



Naturally derived cancer treatments



Professor Dr Regine Schneider-Stock and collaborators are working to develop new drugs to be used in the battle against cancer. She discusses the advantages of naturally derived treatments, and why her study is on the brink of a breakthrough

Would you begin with an explanation of thymoquinone (TQ) and its function?

The blackseed, also known as black cumin, black caraway seed, habbatul-baraka (the blessed seed) and by its botanical name, *Nigella Sativa*, has been used in traditional medicine in Asia, the Middle East and Africa for more than 2,000 years to promote health, fight diseases, and boost immunity. TQ is a small molecule that was first isolated from blackseed in 1963. It exerts broad antineoplastic effects, and its strong anti-inflammatory activities are primarily responsible for its potent anticancer properties.

How can TQ be used in human medicine?

At the beginning of the 21st Century, a great deal of promising evidence showed that TQ has anti-inflammatory, antioxidant and anticancerous properties. However, when my research group began testing this molecule in 2001, there were no studies on TQ's effects on colon cancer. Such studies are warranted, especially given that TQ's natural origin and the widespread human consumption of blackseed point to the likelihood of this drug producing fewer cytotoxic side effects when consumed over long periods of time. TQ also has considerable effects for treating diseases such as microbial and parasitic infections, as well as epilepsy and other neurological disorders.

What are the principal aims and objectives of your current research?

Our principal aim is to identify the specific targets of this interesting molecule and

to determine the cellular and molecular mechanisms of its anticancer activity. Identifying the molecular targets of TQ is the first instrumental step for recommending its use in the clinic. Additionally, only by identifying and characterising the specific signalling pathways that TQ is targeting can we envisage novel therapeutic approaches using this compound. We have investigated TQ's action in several cancer systems including colon cancer, skin cancer, osteosarcoma and leukaemia, and it showed very promising anticancer effects against each of these. However, its mechanism of action differed depending on the cancer type.

We have also attempted to combine TQ with other chemotherapeutic agents used in the clinic against colon cancer and leukaemia and found that the addition of this natural compound enhances the efficacy of the clinical drug even in resistant types of cancers. Our ultimate aim is to push TQ into the clinical setting, since so far only one study investigated its efficacy in adult patients with solid tumours or haematological malignancies who had failed or relapsed from standard therapy.

Has sufficient data from animal testing been accumulated to transfer the drug into a clinical setting?

Our high-impact work has initiated worldwide interest in TQ, especially in the last five years. We have already shown in a rat model of chemically induced colorectal cancer that TQ significantly reduced the numbers and sizes of aberrant crypt foci. Moreover, sub-cytotoxic doses of TQ decreased cell invasion by 50 per

cent and suppressed growth in three-dimensional spheroids. Other investigators found in animal models that TQ effectively protected against transient forebrain ischaemia-induced damage in the rat hippocampus, and healed in a model of bone marrow in benzo(a)pyrene [B(a)P]-induced chromosomal aberrations. Furthermore, TQ is a protective agent for gastric mucosa from acute alcohol-induced mucosal injury, protects lung tissue from hazardous effects of human gastric juice and seems to be a promising prophylactic agent, increasing the activities of quinonereductase and glutathione transferase. Altogether these data encourage further testing in humans, and support the potential use of TQ as a therapeutic agent in cancer.

At what stage are you at in your investigation? What areas do you plan to perfect over the coming year?

We are at a very advanced stage in our research, and quite close to identifying TQ's molecular targets. The next step is to unravel a new kinase signalling pathway that is a target of TQ, which will provide detailed molecular and biochemical characterisation under single and combination drug treatment in leukaemia and colorectal cancer cells. So far, there have been no controlled clinical trials on the application of TQ in human gastrointestinal tumours. In a phase I clinical trial we plan to assess the safety, tolerability and maximum tolerated dose of a combination of TQ with 5-Fluorouracil based chemotherapeutic regimens in patients with advanced colorectal cancer. Secondary endpoints will be overall survival, time to progression and overall response rate.

Moving towards combination therapy

An ongoing international study based at the Institute of Experimental Tumor Pathology at the **University of Erlangen**, Germany, is breaking new ground in the development of novel cancer treatments. By championing the use of combination therapy, the project is likely to lead to more effective treatments, with fewer side effects

IN RECENT YEARS, natural and synthetic drugs have been, and continue to be, employed in the fight against cancer. During the last 20 years, more than 25 per cent of drugs used to treat cancer have derived directly from plants, with another 25 per cent comprising chemically altered natural products. One advantage of using naturally derived drugs is that they are likely to be relatively non-toxic, and are more widely available in digestible forms than their synthetic counterparts.

One such natural potential basis for anticancer drugs is *Nigella Sativa*, or blackseed, which has long been known to have qualities which improve health, fight disease and boost immunity. More recently, discoveries have also been made into the specific anticancer properties of thymoquinone (TQ), a compound molecule which naturally occurs in blackseed. It is these properties, and their potential application in the fight against cancer, which is forming the basis of a groundbreaking study at the University of Erlangen, Germany. Professor Dr Regine Schneider-Stock of the Institute of Experimental Tumor Pathology at the University, along with her chief collaborator Dr Hala Gali-Muhtasib from the Department of Biology at the American University of Beirut, has been awarded three separate grants by the Deutsche Forschungsgemeinschaft (DFG) to study the anticancer activity of TQ.

A MULTIFACETED STUDY

Despite the fact that TQ has a potential application in combating a variety of cancers, for the moment Schneider-Stock is focusing her studies on colorectal cancer, whilst Gali-Muhtasib has been concerned chiefly with

leukaemia. Their collaboration, which dates back to 2003, has already culminated in 10 published research articles and two reviews, seven of which are on TQ, with a mean impact factor of 4.5. The collaboration has also resulted in four graduate students travelling from the American University of Beirut to conduct research in Schneider-Stock's lab, thanks to funding from the German Academic Exchange Service (DAAD). Schneider-Stock argues: "Our collaboration is one of few successful long-term scientific partnerships, and would not have been made possible without the continued support of DFG and DAAD".

In a particularly interesting 2008 paper which featured in the prestigious journal *Cancer Research*, Schneider-Stock demonstrated that upon application of TQ, the tumour suppressor p53 transcriptionally represses the DNA damage sensor and pro-survival protein CHK1. This leads to significant levels of cell death in colorectal cancer cells that are wildtype for p53, but not in those in which p53 is inactivated. The study also confirmed this link between CHK1 and p53 in *in vivo* colorectal cancer patients, showing that tumours lacking p53 had higher levels of CHK1, and that CHK1 overexpression correlated with more advanced tumour stages and a worse prognosis. Schneider-Stock elaborates: "We believe that the inhibition of the stress response sensor CHK1 might contribute to the antineoplastic activity of specific DNA-damaging drugs, including TQ". Indeed, CHK1 inhibitors have been the recent focus of pharmaceutical development, and there are currently clinical trials in which they are being tested for the treatment of non-small cell lung cancer and pancreatic cancer.

GENETIC MARKER IDENTIFICATION TECHNIQUES

Schneider-Stock and her collaborators have used a variety of techniques over the course of their research. In a relatively early study, they used a complementary DNA (cDNA) microarray to identify targets that are transcriptionally dysregulated by TQ. In doing this, it was possible to identify the G2/M checkpoint and damage sensor CHEK1 to be involved in TQ-induced apoptosis, results which were also published in *Cancer Research* in 2008. More recently, they used a phosphopeptide array analysis to identify new phospho-proteins that are kinase targets after TQ treatment.

Protein kinases in a cell are key regulators of diverse essential functions. By adding phosphate groups to their substrate proteins, they directly influence the activity, localisation and cellular function of many proteins. Schneider-Stock reveals: "Because their dysregulation is crucial for the malignant phenotype, it is no wonder that kinases are one of the key targets for personalised therapy. We believe that, through analysis of the phosphorylation patterns of the peptides, the molecular signatures of activated signalling pathways in the cell will be directly obtained". The studies have identified a candidate list of signalling pathways that are activated after TQ treatment.

Going forward, Schneider-Stock and her team plan to use a variety of molecular and conventional protein biochemical investigations to verify the peptide array data. They hope that TQ targets can then be identified through functional analyses using different apoptosis, inflammation, migration and kinase assays, as well as damage and cell cycle analyses.

COMBINING NATURAL AND SYNTHETIC APPROACHES

It is believed that the wide variety of compounds produced by plants, referred to under the umbrella term 'phytochemicals', will play an important role in the future of medicinal research. Schneider-Stock observes: "For centuries, herbal medicine

Recently, discoveries have been made into the specific anticancer properties of thymoquinone (TQ), a compound molecule which naturally occurs in blackseed

has targeted numerous diseases such as heart disease, diabetes and high blood pressure, and has proven to be successful in the prevention and treatment of cancer". Phytochemicals contribute to cancer prevention via a number of mechanisms, including blocking the formation of potential carcinogens, blocking the effects of carcinogens on their target organs or tissues, or acting directly on cells to suppress the initiation and promotion of cancer. "The main gap in our knowledge is to identify the exact mechanism or mechanisms by which these plant-derived compounds inhibit cancer, and determine their specific targets in the cell," she adds.

Current primary cancer treatment consists chiefly of chemotherapeutic drugs, despite their many unpleasant side effects. They are administered independently, or in combination with other chemically synthesised compounds. One example is 5-Fluorouracil (5-FU), currently the leading antineoplastic agent used against colon cancer. High resistance levels mean that when administered independently, the drug has a success rate reported to be less than 30 per cent. Recent studies have shown that combining TQ with clinically used anticancer drugs has led to improvements in their therapeutic index. "In this regard," Schneider-Stock reveals, "we have shown that TQ reduces the toxicity of anticancer compounds by protecting organs, such as the heart, nervous system and liver, from the toxicity of chemotherapeutic agents." The team has also tested the efficacy of TQ in combination with another clinically used synthetic drug, doxorubicin, which is used to combat leukaemia cells. The findings indicate that there could be a therapeutic role for TQ in this instance, too – sensitising HTLV-1-negative T-cell lymphomas to doxorubicin.

Indeed, combination therapies are being increasingly employed in the battle against cancer, particularly after tumours have developed resistance to traditional treatments, and Schneider-Stock's lab is at the forefront of championing such an approach; as she reveals: "It would be ideal to combine the toxic chemotherapeutic drugs with non-toxic natural drugs". In this way, it appears that more effective cancer treatments, with fewer unpleasant side-effects, might be just around the corner.



PROFESSOR DR REGINE SCHNEIDER-STOCK WITH RESEARCHERS

INTELLIGENCE

THE CLINICAL POTENTIAL OF THYMOQUINONE IN TUMORS: IDENTIFICATION OF NOVEL MOLECULAR TARGETS AND TESTING ITS EFFICACY IN COMBINATION THERAPY

OBJECTIVES

The goal of this research is to dissect the molecular mechanisms of TQ action to provide a basis for recommending its use as a promising agent against cancer.

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