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Role of IDDRC Network in Recent Developments in the Treatment of Urea Cycle Defects

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Part 1: An Introduction to Ammonia and the Urea Cycle

Ammonia is a nitrogen-containing substance that is generated from the breakdown of proteins, and is very toxic to our bodies. An adult who eats 70-100 grams of protein each day will generate 10-20 grams of ammonia nitrogen. Ammonia is extremely toxic to the brain, and an elevation of blood ammonia (“hyperammonemia”) can cause confusion, vomiting, severe headaches, convulsions, brain swelling and even coma. The developing brain of a human infant is especially vulnerable to ammonia accumulation. Infants with hyperammonemia often suffer irreparable brain damage, including severe learning disability, epilepsy and serious limitations of speech and motor function. Therefore, it is very important to remove ammonia from the body to avoid its harmful effects.

The urea cycle is a series of chemical reactions within cells that prevents hyperammonemia by converting ammonia into non-toxic urea, which is then excreted into the urine and discarded from the body. Virtually all urea production through the urea cycle occurs in the liver. Inherited defects in the urea cycle usually are caused by mutations in genes that code for enzymes within the urea cycle. The estimated incidence of urea cycle defects is about 1:35,000 births, or about 113 cases annually in the United States. The Urea Cycle Disease Consortium (UCDC), an initiative supported by the National Center for Advancing Translational Science of the NIH, estimates that more than 800 people in the US have a urea cycle defect with at least 1,000 additional people in the European Union.

Part 2: Treatment – Minimizing Ammonia Production from Protein

Ammonia is formed mainly from the breakdown of proteins, both dietary proteins and proteins that are part of the structure and function of organs such as muscle, liver and brain. Limiting dietary protein intake is an important aspect of treating urea cycle defects, but this approach is challenging in a rapidly growing newborn with a relatively high minimal daily protein requirement that is above the threshold for developing hyperammonemia.

Another treatment for these disorders seeks to maximize body protein production and inhibit protein breakdown. Such an intervention is crucial when someone with a urea cycle disorder has an acute infection that results in a tendency for body proteins to break down and release ammonia. Under these conditions, the person with the urea cycle disorder must receive extra glucose, which causes insulin release from the pancreas. Insulin is a potent hormone that favors the production of body proteins and inhibits their degradation.

Part 3: Acylation Therapy – A Revolution in the Treatment of Urea Cycle Defects

Dietary protein restriction often fails to prevent all episodes of hyperammonemia. In addition, strict adherence to a diet ultra-low in protein creates a major challenge to both those with urea cycle defects and their families.

In the late 1970's, at the John F. Kennedy Center (now the Kennedy Krieger Institute) of the Johns Hopkins Medical School, Drs. Saul Brusilow and Mark Batshaw were studying the benefits of substances called ketoacids on a specific urea cycle defect (citrullinemia). They discussed their findings with a colleague, Dr. Norman Radin, who referred them to a 1914 scientific publication which showed that treatment with sodium benzoate reduced urea excretion in healthy young men. In careful clinical studies, Drs. Brusilow and Batshaw showed that benzoate and a similar compound, phenylacetate, were very effective in lowering blood ammonia of those with urea cycle defects.

Dr. Batshaw, who was the founding Associate Director of the Developmental Disabilities Research Center at the Kennedy Krieger Institute (and later established an Intellectual and Developmental Disabilities Research Center (IDDRC) at both the University of Pennsylvania/Children's Hospital of Philadelphia (CHOP) and Children's National Health System (CNHS) in Washington, D.C.) recalls these events: "We are indebted to Norm Radin for directing us to the 1914 publication. Of course, at that time nobody even knew of the existence of urea cycle defects, but we appreciated the therapeutic potential of acylating agents (benzoate and phenylacetate), and we set about testing them in children with urea cycle defects. The establishment in 1987 of an IDDRC at the Kennedy Krieger Center proved an invaluable asset by providing a core facility for studies in both humans and animals as well as support for experimental design and statistical analysis."

This discovery greatly improved the outcome for nearly all of those with urea cycle defects. Benzoate and phenylacetate could be given as a preventative measure – before they developed hyperammonemia. Furthermore, when they did become hyperammonemic, the acylating agents rapidly lowered blood ammonia levels, thereby shortening both the hospitalization and the time of exposure of the their brain to this toxin.

Over the decades since its initial discovery, acylation therapy has been improved. Phenylbutyrate, which the liver converts to phenylacetate, was substituted for phenylacetate (which smells bad). In addition, it was found that phenylbutyrate could be given in a form that didn't have a heavy salt load. Development of this new form resulted in a drug (Ravicti) in which phenylbutyrate is not bound to sodium but to glycerol. The IDDRCs at UCLA, Baylor College of Medicine, and the Children's National Medical Center were critical to creating this new medication. The new drug avoided a high salt load, and it also provided a more stable, 24-hour blood concentration of phenylacetate, the active form of the medication.

Part 4: Carbamylglutamate Therapy

The first reaction in the urea cycle occurs in liver mitochondria and converts ammonia to carbamoylphosphate. This reaction is performed by an enzyme called carbamoylphosphate synthetase (CPS), which is the most abundant protein in liver mitochondria. Although abundant, CPS is inactive – that is, it does not convert ammonia into carbamoylphosphate unless it receives a signal when a helper molecule called N-acetylglutamate (NAG) binds to it.

It may seem surprising that the most abundant protein in liver mitochondria should be “silent” until activated. Dr. Mendel Tuchman, a member of the IDDRC at the Children's National Medical Center and an expert on the genetics and function of this enzyme, made this comparison about the way the CPS enzyme works: “A classic military formation of an ancient Greek army was arranged with hundreds, at times thousands, of spear-bearing soldiers standing together, each joining his shield to that of his fellow to form a protective phalanx and each holding his spear in the other hand, all the while awaiting the commander's order to launch the spear against the opposing enemy. Once the command to launch was received, each spear would be [thrown] with terrible force and at high velocity. In seconds the enemy would find himself confronted by a massive barrage of deadly missiles.” Similarly, the CPS enzyme can move from low to high activity very quickly when an increase in ammonia level occurs, such as during a high protein meal or metabolic stress.

Such tight regulation of the urea cycle probably was important to the evolution of humans, who often ate protein sporadically, such as in pre-historic times following a successful hunt for animal prey. Consuming the meat with its protein load results in a sudden – and potentially toxic – flood of ammonia. Fortunately, the liver is prepared: consumption of large amounts of protein does not cause hyperammonemia. Our liver has a remarkable ability to efficiently convert ammonia into carbamoylphosphate.

The ability of N-acetylglutamate (NAG) to stimulate the CPS reaction prompted researchers to find an artificial form of NAG that might treat individuals who don't have the liver enzyme that normally produces NAG. These individuals could not be successfully treated with NAG because cells quickly degrade it before it reaches its target in liver mitochondria. An alternate drug might be carbamylglutamate, structurally similar to NAG, but which is safe and not broken down in cells. A 2002 study by investigators in Israel and Switzerland showed that those with NAGS deficiency could be successfully treated with carbamylglutamate.

In a later study (2004), a team led by Dr. Tuchman showed that even a brief 3-day treatment with carbamylglutamate could dramatically improve ureagenesis (urea generation) in those with NAGS deficiency. The investigators at the IDDRC at CNMC used a novel method that had been developed at the University of Pennsylvania/Children's Hospital of Philadelphia (UPenn/CHOP) by Drs. Marc Yudkoff and Itzhak Nissim. The new method, which was developed with the support of the IDDRC at UPenn/CHOP (where Dr. Yudkoff was IDDRC Director) used a stable (non-radioactive) version of the carbon atom to trace the rate of urea synthesis in both healthy individuals and in those with urea cycle defects. The new method allowed a precise, before-and-after measure of the amount of urea formed in response to treatment with carbamylglutamate. In later investigations, the CNMC team was joined by Nicholas Ah Mew, who wondered if carbamylglutamate might improve ureagenesis (and lower toxic ammonia levels) in situations other than primary defects of the urea cycle. One of these is propionic acidemia, a condition where there is a build-up of propionic acid, a compound in the breakdown of certain amino acids. Affected individuals often have hyperammonemia as newborns. Dr. Ah Mew again utilized the stable isotope methodology to demonstrate that carbamylglutamate could improve defective urea production in some individuals with propionic acidemia. He comments: "Propionic acidemia causes developmental disabilities and other neurologic problems in almost all youngsters with early-onset disease. During an episode of metabolic decompensation, many children have hyperammonemia, which can cause brain injury. Administration of carbamylglutamate, which can lower blood ammonia levels during a metabolic crisis, may prove a valuable treatment that improves overall cognitive outcome."

Part 5: Gene Therapy

For more than 50 years, it has been known that viruses can deliver new genes into host cells. The first efforts to utilize this technology to treat an inherited disorder occurred in 1990, when two children with an immune disorder received a transfusion of their own white blood cells in which the absent gene had been introduced. This pilot study failed to demonstrate obvious benefit. Nonetheless, medical interest in gene therapy grew until 1999, when a young man with a urea cycle defect (ornithine transcarbamylase deficiency) tragically died after receiving a virus that carried the missing gene. The cause of death was an overwhelming immune system reaction in response to the viral particles.

This tragedy increased the efforts to create safer viruses to introduce a healthy version of a gene that either is missing or defective in people with inherited disorders. Today, nearly 2000 such trials are in progress for a number of diseases, including urea cycle defects. As an example, research is now underway to treat people with a deficiency of arginase, the enzyme that converts the amino acid arginine to urea in the final step of the urea cycle. People with argininemia (elevated levels of arginine) have cognitive impairment, as well as a movement disorder similar to cerebral palsy. To research improved therapies for this disease, the IDDRC at UCLA has supported research in mice that were bred to have an arginase deficiency similar to the human disease. These animals typically die within the first two weeks of life. However, when they are treated in the newborn period with a virus that carries the missing gene, they not only survive into adult life, but also display normal cognitive ability. Dr. Gerald Lipshutz, who supervises this research, offers this observation: “Our research strongly suggests that viral-mediated gene transfer one day may be successfully given to human[s]. An important conclusion is the finding that we were able to improve lifespan and outcome in the mice even with very small increases in the activity of arginase in the liver. We worked with our colleagues at the IDDRC of the Children’s Hospital of Philadelphia to show that seemingly tiny elevations of arginase – for example, less than 10% - resulted in visible increases in the rate of urea synthesis. In other words, complete correction of the defect is unnecessary. This observation may be relevant in terms of the ‘load’ of viral particles that have to be given to humans. It may be that we can reach a therapeutic effect with fewer viral particles than we had originally anticipated. This would be a most welcome result.”

Gene transfer is being tested to treat yet another disorder, ornithine transcarbamylase deficiency, the most common of the urea cycle defects. Studies in a mouse model of the disease have been promising. Not only did viral delivery of the gene normalize metabolism in the animals, it also prevented liver scarring that recent research has shown is a common occurrence in humans with the condition. This collaborative effort has been supported by many IDDRCs, including those at Boston Children’s, CHOP and CNHS. Several venture capital firms have joined with partners in medical schools to bring this “cutting edge” technology to a bigger market. According to Dr. Mark Batshaw, “The effort to make gene therapy a clinical reality has a history that extends back over decades and represents the talent and hard work of hundreds of dedicated investigators. It has cost billions of dollars. Fortunately, this investment seems finally to be leading to a favorable return. The FDA has approved viral gene-transfer as a treatment for eye disease that otherwise results in blindness. The road ahead is long and likely will be filled with unexpected twists and turns, but for the first time, our generation can hope for a treatment – even a “cure” – of inherited diseases like the urea cycle defects”.