Background information on
the Paul Ehrlich and Ludwig Darmstaedter Early Career Award 2024

Remote-controlled magic bullets

Cisplatin and two of its derivatives are the world's most common cancer drugs, used in around half of all chemotherapy treatments. While they are impressively effective against certain types of cancer, they also attack healthy body cells, with serious side effects, and quickly bring about resistance. That is why attempts have long been made to convert these drugs' non-toxic precursors (prodrugs) into their effective form exclusively in the cancer cells themselves. By integrating and further developing existing approaches into a new whole, Dr. Johannes Karges has succeeded in doing just this. Together with his team, he has constructed tiny beads (nanoparticles) that transport platinum preparations or their prodrugs solely into the tumor tissue, where they can be activated through irradiation with light or ultrasound. Karges has already proven the effectiveness of these methods in pre-clinical trials, thereby revitalizing Paul Ehrlich's vision of a magic bullet that can eliminate a specific disease without harming the body.

Paul Ehrlich first formulated this vision of "magic bullets that are only directed at the foreign pathogen but do not affect the organism itself or its cells"[1] during the years in which he discovered the world's first chemotherapeutic agent, compound 606. Developed together with Farbwerke Höchst, the arsenic complex arsphenamine was introduced onto the market under the tradename "Salvarsan" in 1910 as a treatment for syphilis. Ehrlich was aware that he had not yet found the ideal magic bullet. But to his mind, the therapeutic effect of his compound 606 far outweighed its side effects. "Therefore, in my opinion, there is no reason to look for a compound '607' now and neglect the existing good in the pursuit of future improvement.[2]" All told, the discovery of arsphenamine was not only a milestone in medical history. It also constitutes the first structurally defined synthesis of a metal complex for therapeutic purposes, even if – strictly speaking – arsenic is only a semi-metal. Advanced ancient civilizations also intuitively used metals such as gold, iron and copper as medicines.

The reactivity of metal complexes

Metal complexes are compounds whose core consists of a metal that lacks electrons. The molecules that bond with this metal compensate for this lack by donating

electron pairs to it. In contrast to "normal" covalent bonds, the bonding electrons of a metal complex therefore only come from the ligands, which oxidize when a metal complex is formed. The central metal absorbs electrons and is reduced. Since these redox properties give metal complexes a special reactivity that favors the exchange of their ligands, they play an important role as catalysts in organic chemistry. The best-known biological metal complex is hemoglobin, with its central iron atom, which transports oxygen in the blood.

A double-edged sword

Of all the metal complexes in medicine, cisplatin is the most prominent. In the 1960s, physicist Barnett Rosenberg of Michigan State University accidentally discovered its effectiveness against tumors, when he wanted to investigate the influence of an electric field on the growth of *E. coli bacteria*.[3] He applied the necessary voltage to the bacterial culture using platinum electrodes. To his astonishment, the bacteria did not multiply, but instead grew into long filaments. In other words: They grew but did not divide. After careful examination, Barnett and his team discovered the surprising reason why: the platinum in the electrodes had reacted with the ammonium chloride in the bacterial culture's nutrient solution, thereby forming the metal complexes cisplatin and transplatin. In both complexes, the platinum forms a compound with two chloride ions and two ammonium ions in a spatially mirror-image arrangement. Cell division is only inhibited by cisplatin. In clinical trials, cisplatin initially showed a high level of efficacy, particularly in the treatment of testicular cancer, where the survival rate of patients treated with the complex rose from 10 to 90 percent. Cisplatin was approved by the US Food and Drug Administration (FDA) in 1978 and has since proven effective in the treatment of many types of cancer – as a cytostatic drug that inhibits DNA replication during cell division and causes programmed cell death (apoptosis). However, this action mechanism does not stop at normal body cells, explaining cisplatin's severe side effects, which range from nausea and vomiting to kidney, hearing and nerve damage, and even inhibition of blood formation in the bone marrow. Although the cisplatin derivatives carboplatin and oxaliplatin are better tolerated, they are also associated with considerable side effects.

The courage to be interdisciplinary

During his master's degree in chemistry in Marburg and London and his subsequent doctorate in Paris, Johannes Karges increasingly specialized on the medical application of metal complexes, which he researched by applying a wide-ranging interdisciplinary approach involving biological, chemical and physical methods. He came to the conclusion that their therapeutic potential was being vastly underestimated, partly for fear of their possible toxicity[4] . The key question that has guided his research since is: How can we make metal complexes work exclusively in the tumor they are supposed to destroy? Of course, Karges was not the first to ask himself this question. Many researchers before him had already answered it in principle, namely by administering such complexes as inactive precursors (prodrugs), which are only activated in the tumor. In the case of platinum complexes, the central atom of such precursors would consist of fourfold positively charged platinum (platinum IV), which would then be activated by

reduction. This is because cisplatin and its derivatives are platinum II preparations. It was hoped that natural reducing agents such as glutathione or ascorbic acid, which are particularly prevalent in cancer cells, could be used as activators of such precursors. However, since low concentrations of these reducing agents are also present in normal cells, undesirable effects continue to occur if the prodrugs do not reach the targeted cancer cells. Supported by his doctoral supervisor Prof. Gilles Gasser and inspired by a doctoral student exchange with Sun Yat-Sen University in Guangzhou , China, Johannes Karges set himself two goals: to selectively enrich the active substance or its precursor in the tumor, and then activate it there using an external trigger.

Targeting the core of the cancer cell

Together with his Chinese mentor Prof. Haihua Xiao, Karges initially achieved this goal using the active ingredient oxaliplatin and long-wave light as an external trigger[5] . Located near the infrared range, this light penetrates deeper into the tissue than the blue light that had previously been tried and tested in many places. As part of a multi-stage process, the team surrounding Xiao and Karges packaged the active ingredient into nanoparticles, beginning by coupling it with a suitable photosensitizer, i.e., a molecule that can chemically convert the energy of absorbed light and introduce it into redox reactions. They then bound the active ingredient and light receptor into a fat-soluble polymer, onto whose ends they attached water-soluble peptides. The resulting long molecules then self-assembled into spheres with a diameter of 80 nanometers each. This meant the nanoparticles were too large to penetrate healthy tissue, whose cells are closely knit together, but small enough to squeeze between the cancer cells, whose connection is patchy due to their very rapid growth. The peptides on the surface of the particles in effect acted as address labels for the nucleus of the cancer cells. Once there, the particles remained stable in the dark. Only when irradiated with red light did they disintegrate and release oxaliplatin as well as highly aggressive oxygen, thereby destroying the cancer cells. Karges and Xiao were able to confirm the findings obtained in cell cultures in animal experiments. The tumors of mice in which the oxaliplatin-containing beads had accumulated disappeared almost completely just a short time after having undergone external irradiation with red light.

Ten times as far with ultrasound

Given that red light hardly penetrates more than one centimeter deep into an organism, most human tumors cannot be reached with it. Ultrasound waves, on the other hand, can travel ten times the distance in the body. The question thus became: Would it be possible to convert precursors of cisplatin into their active form using ultrasound irradiation? The answer is yes, as Johannes Karges discovered in *computer-aided design* using density functional theory methods: “This could work if we used the hem group of hemoglobin as a sonosensitizer, i.e. as a molecular antenna that reduces the prodrug Pt1 to active cisplatin by electron transfer after sonication.” After spending some time as a post-doc at the University of California in San Diego, Karges became junior research group leader at Ruhr University Bochum. In keeping with the proven method for red-wavelength light, Karges, Xiao and their working groups constructed nanoparticles containing hemoglobin

and Pt1. In this case, too, the particles selectively accumulated in cancer cells. While they remained stable under physiological conditions, within three minutes after sonication in the presence of ascorbic acid, the prodrug was completely converted into cisplatin. In the ensuing animal experiments, intestinal tumors in mice almost completely regressed even when sonication was carried out through a two-centimeter-thick piece of chicken breast. [6]

Promising prospects

Together with his research group in Bochum and his Chinese partners, Johannes Karges is now pursuing an even more ambitious goal of fighting cancer with the help of metal complexes: He wants to provide them with address labels that direct them into the endoplasmic reticulum (ER). Once here, they should catalyze the formation of aggressive oxygen, thereby causing immunogenic cell death, which in turn is communicated to the entire immune system. This would enable the immune system to attack not only the primary tumor, but all cancer stem cells and metastases of a patient. It is not yet clear which metal complex is best suited for this purpose. While corresponding research is still at a very early stage, it holds clear prospects for Johannes Karges.[7]

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[1] Paul Ehrlich. Über den jetzigen Stand der Chemotherapie (Reports of the German Chemical Society 1909). quoted from: Axel C. Hüntemann. Paul Ehrlich. Göttingen 2011, p. 166

[2] Paul Ehrlich. The treatment of syphilis with Ehrlich's preparation 606, Dtsch. Med. Wochenschrift 1910, 41, 1893-1896

[3] B. Rosenberg, L. van Camp, T. Krigas. Inhibition of Cell Division in Escherichia coli by Electrolysis Products, Nature 1965, 205, 698-699.

[4] J Karges. Combining Inorganic Chemistry and Biology: The Underestimated Potential of Metal Complexes in Medicine. ChemBioChem 2020, 21, 3044-3046

[5] D. Wei, Y. Huang, B. Wang, L. Ma, J. Karges, H. Xiao. Photo-Reduction with NIR Light of Nucleus-Targeting Pt^{IV} Nanoparticles for Combined Tumor-Targeted Chemotherapy and Photodynamic Immunotherapy. Angew.Chem. Int. ed. 2022, 61, e202201486

[6] G. Liang, T. Sadhukhan, S. Banerjee, D. Tang, H. Zhang, M. Cui, N. Montesdeoca, J. Karges, H. Xiao, Reduction of Platinum (IV) Prodrug Hemoglobin Nanoparticles with Deeply-Penetrating Ultrasound Radiation for Tumor-Targeted Therapeutically Enhanced Anticancer Therapy, Angew. Chem. Int. ed. 2023, 63, e202301074

[7] L. Zhang, N. Montesdeoca, J. Karges, H. Xiao, Immunogenic Cell Death Inducing Metal Complexes for Cancer Therapy, Angew. Chem. Int. ed. 2023, 62, e202300662.