CONTROVERSIAL SELENIUM: UNIQUENESS IN THE SHADOW OF TOXICITY

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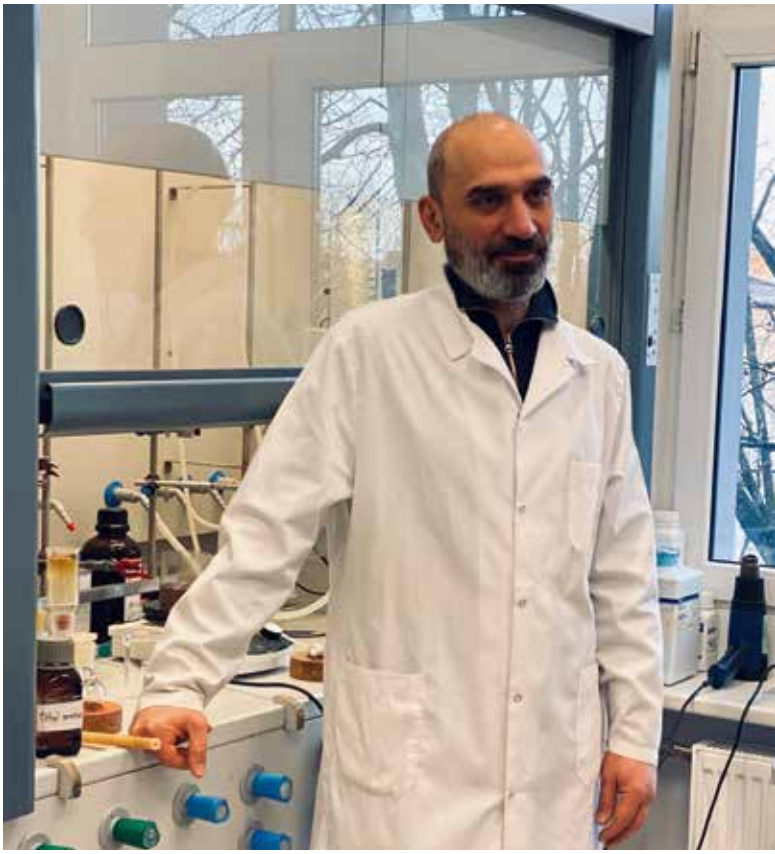

Two hundred years ago, a new element was discovered by the Swedish chemist Jöns Jacob Berzelius. He named the new element **Selenium**. It was very farsighted, because 45 years ago a brilliant biochemist Prof. Thressa Stadtman found the 21st proteinogenic amino acid – selenocysteine in the active site of glutathione peroxidase (GPx). And since then, interest about selenium increased in geometrical progression. Over the past 20 years, scientific studies have clearly demonstrated that selenium is an irreplaceable microelement with essential properties for human health. Indeed, relationships between the level of selenium in the daily diet and the risks of developing various types of cancers have been established. Selenium is an active component of GPx, which is a basic enzyme involved in cell redox homeostasis. Selenium has antioxidant properties and is effective in physiological and pathological processes linked to increased intracellular free radical and reactive oxygen species (ROS) generation. Selenium is needed for proper functioning of the immune system, since a key nutrient inhibits HIV progression to AIDS, decreases a risk of cardiovascular diseases, and elevated intake of this microelement is associated with reduced cancer risk. But the main negative issue about selenium is a narrow dose range between therapeutic and toxic doses. Selenium-containing compounds used in industry are quite toxic, e.g., LD_{50} of a widely used nutrient – sodium selenite – varies from 8.1 to 12.1 mg/kg, which is comparable with potassium cyanide toxicity.

During the last decades, introduction of selenium into biologically active molecules have attracted increasing attention. Some compounds exhibit excellent activity as antioxidants, redox modulators, antitumor, antihypertensive agents, etc. Regarding the biochemical role of both naturally occurring and synthetic selenium compounds, based on their action mechanism, they could be divided in three major groups:

1) Se-compounds that can be metabolised to hydrogen selenide and, therefore, be able to serve as Se source to be incorporated in Se-proteins;
2) Se-compounds possessing bioactivity that is not directly related to selenium itself;
3) Synthetic mimics of known Se-enzymes.

Unfortunately, of thousands of synthesised and studied selenium-containing compounds no one has been approved as a drug so far.

Since Na_2SeO_3 is the most common additive in nutrients, we started our research preparing several series of ammonium selenites, hydroselenites of ethanolamines, amino acids and gamma-butyrobetaine (GBB) derivatives. According to cytotoxicity and *in vivo* antitumor activity it was found that triethanolammonium and diisopropyl GBB are able to suppress sarcoma growth *in vivo* up to 100%. Acute toxicity decreased more than 10 times compared with sodium selenite. However, toxic effects during *in vivo* experiments were detected as well. Attempts to find promising antitumor agent using inorganic selenium failed. Notably, the main problem in this field remains the high toxicity and unpredictable selectivity of selenites against cancer cells *versus* normal cells.

The introduction of selenium atom in already known drugs in place of sulphur, oxygen and nitrogen was very popular, medicinal chemists desired to endow positive properties of selenium to drugs with the aim to lower toxicity, increase selectivity, etc. One of our projects was connected with modification of selective estrogen receptor modulators (SERM), which are widely used to prevent osteoporosis, reduce breast cancer risk. These drugs lower LDL cholesterol level and prevent prostate cancer as well. Of course, as for all synthetic drugs they have a long list of side effects. Raloxifene molecule contains benzothiophene ring, and our idea was to introduce selenium instead of sulphur in purpose to compare both compound activities. Both compounds were not toxic, but selenium analogue inhibited breast cancer growth by 30% (15.0 mg/kg), besides, Raloxifene was ineffective in triple negative breast cancer model (4T1 carcinoma). It could be accepted as a positive result, but more profound studies let us to conclude that we should focus on other projects.

Based on many scientific reports we decided to go in a little opposite way as other researchers.

Coumarin core is very common in natural product structures, which are widely used in Indian (Ayurveda) and Chinese medicine. Ayurveda medicine lies on a different principle than Western techniques; it is based on the treatment of a patient with a mixture of natural herbs containing biologically active substances with the aim to create a balance between body and soul. In other words, it helps the soul remedy a body and *vice versa*. This therapy is always slower, but usually do not harm organism with side effects. Western scientists also paid attention on Ayurveda: coumarin derivatives as medicines with substantial activity *in vitro* and *in vivo* have been discovered (*Psoralen*, *Angelicin*, *Xanthotoxin*, *Bergapten*, *Nodakenetin*, etc). *Imperatorin* shows the ability to inhibit tumour growth. *Osthole* inhibits the migration and invasion of breast cancer cells and effectively blocks matrix metalloproteinases promoter and enzyme activity. In a long list of coumarins, furocoumarins always make a separate group, because these natural products are very effective poisons in up to femtomolar concentration, because of their phototoxicity. To our knowledge, the introduction of a heavy element in the molecule lead to bathochromic shift of emission, reducing quantum yield and depleting fluorescence. We decided to use this effect by the introduction of selenium instead of oxygen atom. In a series of more than 150 various derivatives we have found the leading compound (**PA-27**) with no phototoxic properties. **PA-27** exhibit outstanding antimetastatic activity. Moreover, pre-treatment of laboratory animals with this compound led to impressive suppression of metastasis and malignant tumour formation in *in vivo* experiments. Cancer development (breast cancer model) results with no signs of tumour in three mice out of six, but two had less than 7% of occupied with tumours areas (total: 90%, p=0.001). In a pre-treatment model, four mice had no signs of tumour (100% inhibition); however, treatment did not influence on tumour growth in two mice (total: 58%, p=0.122). Beginning of the treatment right after metastatic melanoma cells inoculation resulted in 75% inhibition. Notably, three mice out of six had no melanoma nodules in lung after the end of the experiment on day 22. Even more interesting results were received from pre-treatment experiment (treatment in advance from day 19th till 3rd before cancer cell inoculation): four mice from a group of seven animals had no tumour nodules in lung and one animal had only

one small nodule. LLC1 lung carcinoma growth suppression caused by **PA-27** in dose 20 mg/kg was completely (100%, *p*=0.050) prevented. Moreover, only one mouse out of five had only 1 tumour nodule (97%, *p*=0.056) performing the pre-treatment (treatment in advance from day 14th till 0 before cancer cell inoculation). Those values were particularly encouraging, bearing in mind that **PA-27** did not induce any major side effects either: all animals looked healthy and active and weight change was visually non-existent. This unexpected discovery makes **PA-27** very promising immunomodulating drug candidate for the suppression of cancer metastasis development and prevention of tumour formation. PK and safety studies of **PA-27** were performed. Bioavailability (AUC) was determined after intravenous (IV), subcutaneous (SC) and peroral (PO) administration of **PA-27** at a dose of 20 mg/kg in adult Balb/c female mice. According to our results **PA-27** bioavailability is high, 85% after s.c. administration and 38% after p.o. administration. Dose linearity of **PA-27** was determined after peroral (PO) administration at doses of 20–1000 mg/kg. The disposition a **PA-27** is linear at doses from 20 mg/kg to 400 mg/kg. Single maximal peroral tolerable dose (MTD) of **PA-27** is 1000 mg/kg. Same dose is also No Observable Adverse Effect Level (NOAEL).

Twenty-eight-day safety studies were determined on Balb/c female mice. **PA-27** administered at a dose of 400 mg/kg (PO). Animals weight, behaviour and clinical signs were monitored during 28-day treatment, plasma collected for PK after the last dose; organ (spleen, kidney, liver, heart) weight recorded, marker measurements in plasma and urine. Liver function tests show significantly elevated levels of ALAT and ASAT although the observed increase does not indicate on marked liver damage. Notably, PK profile and exposure of **PA-27** was similar after 28 days of administration and single administration.

Notably, at the moment there is no effective treatment for cancer metastasis suppression. Monoclonal antibodies are widely available, but they are characterised by serious side effects. Therefore, novel drug candidate molecules are required for treatment of cancer metastasis development and prevention of tumour formation. Based on previously received data, **PA-27** can be regarded as a promising novel drug candidate, characterised by a wide range of therapeutic effects and fewer side effects compared to currently available drugs.

Thus, masking of selenium pharmacological properties in the development of drug candidate, gave an impressive result and may be accepted as new direction in the search of anticancer drugs in **Unmet Medical Need** category.