skin in the lab.

Certainly, moderating sunlight exposure and sugar consumption could help to prevent hyperpigmentation or glycation in our skin over time, says Trotsenko. But it is impossible to always avoid sunlight and sugar in life, says Kolbe. "We do not want to hide in the basement all day and only come out at night," he says, "So we need to have some more sophisticated ways to protect our skin." ■

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