1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, *Fakulteta za farmacijo* University of Ljubljana, *Faculty of Pharmacy*

2. Ime in priimek mentorja (*Name and surname of a mentor*):

Marija Sollner Dolenc

3. Področje znanosti iz šifranta ARRS (*Primary research field*):

1.09 - Naravoslovno-matematične vede / Farmacija 1.09 - Natural sciences and mathematics / Pharmacy

4. Kontaktni e-naslov mentorja (*Contact of a mentor)*:

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5. Kratek opis programa usposabljanja (*Short description of the program)*:

SLO

Vpliv endokrinih motilcev na delovanje imunskega sistema in celične adhezijske procese

Predstavitev problema
Mnogi farmacevtski materiali, zdravilne učinkovine, kozmetične sestavine ter aditivi in njihovi presnovki imajo izražene neželene učinke na različne sisteme v živih organizmih. Zato je raziskovanje mehanizmov teh učinkov zelo pomembno, še posebno, ker so v telesu praviloma prisotni v majhnih količinah, mnogim pa smo izpostavljeni kronično zaradi uživanja hrane in vode, v katerih se kot okoljska onesnaževala lahko nahajajo. WHO navaja na osnovi objavljenih in nacionalnih študij, da se tako zdravila za humano in veterinarsko uporabo, kot tudi zdravila brez recepta nahajajo v površinskih vodah in podtalnici v razponu od ng/L do 0,1 µg/L (Pharmaceuticals in drinking-water, World Health Organization, 2012). Podobno velja za ostale prej omenjene skupine snovi. Poznavanje njihovega toksikološkega oz farmakološkega profila omogoča po eni strani oceno varnosti izpostavitve živih organizmov tem snovem, po drugi strani pa lahko služijo kot spojine vodnice za pripravo novih terapevtsko sprejemljivih (zdravilnih) učinkovin. Za mnoge spojine iz navedenih skupin in njihove presnovke je zelo malo podatkov o njihovih neželenih/toksičnih učinkih na endokrini sistem in ali modulirajo delovanje ostalih jedrnih oz transkripcijskih faktorjev ter integrinskih receptorjev.

Cilji usposabljanja mladega raziskovalca in metode, ki jih bo pri tem uporabil
Posledice delovanja zgoraj omenjenih spojin v endokrinem sistemu povezujejo z različnimi obolenji: od rakavih, neplodnosti do sladkorne bolezni. Snovi- endokrini motilci- modulirajo endokrini sistem in ostale receptorje preko različnih mehanizmov: vplivajo lahko na sintezo, sekrecijo, transport, vezavo na receptorje ter eliminacijo endogenih hormonov, ali pa je učinek epigenetski (Diamanti-Kandarakis, E., et al. Endocr Rev, 2009, 30, 293-342). Vse več študij kaže, da mnoge hormonsko aktivne spojine modulirajo procese adhezije celic preko aktivacije/inhibicije vitronektinskega receptorja (Davis et al., 2013, Horm. Cancer 4, 335-342). Predpostavljajo, da se vežejo v bližino RGD-vezavnega mesta, preko katerega se z največjo afiniteto med vsemi proteini ekstracelularnega matriksa veže vitronektin. S tem pa lahko bistveno prispevajo k zaviranju/razvoju rakavega obolenja. Vitronektinski receptor je namreč v nekaterih vrstah raka še posebno ekspresiran, ima pa pomembno vlogo pri metastaziranju in razrastu krvnih žil (angiogenezi)- nujnemu pogoju za preživetje rakavega tkiva. Tako je

vitronektinski receptor tudi zanimiva tarča za razvoj novih protirakavih učinkovin (Perdih et al., 2010, Curr. Med. Chem. 17, 2371-92).V namen bolje razumeti učinke spojin bo maldi raziskovalec skušal razviti ustrezne in vitro metode (celične linije, računalniške (in silico) metode) za preverjanje mehanizma tovrstnega delovanja ter z obstoječimi in vitro/in silico metodami (npr. luciferazni reporterski test na MDA-kb2, GH3.TRE.luc celičnih linijah, Endocrine disruptome) ugotavljati endokrino delovanje posameznih skupin zgoraj omenjenih spojin, kjer bo posebno pozornost posvetil delovanju spojin na glukokortikoidni in tiroidni sistem.
Družina hormonskih jedrnih receptorjev vključuje estrogenske, androgene, progesteronske, glukokortikoidne, mineralokortikoidne, tiroidne ter retinoidne receptorje, na katere lahko delujejo snovi (hormonski motilci) agonistično ali antagonistično. Najbolje je raziskano moduliranje estrogenskega endokrinega sistema. Iz literturnih podatkov (Rogers, et al., 2013. Mol. Immunol. 53, 421-430), kot tudi lastnih preliminarnih raziskav (Švajger et al., 2016, International Immunopharmacol. 34, 146-154) je razvidno, da snovi, tako eksogene (npr. bisfenol A), kot endogene (endogeni estrogeni), ki modulirajo estrogenske receptorje, vplivajo tudi na delovanje imunskega sistema. Mladi raziskovalec bo skušal ugotoviti, kakšni so molekulski mehanizmi teh učinkov na nekaterih najbolj poznanih hormonskih motilcih, pri čemer bomo uporabili različne celične linije in humane celične modele (npr. THP-1, Jurkat celične linije, makrofagi, dendritske celice).
Toksikološke in farmakološke študije neke snovi potekajo trenutno z izpostavitvijo samo eni (testirani) snovi. V realnem življenju pa so živi organizmi izpostavljeni več snovem hkrati, zato bo mladi raziskovalec skušal ugotoviti, kako zmesi hormonskih motilcev interagirajo med seboj in tako izražajo aditivni/ sinergistični učinek na prej omenjene učinke, ki jih lahko izražajo.

Izvirnost rezultatov
Predlagane raziskave bodo prispevale k razumevanju vpliva endokrinih motilcev na celično adhezijo ter imunske procese in tako osvetlile mehanizme, preko katerih endokrini motilci zaviranjo/pospešujejo razvoj rakavega obolenja. Napovedovanje z in silico in ugotavljane z drugimi in vitro metodami endokrinih, zlasti glukokortikoidnih učinkov posameznih spojin iz zgoraj omenjenih skupin, bo dala nove informacije o resnosti učinkov teh spojin sproščenih v okolje za vodne organizme in človeka.

ANG

Influence of endocrine disruptors on the modulation of the immune system and adhesion processes
Many pharmaceutical materials, drugs, cosmetic ingredients, additives and their metabolites have expressed adverse effects on the various systems in living organisms. Therefore, the research of mechanisms of these effects is very important, especially because they are in the body present in small amounts. We are chronically exposed to them through consumption of food and water, where may be found as environmental pollutants. WHO states on the basis of published studies and national studies that medicinal products for human and veterinary use, as well as non-prescription medicines are presented in surface waters and groundwater in the range of ng/L to 0.1 mg/L (Pharmaceuticals and drinking - water, World Health Organization, 2012). The same is true for the majority of the above-mentioned groups of substances. Knowledge of their toxicological and pharmacological profiles allows on the one hand, the safety assessment of exposure of living organisms to these substances, on the other hand, can serve as lead compounds for the development of new therapeutically acceptable (active) drugs. For many compounds from those groups and their metabolites, there are very little data on their adverse / toxic effects on the endocrine system and whether modulate the activity of nuclear or other transcription factors and integrin receptors.

Objectives of young researcher training and the methods that will be used
The modulation of endocrine systems has been associated with a variety of diseases from cancer, infertility to diabetes. Substances - endocrine disruptors - modulate the endocrine system and other nuclear receptors (e.g. AhR, PPARγ, PXR) through different mechanisms: they can affect the synthesis, secretion, transport, binding to receptors and elimination of endogenous hormones, or they have the epigenetic effect (Diamanti - Kandarakis, E., et al. Endocr. Rev., 2009, 30, 293-342). More and more studies show that many hormone-active compounds modulate cell adhesion processes through the activation/inhibition of vitronectin receptor (αVβ3) (Davis et al., 2013, Horm. Cancer 4,

335-342). It is assumed that they bind in the vicinity of the RGD-binding site (recognition site for EMC proteins, e.g. vitronectin) on the vitronectin receptor. This could significantly contributes to the prevention / development of cancer, since vitronectin receptor is especially expressed in certain types of cancer (Perdih et al., 2010, Curr. Med. Chem. 17, 2371-92). It has an important role in metastasis and regrowth of blood vessels (angiogenesis) - a necessary condition for the survival of cancerous tissue. The young researcher will try to develop appropriate in vitro methods (cell lines, computer methods) to verify the activity of selected endocrine disruptors on the adhesion processes. With the screening in vitro/in silico methods (eg. luciferase reporter gen assay on MDA-KB2, GH3.TRE.luc cell lines, Endocrine disruptome,), evaluation of the endocrine function of individual groups of compounds mentioned above will be studied, where the young researcher paid particular attention to the functioning of the compounds on the glucocorticoid and thyroid system.

The family of hormone nuclear receptors includes estrogen, androgen, progesterone, glucocorticoid, mineralocorticoid, thyroid and retinoid receptors, which may act agonistic or antagonistic. It is best studied modulation of estrogen endocrine system. From literature data (Rogers, et al., 2013. Mol. Immunol. 53, 421-430), as well as from our own preliminary studies (Švajger et al., 2016, International Immunopharmacol. 34, 146-154), it is clear that exogenous compounds (e.g. bisphenol A) and endogenous estrogens (e.g. estradiol) which both modulate estrogen receptors, may also affect the immune system, but their immunomodulating activity is poorly characterized. The young researcher will try to figure out what are the molecular mechanisms of these effects in some of the best known endocrine disruptors. For the evaluation of their immunomodulatory activity the variety of cell lines and human cell models (eg. THP-1, Jurkat cell lines, macrophages, dendritic cells) will be used.

Toxicological and pharmacological studies of some substance are currently investigated and evaluating by exposing only to one substance. In real life organisms are exposed to multiple agents simultaneously, so the young researcher will evaluate, if mixtures of endocrine disruptors interact with each other and thus exhibiting an additive/synergistic effects on immune system or adhesion processes.

Originality of the results

The proposed plan of work, in which young researcher will try with appropriate in vitro methods (cell lines, computer (in silico) methods) to verify the activity of selected endocrine disruptors on the adhesion processes and immune system is an new area of research and will give a new insight how the endocrine disruptors may contribute to cancer development or suppression. The evaluation with in silico and other in vitro methods (eg. luciferase reporter gen assay on MDA-KB2, GH3.TRE.luc cell lines) the endocrine, particularly glucocorticoid function of individual compounds from groups of chemicals mentioned above will give new data in the field of exploring the subtle effects of endocrine disruptors released to environment to aquatic biota and humans.