OPONENTSKÝ POSUDEK

Disertační práce:

„Microparticular carriers for construction of targeted drugs and vaccines"

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Předložená disertační práce se zabývá vývojem stabilních lyofilizovaných preparátů cytostatik (paklitaxelu a analogů vitaminu E - α-tokoferyl sukcinátu a α-tokoferyl amidomalátu) enkapsulovaných do lipozomů. Tato aplikační forma vede k výraznému zvýšení selektivity působení cytostatik vůči nádorovým buňkám při maximálním snížení toxicity vůči ostatním tkáním (kostní dřeň, leukocyty), což umožňuje použití vyšších dávek cytostatik potřebných pro účinnou protinádorovou léčbu. Použitím vhodných metodik přípravy preparátů bylo dosaženo vytvoření kladně nabitých lipozomů zaměřených na endoteliální buňky tumoru, což je velice důležité pro cílení účinku transportovaných léčiv.

Těžištěm předložené disertační práce jsou tři publikované články. První z těchto publikací se zabývá vývojem a přípravou lipozomů s vysokou enkapsulační kapacitou pro fázově specifické cytostatikum paklitaxel, a testováním jeho protinádorového účinku *in vivo* na myších modelech melanomu. Další práce je věnována přípravě a fyzikálně-chemické charakterizaci lyofilizovaného lipozomálního preparátu transportujícího α-tokoferyl sukcinát, semisyntetický analog α-tokoferolu se selektivní toxicitou vůči nádorovým buňkám. Ve třetí publikaci je popsána příprava lipozomálního preparátu transportujícího α-tokoferyl amidomalát, který vykazuje 10x vyšší účinnost proti nádorovým buňkám než dříve použitý analog vitamínu E, a testování jeho cytotoxicity proti rakovinným buňkám *in vitro* i *in vivo*. Vyvinutím lyofilizovaného lipozomálního preparátu bylo dosaženo odstranění nežádoucích účinků neurotoxicity a imunotoxicity volného α-tokoferyl amidomalátu i jeho nízké rozpustnosti při zachování cytostatického účinku na nádorové buňky.

Disertační práce ing. Štěpána Koudelky je velice kvalitně zpracovaná, předložená v angličtině (včetně velmi pěkně zpracované teoretické části). Autor využil nového, racionálního způsobu vytvoření disertační práce prezentací publikovaných prací s doplněním úvodu, cílů a závěru. Uvedené práce jsou pochopitelně týmovými pracemi, proto je třeba, aby autor specifikoval svůj podíl na řešení jednotlivých problematik.

K disertační práci mám jen několik dotazů:

1. Paklitaxel lze v současné době použít např. při léčbě nádorů prsu, plic, mediastina. Rozšíří Vámi vyvinutá forma preparátu spektrum diagnóz při kterých je tato látka účinná?

2. Jaký je mechanizmus proapoptotického účinku použitých analogů vitamínu E?

Závěr: Předloženou disertační práci považuji za vynikající, splňující všechny požadavky na ni kladené, a proto ji doporučuji k obhajobě.

V Pardubicích 2. 3. 2010

MUDr. Vladimíra Mužáková, Ph.D.

Assessment of the PhD Thesis of Mgr. Stepan Koudelka

General: In this thesis, the PhD candidate focused on the development of microcarrier systems for potential efficient delivery of anti-cancer drugs, as exemplified by an established agent paclitaxel (PTX) and an emerging group of novel anti-cancer drugs, vitamin E (VE) analogues. This is a very intriguing and important area of research, since often the unfavourable logistics of efficient delivery of anti-cancer drugs to the site of malignancy precludes development of frequently very promising compounds into drugs applicable for human clinical medicine. Therefore, in general terms, this thesis contributes to this area of research without which the 'quantum' leap from the bench to the bed-side would not be possible.

The thesis is well written, with neat usage of the English language. The first part nicely documents the problem at hand. Enough space is dedicated to the basics of chemistry and physics of liposomes and their use as vehicles for drug delivery. The candidate also provides sufficient information about the two groups of anti-cancer drugs that are studied, i.e. PTX and VE analogues. In general terms, there is no major problem with this part of the thesis and the few minor issues are detailed below. The thesis itself contains about 50 pages plus some 15 pages of references. Between the body of the text and the references, the candidate inserted three publications, on which he is the first author or a co-author and which are relevant in terms of the topic of the thesis. The three papers are published in solid pharmacological journals with impact factors of about 4, which is rather good for this area. It is commendable that the candidate arranged his thesis in this way, rather than writing a long thesis with a lot of text. It is my position that this way of putting a PhD thesis together is economical from the point of view of the resources as well as time. There is no better way to document the quality of a PhD candidate than having several original publications in good-impacting journals.

Some details on the PhD thesis are provided below. It is advisable that the applicant modifies his thesis accordingly.
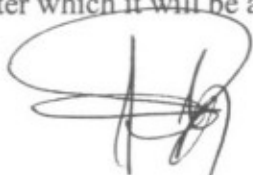
Detailed analysis:

1. I have a problem with the title of the thesis and its contents, the title being "Microparticular carriers for construction of targeted drugs and vaccines". The problem is that nowhere nowhere in the thesis the candidate mentions anything about vaccines! While I do appreciate that the microparticular carriers that are the major thrust of the thesis can be used for delivery of vaccines, the term 'vaccines' should not be in the title of the thesis. Similarly, the carriers can be used for delivery of other classes of therapeutic agents.

2. The candidate duly thanks his supervisors and colleagues, as well as several agencies that provided him with funds allowing this work to go ahead and be completed. However, there should be some acknowledgement of others who would have been involved in the experiments that are included in the thesis and that were not carried out by the candidate or his immediate colleagues. This is particularly the case of the mouse work *in vivo*, with the experiments with the FVB/N *c-neu* transgenic mouse and their treatment with liposomal α-TOS and α-TAM were abroad, where the ultrasound imaging technique to visualize and quantify tumours was also used. It is fair to acknowledge the origin of these results; at the same time it does not take anything from the quality of the work included in the thesis. On the contrary, it is commendable that the candidate was capable of initiating such collaboration(s), and this is a promise for the future career of the candidate that he will actively seek collaborations that are essential for high quality research.

3. Concerning the Theory, part 1: a lot of space is given to liposomes – their physico-chemical properties. However, the part on the use of liposomes as drug carriers is a bit too short in my mind. I would expect more here, since the major thrust of the thesis is the application of liposomal preparations of PTX and VE analogues in mouse cancer models

4. When it comes to chapter 2.2, there is a lot of data included (and rightly so), on the various preparations of PTX, most of them involving liposomal systems. One of the major points is the preparation of liposomal PTX for delivery to mice with tumours. The results pertinent to

this are included in Paper 1. While it is explained there, I think it would be a good idea to summarise these results in the main body of the thesis – otherwise, it is a bit awkward to try find them in the published paper(s). So, I would welcome a chapter, perhaps preceding the three papers, that would in some abbreviated manner describe what was published in the papers that makes a progress in the field. Otherwise, it is not clear (unless one really tries hard to fine the information in the papers), what was the improvement of the liposomal preparation developed in the course of the thesis when compared with the previously published papers.

5. Similar criticism can be used when it comes to part 3, where some data on the preparations of VE an analogues, as published by others, are mentioned. Again, it is not clear what the contribution of the candidate is without detailed reading of papers 2 and 3.

6. It should be also mentioned somewhere why the candidate chose PTX and VE analogues. These are two groups of drugs with rather different modes of action, so some logistics for their choice is needed to be included.

7. The choice of some of the references concerning VE analogue is not exactly correct and needs to be re-checked by the candidate. There are small mistakes (eg. on p. 42, bottom, the reference should be 'Prasad and Edwards-Prasad' rather than 'Prasad and Edwardsprasad').

8. I am wondering about the choice of animal models to show the anti-cancer efficacy of the liposomal preparations of PTX and VE analogues. The models for PTX and those for the VE analogues differ considerably. For both groups of drugs, the 'hollow fibre implant mouse model' was used. I find this model rather cumbersome and difficult to really comprehend. To me, this model present only a small improvement over testing the effect of drugs on cell lines. Maybe one of the reasons for my skepticism about this model is that the thesis lacks a Methods section, where the advantages of this model (except that nude mice do not have to be used) would be explained. Liposomal PTX was also tested on a syngeneic metastasis model. For the effects of liposomal formulations of VE analogues the hollow fibre model was used again while the metastasis model was not included. A transgenic model of breast cancer was also used, however this was done in another laboratory. Again, it would be nice to have some information in the main body of the text comparing the effects of the liposomal formulation prepared by the candidate when compared to the published data. Such as, what are the differences/similarities, is there an improvement over the published data etc.

9. The Thesis lacks a conclusions part. In fact, after the first part (Theory), which is pretty good, the thesis somehow 'fades away'. 'Aims of This Study' are mentioned on page 49. This is followed by the three nice papers, after which the References to the thesis follow, and the whole document is finalized by inclusion of a list of presentations of the candidate. What I really lack is a part where the candidate would summarise his findings and propose, hopefully, some further strategy – such as what these data may lead to, what other studies may follow etc. So, not only to repeat the major findings in the part, which is called 'Conclusion', on pages 84-86. This is even more important, since the Thesis does not contain the Discussion section where one would analyse the data in the light of data published by others. In any case, this would definitely improve the quality of the thesis and would give it a clear position within the 'translational' field of biomedical research.

10. The Annotation to the Thesis is a good idea. It is, principally, based on the three papers by the candidate. However it is put together in a somewhat sloppy manner. The first part lists Figures and there are Legends to individual figures – with the exception of Fig.3, which does not have a legend. Part two contains legends to figure, while part 3 does not. This ought to be fixed.

Conclusion: I recommend the candidate that he modifies the thesis according to the points mentioned above, after which it will be a good basis for awarding him the title of Doctor of Philosophy (PhD).