Deutscher Akademischer Austauschdienst
German Academic Exchange Service

Abschlussbericht

über Ihre Stipendienzeit

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Stipendienprogramm	DAAD Rise WW
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Gastinstitution	NDMC

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Verlauf des Vorhabens *(Textfeld erweitert sich nach Eingabe automatisch; Formatierungen sind jedoch nicht möglich. Für eine bessere Strukturierung können Sie dieses Formular als Deckblatt nutzen und den Bericht als Anlage beifügen.)*

Taiwan during Covid19

A sweaty trip from fried chicken feet at the night market to hematopharmacology

1.1 Introduction

It was the 17th of February, early in the morning, I just went out of the hotel in Palma to start a hiking trip, that I’ve planned for the day and *Beeeeeeeep*. I received an e- mail. I turned on my phone and saw that I got a message from the German Academic Exchange Service (DAAD). My heart was beating faster and with sweaty hands I clicked on it and read the few words: “Congratulations! We

are delighted to inform you that your application has been successfully matched“. I’ve made it! I got the internship! The day, what was already beautiful became even better. I put my phone back in my pocket with a smile and started to hike up the mountain - the mountain of discoveries and difficulties, as it turned out later.

1.2 Organizational preparations

First, I tried to make a contact with the local Taiwanese professor. I wrote an e- mail and introduced myself personally. A few days later I got an answer and he said that he is very happy that I have chosen his lab and that I have interest in exploring how life and scientific research is in Taiwan. From that moment on, I could always message him with any concern, and he tried to answer me within a week. He was my only contact person in Taiwan, and it was fine for me, because he knew about everything concerning my stay. A challenge nevertheless was the time difference, and it was the reason why it took quite a long time until everything was discussed and planned.

The things that had to be arranged were: to get a visa, to book a flight and a quarantine hotel and to find a place to sleep.

The first thing on my to do list was the biggest problem and the reason why I almost had to cancel respectively to postpone my internship. Due to the COVID- 19 pandemic, Taiwan has imposed entry restrictions for foreigners since 2020 and therefore it was not possible to enter without a visa until the 25th of July (just 2 weeks before my internship should have started!). After that date I was able to apply for visitor visa with special permissions and even more short- term, I was granted one. I contacted the consulate in Munich and the civil servants there were very friendly and helpful. But attention: Before I contacted Munich, I had some correspondence with the consulate in Frankfurt and there, they were very unfriendly and incompetent! Therefore, every student no matter where they are from, should address the consulate in Munich! Normally, the requirement for getting a visa, to be able to do an internship, was to get a Letter of Approval from the Ministry of Education of Taiwan. Thus, you should contact your professor and ask him to contact the International Office of the university for you. But in my case, I had to deal with the Ministry of Defense since I was planning to go to a military medical school. And the Ministry of Defense was not willing to give me the required letter. At the end of a long e- mail conversation it turned out, that an official letter from my professor was enough for my visa application. I can recommend, that you ask very specifically what is necessary to get what you want.

After the misery and the drama with the visa, I booked the flight. I chose the Economy Class from Emirates and because it was very short term, nearly last- minute, the flight was more expensive than in April or June. But the money for the outbound and return flight was well calculated from DAAD and I didn’t have to pay on top. At least something that worked out without problems. The flight was long, but the excitement about finally starting my internship let my forget about it. I can recommend Emirates and the Economy class, nevertheless. I got there well and in a whole.

During the summer 2022, there also was a quite tense situation between Taiwan and China. I decided to go there nevertheless, and I have to say, that in Taipei, there was no sign of stress or fear. Everything was normal and additionally, the professor told me, that the Taiwanese people got used to the “China thing” and live on as normal.

1.3 First steps in Taiwan and the accommodation

The process of getting out of the plane, into the airport building and through all the registration and checking stations was well organized but nevertheless it took me 2,5 hours. Before the outbound flight, I made a PCR Test and registered myself on the following website of the Center of Disease Control (CDC) respectively the Central Epidemic Command Center (CECC): https://hdhq.mohw.gov.tw/Default1?openExternalBrowser=1 . In the airport I had to buy a new sim card for my stay (90 days for approximately 72 €), so the CDC could contact me. Therefore, I had to update my new Taiwanese phone number in the document from the CDC and I got a SMS with the declaration that I was cleared for entering. The last step was to make a saliva PCR test at the airport.

The regulations at that time stipulated that everybody who enters Taiwan must go to quarantine in a special apartment or hotel for 8 days. There is a list on the internet where all officially approved quarantine hotels are shown (https://docs.google.com/spreadsheets/d/1fMSZMhbTSOCKG_8DZYycGu6Y-hBFlJCIH3HNI2NTKvE/edit?usp=sharing), and I found mine on booking.com (Quarantine Hotel – Hotel B). It costed something about 700€ and there I got breakfast, lunch, and dinner. It was typical Taiwanese food for Take- Away from local restaurants and it was very nice. During these 8 days I relaxed a lot, read books, and prepared myself for the project. In conclusion I could get used to the new climate and time very calmly. From the airport I had to take a taxi to get to my hotel.

After these 8 days I was picked up by an assistant from my professor and a master student and was brought to my accommodation. In advance of the internship, my professor told me that the military medical school has dormitories within the campus, and he offered me to stay there. I accepted his offer, and I got a room, which I shared with an Indonesian exchange student. He was also doing an internship there (scientific research in the School of Dentistry). Our room and the bathroom were very dirty, and I hadn't much privacy, but at least I wasn't alone and it was for free. One big advantage was, that I could discuss problems with my roommate, e.g., when an experiment didn't go well. In the campus of this military base there were the dorms, the medical school, a hospital, and a training center. For example, I could swim in the pool and train in the gym for free after work.

1.4 Life in Taipei

I want to add a few annotations about things I took into consideration: First, I decided to take clothes for 10 days (10 underpants, 10 socks, 10 T- shirts, 3 shorts...) so that I would have enough clothes and time to do my laundry. During the summer it is very hot in Taiwan, approximately between 30 and 40 degrees with high humidity, so I was wearing shorts and t- shirts all the time. However, one hoodie as well as one water coat were necessary.

The second thing is the problem with the infectious diseases. In Taiwan, Malaria is not a problem, but You should be aware of COVID- 19, Dengue- Fever, HIV/AIDS, Hepatitis A and B, Scrub Typhus, Hand-Foot-Mouth-Disease and Japanese Encephalitis (JE). You can protect yourself with basic hygiene, the mnemonic: *peel it, wash it, boil it, cook it, or LEAVE IT* (concerning food or other oral intake) and vaccination against Hepatitis and JE. Because I am a medical student, I already had the vaccination against Hepatitis A and B and I decided myself against the JE- vaccination, because the specific mosquitoes are only active at night, and they live mainly near water (rice growing areas...).

Consequently, in Taipei and even during day trips in the mountains there wasn't a high risk. Nevertheless, I bought an anti- mosquito- spray with DEET for the skin and one for the clothes.

The third thing: Taiwan is called the *Kingdom of Insects*, concerning e.g., beautiful butterflies but also small crawling animals like spiders. However, at least in the cities they aren't poisonous.

I hadn't had a kitchen in the dorm, thus I had to grab the meals out- of- the- house. For breakfast I ate basic porridge, for lunch I could go to the restaurants in the medical school respectively the hospital and for dinner I went to the city center to night markets or other restaurants. Appeasing hunger was not cheap but not expensive either, meaning you can expect 150 – 400 NTD (between 5 – 14 €) per meal. For me, on the one hand I could live rent- free but on the other hand I haven't had the chance to cook cheaper for myself. Overall, eating outside and the fees for the quarantine hotel was more expensive than expected.

If you have an apartment with a kitchen and you want to cook your food, then you can buy groceries in *seven eleven (7/11)* (=ALDI), *Family Mart* (=LIDL) and *Watsons* (=DM). Except the latter, the stores also provide fresh food to eat there, like meat balls, eggs, and noodles. So, it is something like a supermarket and a cheap restaurant in one (for late night hunger).

In Taipei the public transport system is modern, on time and cheap. First you need to buy a public transport card, called *easy card* and then you can load money on it (e.g., in *seven eleven*) and then pay for the buses, MRT (Metro) and the rental bikes (something like *next bike* in Germany). I think it was about 3 - 5 €, when I used the MRT and bus a hole day.

The younger generations speak at least basic English, and the older ones normally understand the most important words. Even when I got lost in the forest during a hike, I could ask some tea farmers to help me find the right path. On the other side, you can use a few important Chinese words to make communication easier and to show interest in the Taiwanese culture. The most important ones are: "Hello" = "Nǐ hāo" (spelled: Ni hau in German) and "Thank you" = xièxiè (spelled: schesche in German).

In conclusion, I can only repeat what many others before me have already said: It is a beautiful island with very friendly and open- minded people and the perfect place to learn how live is in an Asian country and how scientific research is in a modern and internationally- connected lab! And don't worry about the Chinese signs: everything very important is also written in English (and if there is no English translation, then there will be just a low risk to do something forbidden).

1.4 Laboratory Environment

I was in the Department of Pharmacology, and I was mainly in the lab of my professor. There were a few bachelor and master students and one PhD student. All were very helpful and interested in teaching or showing me techniques and methods. The research center had a restaurant where we could have lunch together. I had an own desk in a bureau with other students, where I could do my research and the evaluation and analyzation. The different employees had different projects, so I had the chance to get an insight view on a variety of fields of research. That's the reason why I also got the chance to decide on what field and what project I want to do exactly. With my professor I discussed what is possible for me to realize and to do on my own and I decided to participate in his field of interest: the research of LVV- Hemorphin- 7.

1.5 Project: Isolation, purification, and quantification of LVV- Hemorphin- 7 and LVV- H7 filled Exosomes in venous and artery blood from mice and human

1.5.1 Introduction

“You have to know where you come from if you want to know who you are […]“ (Fulbert Steffensky *1933). Regarding this quote, our aim was to find out more about the point of origin and the mechanisms of release of a peptide in the tissues from living creatures.

Hemorphins are small endogenous peptides consist of just a few amino acids (between 4 and 8) and are also known as “atypical opioid peptides”. There is a variety of different sequences of the amino acids, leading to different biological molecules that have different targets (e.g., receptors) on different eucaryotic cells. Some targets are e.g., the μ-/ γ-/ κ- opioid receptors, the angiotensin IV- receptor, the bombesin- subtype 3-receptor or the corticotropin releasing factor (CRF)- receptor (Wei et al. 2020; Lammerich et al. 2003). The physiological and pathophysiological effects in the body range from effects on learning, memory, emotions, pain to the pathophysiology of cancer, Alzheimer’s disease, and diabetes (Lee et al. 2004; Cheng et al. 2012).

These Hemorphins are derived from Hemoglobin, the main protein in red blood cells (RBC, Erythrocytes) for transporting the needed oxygen (Nyberg et al. 1997). The data indicates that Proteasomes and Oligopeptidases play an important role in the metabolism of the Hemoglobin fragments (Ferro et al. 2014). Denatured Hemoglobin chains are processed, and the fragments are transported in intracellular vesicles, according to recent studies (Conigliaro et al. 2017; Aryani et al. 2016). The most likely candidates are the exosomes. These are small vesicles that are produced in the cells in multivesicular bodies and then are released into the extracellular space (the blood) through exocytosis (Van Niel et al. 2006). These exosomes could transport the Hemorphins over a long distance to the brain and even cross the blood- brain- barrier (Yang et al. 2015). They would be then able to function as transmitters respectively hormones.

The presence of oxygen respectively oxidative stress with Reactive- Oxygen- Species (ROS) could affect a change in degradation of Hb and the releasing rate of the Hemorphin- filled exosomes. Some studies (Benedikter et al. 2018) showed that there is a significant change in the activity of responsible enzymes in the pathways before and after oxidation (or under different oxygenation levels).

During my internship, we focused on LVV- Hemorphin 7 (LVV- H7), a Hemorphin with 7 amino acids (Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe). This peptide is the most stable form of Hemorphins (Ali et Al. 2019) and a candidate for drug therapy for a variety of diseases. For example, it is also tested if there is an improvement in cognitive performance after Traumatic Brain Injury (TBI). However, the presence of Hb- derived opioid-like peptides in RBC- derived vesicles is still unverified (Conigliaro et al. 2017).

1.5.2 Hypothesis

1. The higher the concentration, the nearer to the point of origin. The concentration of LVV- H7 in the exosomes should be the highest, in the cytosol it should be lower, and the plasma should have the lowest level.

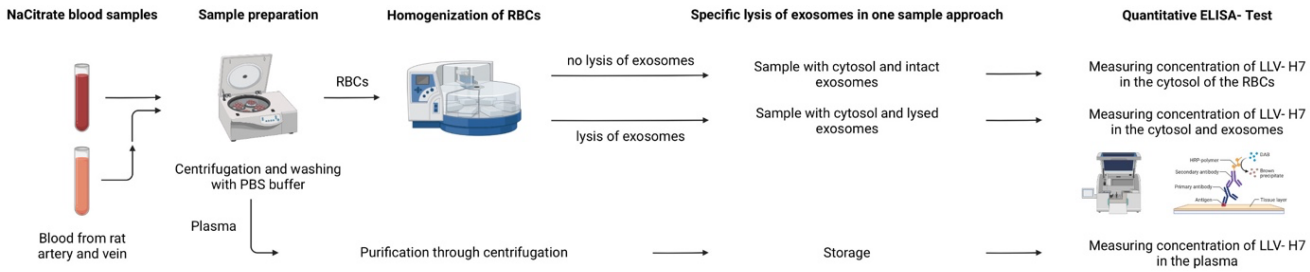
2. There are RBC- derived LVV- H7- filled exosomes.

3. There is a different concentration in arterial and venous blood from rats, because of the changing oxygenation- level.

1.5.3 Methods

First, we collected the human samples from healthy blood donors and the rat blood from animals, that were old and had to be sacrificed. We separated the plasma from the RBCs with centrifugation. The RBCs were then washed for several times with physiological PBS buffer to reduce the remaining LVV- H7 and were suspended 1:2 in PBS to get a physiological hematocrit of 40 %. To be sure that all the exosomes and peptides come from the inside of the cells, we had to check the cells for being intact under the microscope. After this washing process nearly all the cells were still intact. The next step was to lyse the cells without destroying the smaller vesicles, mainly the exosomes. For that task we used glass beads with three different diameter sizes (0,1 mm; 0,5 mm; 1,0 mm) for mechanical hemolysis. The success rate was measured and with this protocol we gained a rate of over 95 %. At this point, we got the cytosol out from the erythrocytes with intact vesicles and exosomes.

Then, we lysed halve of the cytosol with a chemical lysis buffer, that destroys every membrane in the sample (every vesicle and exosome). Later we compared the concentrations between the cytosol before and after lysis. We wanted to prove that the LVV- H7, that was assumed to be stored in the exosomes, was released due to the lysis and the concentration should increase consequently. These results were also put into comparison with the measured levels in the blood plasma. For the rat blood, we also compared the arterial and venous results with each other.

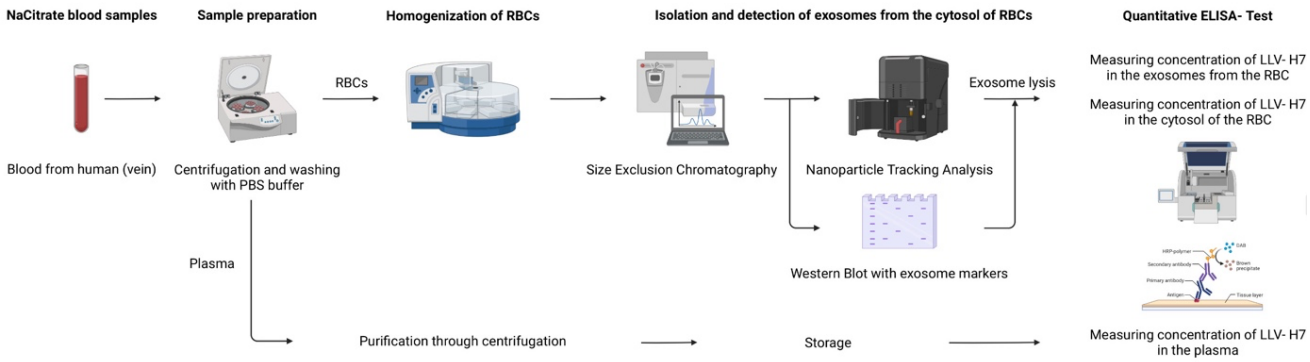


To get more specific results of the exosomes, we isolated them with Size exclusion chromatography (SEC). The Automatic- Fraction- Collector (AFC) from IZON Science LTD separates molecules and vesicles by their size. Bigger molecules and cell debris were filtered out. To not just get purified, but also enriched samples of exosomes, we used membrane filters, to concentrate all the small vesicles. At this point, we got a small amount of exosome isolate.

In the next step we lysed halve of the isolated exosome samples also with a chemical lysis buffer, that destroys every membrane in the sample (every exosome was destroyed). Later we compared the concentrations between the exosome isolate before and after lysis. We assumed again, that the LVV- H7 should be released due to the lysis and causes an increase of the concentration. With this approach we could prove, that the peptide is in fact stored in small vesicles (exosomes).

To measure the concentration of LVV- H7 in different samples, we used the ELISA- Test- Kit from Enzo Life Sciences Inc. This is a commercial kit, that can be purchased easily, and it detects the concentration of the peptide with a high specificity and sensitivity. At the end of the internship, we performed one ELISA- Test with all the samples and were then able to compare the different levels.

To characterize the vesicles, we used both the Western- Blot and the Nanoparticle Tracking Analysis (NTA). We used the human blood samples. With the Western- Blot we could detect specific markers, that are characteristic for exosomes and with the NTA we could measure the size (between 20 – 100 nm) and the concentration of the exosomes. If both techniques had positive results, we could be sure, that the vesicles are in fact exosomes and that these membrane structures store the LVV- H7.



1.5.4 Results

In humans, we measured a low concentration (nM) in the plasma and a much higher concentration in the cytosol. In rats, the level of LVV- H7 in the plasma is about 3 times higher, than in humans. But there was no difference between the arterial and venous plasma. In the cytosol of the rat's RBCs there was also a higher concentration than in the plasma, but not as high as in humans. In the cytosol of the rat arterial blood, the concentration was about 1,5 times higher than in the venous ones. We could find a difference caused by the different oxygenation level here.

The cytosol lysates and the exosome isolates and lysates couldn't be measured because there was a problem with the lysis buffer.

The results from the NTA take more time because it is a complex machine and it need to be used by special technicians.

With the Western- Blot we could find a marker, that indicates that the vesicles are exosomes.

1.5.5 Discussion

We could find out, that the LVV- H7 comes from the RBCs, because the concentration in the cytosol was much higher than in the plasma. Moreover, we could detect the exosomes with the NTA and Western- Blot and could prove that there are the RBC- derived vesicles. In the future, more samples should be used to get more accurate results.

We had problems with the lysis buffer and the lab in NDMC will repeat this part of the experiments and will send me the results, as well as the NTA results, so that I can analyze it in the future.