8. KONGRES SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

IZZIVI VREDNOTENJA IMUNOTOKSIČNOSTI SNOVI
CHALLENGES IN ASSESSING THE IMMUNOTOXICITY OF SUBSTANCES

Ljubljana, 22. 10. 2026

NACIONALNI INŠTITUT ZA BIOLOGIJO
NATIONAL INSTITUTE OF BIOLOGY

UNIVERZA V LJUBLJANI

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ZBORNIK POVZETKOV

Založnik in organizator/Publisher and organizer:
Slovensko toksikološko društvo, Gerbičeva 60, Ljubljana

Urednici in recenzentki/Editors and reviewers: Marija Sollner Dolenc, Smiljana Milošev Tuševljak

Organizacijski odbor/Organising committee: Marija Sollner Dolenc, Smiljana Milošev Tuševljak, Lucija Peterlin Mašič, Vesna Gorenjak, Bojana Žegura, Alja Štern, Gorazd Drevenšek, Marjan Vračko, Urška Kolar

Kraj in leto izdaje: Ljubljana, 2026

Elektronska objava
Spletna izdaja
Dostopno na spletni strani Slovenskega Toksikološkega društva:
https://www.tox.si/wp-content/uploads/2026/10/Zbornik-8-SloTox-kongres-2026.pdf

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 300853507
ISBN 978-961-91445-8-9 (PDF)

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SPREMNA BESEDA

Spoštovani udeleženci 8. kongresa Slovenskega toksikološkega društva!

Imunski sistem je visoko organiziran in dinamičen sistem, sestavljen iz medsebojno povezanih celic, tkiv in organov, katerih aktivnosti uravnavajo kompleksni regulatorni mehanizmi. Poleg osrednje vloge pri prepoznavanju in odstranjevanju patogenov ter odzivanju na signale poškodbe ali stresa gostitelja pomembno prispeva k homeostazi organizma. Vključen je v številne ključne biološke procese, kot so presnovna regulacija, nevrološki razvoj, delovanje centralnega živčnega sistema, reproduktivni procesi ter prebava in absorpcija hranil. Glede na kompleksnost imunskega sistema ni presenetljivo, da lahko širok spekter snovi, s katerimi se srečujemo v vsakdanjem življenju, vpliva na imunski sistem in potencialno spreminja njegove funkcije. Imunotoksične snovi lahko delujejo prek različnih mehanizmov, vključno z imunosupresijo, imunostimulacijo, senzibilizacijo, razvojem avtoimunosti ter neposrednimi toksičnimi učinki na celice in sestavne dele imunskega sistema. Čeprav je težko oceniti natančno število potencialno imunotoksičnih snovi, so bile le-te potrjene v različnih skupinah snovi, od kemikalij, zdravil, sestavin kozmetičnih izdelkov, aditivov do onesnaževal. Najbolje dokumentirane imunotoksične lastnosti imajo trenutno spojine iz skupine kemikalij. Trenutno ta seznam vključuje per- in polifluoroalkilne snovi (PFAS), policiklične aromatske ogljikovodike (PAH), klorirana topila, pesticide, sestavine plastike. Spomnimo, da je Evropska agencija za varnost hrane (EFSA, European Food Safety Authority) leta 2023 znižala dopustni dnevni vnos (TDI, Tolerable Daily Intake) za bisfenol A za kar 20.000-krat, in sicer s 4 µg na 0,2 ng na kilogram telesne mase ravno zaradi potrjenega vpliva na imunski sistem.
V preteklosti se je ocenjevanje imunotoksičnosti snovi izvajalo predvsem z uporabo živalskih modelov (npr. test odziv protiteles, odvisen od T-celic (TDAR), test lokalnih limfnih vozlov (LLNA)). Premik paradigme v smeri preskušanja toksičnosti brez uporabe živali je spodbudil razvoj novih, za človeka relevantnih metod preskušanja in napovedovanja imunotoksičnosti in vitro ter in silico. To ponuja izjemno priložnost za vzpostavitev in standardizacijo robustnih, zanesljivih in celovitih novih metodoloških pristopov (NAM) za ocenjevanje imunotoksikoloških lastnosti različnih snovi ter s tem oceno tveganja za zdravje ob izpostavitvi tovrstno delujočim spojinam. Kljub izpostavljeni potrebi po takih novih pristopih s strani Svetovne zdravstvene organizacije (WHO) in Mednarodnega programa za kemijsko varnost že leta 2012 pa znanstveno soglasje in splošno sprejeti standardi za preskušanje imunotoksičnosti kemikalij v obdobju opuščanja preskusov na živalih še niso vzpostavljeni. Le omejeno število metod za oceno imunotoksičnosti in vitro je doseglo status testnih smernic OECD. Mednje spadajo TG 444A (preskus IL-2 Luc za ocenjevanje imunotoksičnih učinkov kemikalij na T-celice), TG 442D (testna metoda ARE-Nrf2 luciferaze za ocenjevanje aktivacije keratinocitov) in TG 442E (serija testov za določanje aktivacije dendritičnih celic v okviru kožne senzibilizacije).
Izzivov na področju določanja imunotoksičnosti snovi je tako veliko, in zanimivo bo spremljati v prihajajočih letih razvoj novih metod za določanje spojin, ki modulirajo imunski sistem. Prepričani smo, da program kongresa vključuje tematike, ki bodo za številne udeležence še posebej relevantne, saj se z njimi ali s sorodnimi metodološkimi pristopi srečujejo tudi pri svojem raziskovalnem oziroma strokovnem delu. Ob tem si želimo, da bi predstavljena znanja in izkušnje spodbudili nadaljnje sodelovanje ter nadgradnjo znanstvenega in strokovnega znanja na področju imunotoksikologije v slovenskem prostoru.

Marija Sollner Dolenc
Smiljana Milošev Tuševljak

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PROGRAM/PROGRAMME

8.30-9.00	Registracija/Registration
9.00-9.10	Pozdravni nagovor/Welcome address
9.10-11.10	**Simpozij 1/Symposium 1** **Nove metode določanja imunotoksičnosti snovi/*New methods for determining the immunotoxicity of substances*** Predsedujoči/Chair: Marija Sollner Dolenc
9.10-9.40	Nove metode določanja imunosupresije snovi/*New methods for determining immunosuppressive properties of substances* Etienne Blanc, Univerza v Rennu, Francija
9.40-10.10	Nove metode določanja preobčutljivosti snovi/*New methods for determining substance sensitization* Emanuela Corsini, Univerza v Milanu, Italija
10.10-10.40	Predstavitev nove *In vitro* metode določanja imunotoksičnosti snovi/*Presentation of a new in vitro method for determining immunotoxicity of substances* Nina Franko, Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija
10.40-11.10	Nove metode testiranja razvojne imunotoksičnosti/*New methods for testing developmental immunotoxicity* Nataša Petrovič, Nacionalni inštitut za javno zdravje, Slovenija
11.10 -11.30	Odmor / *Coffe break*
11.30-12.30	**Simpozij 2/Symposium 2** **Dejavniki, ki vplivajo na toksičnost snovi/*Factors affecting the toxicity of substances*** Predsedujoči/Chair: Lucija Peterlin Mašič
11.30-11.50	Vpliv spola in starosti na imunski sistem/*Influence of sex and age on the immune system* Tijana Markovič, Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija
11.50-12.10	Vpliv endokrino delujočih snovi na imunski system/*Influence of endocrine-active substances on immune system* Anja Kodila, Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija
12.10-12.30	Imunoproteasom in inflamasom kot tarča imunoaktivnih snovi/*Immunoproteasome and inflammasome as targets of immunoactive substances* Martina Gobec, Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija
12.30-13.30	Kosilo in ogled plakatov / Lunch and poster viewing
13.30-15.00	**Simpozij 3/Symposium 3** **Primeri imunotoksičnosti izbranih onesnaževal in zdravil/*Examples of immunotoxicity of selected pollutants and drugs*** Predsedujoči/Chair: Smiljana Milošev Tuševljak
13.30-14.00	Neželeni učinki zdravil in imunotoksičnost/*Adverse drug reactions and immunotoxicity* Jasmina Klopčič in Jurij Žel, Javna agencija Republike Slovenije za zdravila in medicinske pripomočke
14.00-14.30	Imunostoksičnost PFAS/*Immunotoxicity of PFAS* Lucija Perharič, Nacionalni inštitut za javno zdravje, Slovenija
14.30-15.00	Cepiva in imunotoksičnost/*Vaccines and immunotoxicity* Nina Grasselli Kmet, Univerzitetni klinični center Ljubljana, Slovenija
15.00-15.30	Odmor in ogled posterjev/*Coffee break and poster session*
15.30-17.00	**Simpozij 4/Symposium 4** **Ocena tveganja izpostavljenosti imunotoksičnim snovem in zakonodaja/*Risk assessment of exposure to immunotoxic substances and legislation*** Predsedujoči/Chair: Mark Stanojević
15.30-15.50	Imunogenost terapevtskih peptidov – regulatorne zahteve/*Immunogenicity of therapeutic peptides – regulatory requirements* Darja Gramec Skledar, Lek d.d., Slovenija
15.50-16.10	Preobčutljivost na sestavine v hrani/*Hypersensitivity to food ingredients* Evgen Benedik, Biotehniška fakulteta/Univerzitetni klinični center Ljubljana, Slovenija
16.10–16.30	Regulatorni vidiki določanja imunotoksičnosti pri pripravi poročila o varnosti kozmetičnega izdelka/*Regulatory Aspects of Immunotoxicity Assessment in the Preparation of a Cosmetic Product Safety Report* Mark Stanojević, BiSafe, Slovenija
16.30-17.00	**Zaključne misli in podelitev nagrade za najboljši poster/*Closing remarks and best poster award ceremony*** Marija Sollner Dolenc, Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija

POVZETKI VABLJENIH PREDAVANJ

Nove metode določanja imunotoksičnosti snovi/*New methods for determining the immunotoxicity of substances*

New methods for determining immunosuppressive properties of substances

Etienne Blanc

University of Rennes, France

Currently, the assessment of chemical immunotoxicity relies predominantly on animal models and *in vitro* assays only addressing specific endpoints, particularly immunosuppression and sensitization. Animal-based approaches are associated with ethical and economic constraints and may have limited predictivity for human health. Moreover, they do not always provide sufficient mechanistic insight into the biological events underlying immunotoxic effects. There is therefore a growing need for human-relevant New Approach Methodologies (NAMs) that can support mechanistic and regulatory assessment within integrated and tiered testing strategies.

Ongoing research initiatives, including the Partnership for the Assessment of Risks from Chemicals (PARC), aim to develop and advance a range of human-relevant *in vitro* systems addressing key events associated with immune system function and dysfunction. These approaches cover major regulatory gaps in immunotoxicity, including immunosuppression, immunostimulation, respiratory sensitization, barrier immunity and developmental immunotoxicity.

This presentation will focus on NAMs aimed at identifying immunosuppressive effects. The development and regulatory applicability of such methods require consideration of several complementary aspects, including their relevance to regulatory endpoints, their ability to address key characteristics of immunotoxicants, and their integration within Adverse Outcome Pathways (AOPs) and Integrated Approaches to Testing and Assessment (IATAs). In parallel, efforts to characterize key events and establish mechanistic relationships between NAM-derived data are also supporting the development of new AOPs. Together, these developments are expected to contribute to more mechanistically informed, human-relevant, and regulatory-ready strategies for the assessment of immunotoxicity.

From Mechanisms to Human Risk: Advancing Immunotoxicity Assessment with NAMs

Emanuela Corsini

University of Milano, Italy

Immunotoxicity assessment is transitioning from conventional animal testing toward mechanistically informed, human-relevant New Approach Methodologies (NAMs). The complexity of the immune system requires strategies capable of addressing multiple functions and distinguishing immunosuppression from inappropriate immunostimulation. This presentation reviews the evolving *in vitro* landscape, including computational models, validated assays such as the Multi-ImmunoTox Assay, and human PBMC-based approaches assessing functional endpoints such as antigen-specific antibody production. Emerging stem cell-derived systems, organoids, and microphysiological models provide opportunities to increase physiological relevance and address complex endpoints, including developmental immunotoxicity. Integration of these approaches with AOPs, Key Characteristics of immunotoxicants, PBK modelling, and QIVIVE offers a path from mechanistic hazard identification toward quantitative human risk assessment. Ultimately, complementary, tiered testing strategies combining functional, mechanistic, and computational evidence can improve predictivity, reduce reliance on animal testing, and support next-generation immunotoxicity assessment.

Predstavitev nove *in vitro* metode določanja imunosupresivnih snovi

Nina Franko[1], Pika Pollak Sicherl[1], Tijana Markovič[1], Marija Sollner Dolenc[1]

[1]Univerza v Ljubljani, Fakulteta za farmacijo, Slovenija

Ocena imunosupresivnih učinkov ksenobiotikov ostaja zahtevna, saj se testiranje imunotoksičnosti na višjih ravneh še vedno v veliki meri opira na živalske modele ali človeški material ex vivo. V skladu z načeli 3R in naraščajočo potrebo po novih pristopnih metodologijah (NAMs) obstaja potreba po robustnih, za človeka relevantnih *in vitro* sistemih, ki omogočajo zajem funkcionalno pomembnih imunskih interakcij. V tej raziskavi smo optimizirali in ovrednotili človeški večcelični kokulturni model, zasnovan za posnemanje ključnih značilnosti polne krvi in zaznavanje imunosupresivnih snovi.

Model vključuje monocite THP-1, T-limfocite Jurkat in limfoblastoidne celične linije (LCL), gojene v mediju z zmanjšano vsebnostjo komponent živalskega izvora, s čimer smo izboljšali relevantnost za človeka ter etično in praktično izvedljivost sistema. Imunosupresivne učinke smo ocenjevali z merjenjem sproščanja interlevkina-8 (IL-8) po stimulaciji in izpostavitvi snovem, pri čemer smo rezultate normalizirali glede na metabolno aktivnost celic. Optimizirana trikomponentna kokultura je izkazala dobro biokompatibilnost in povečano izločanje IL-8 v primerjavi z monokulturami, kar kaže na funkcionalno medcelično komunikacijo in izboljšano imunsko odzivnost.

Za ovrednotenje učinkovitosti modela smo testirali panel 12 znanih imunosupresivnih snovi in 5 ne-imunosupresivnih kontrol v nano- in mikromolarnem koncentracijskem območju. Pri koncentraciji 10 μM je test pokazal visoko napovedno zmogljivost na podlagi izračunanih parametrov, kot so specifičnost, pozitivna napovedna vrednost, občutljivost, točnost in Matthewsev korelacijski koeficient. S tem model potrjuje zanesljivo razlikovanje med imunosupresivnimi in neimunosupresivnimi spojinami. Poleg tega je uporaba modela za bisfenol S in izbrane derivate pokazala, da TGSA pri mikromolarnih koncentracijah deluje imunosupresivno.

Predstavljeni kokulturni model na osnovi človeških celičnih linij predstavlja robusten, ponovljiv in etično sprejemljiv *in vitro* NAM za primarno presejalno testiranje imunosupresivnih učinkov ksenobiotikov ter obetavno orodje za zgodnjo identifikacijo nevarnosti in določanje prioritet pri oceni imunotoksičnosti.

New *in vitro* method for the assessment of immunosuppressive substances

Nina Franko[1], Pika Pollak Sicherl[1], Tijana Markovič[1], Marija Sollner Dolenc[1]

[1]University of Ljubljana, Faculty of Pharmacy, Slovenia

The assessment of immunosuppressive effects of xenobiotics remains challenging, as highertier immunotoxicity testing still largely depends on animal models or human ex vivo material. In line with the 3R principles and the increasing demand for new approach methodologies (NAMs), there is a need for robust and human-relevant *in vitro* systems capable of capturing functionally relevant immune interactions. In this study, we optimised and evaluated a human multicellular coculture model designed to mimic key aspects of whole blood and enable the detection of immunosuppressive substances.

The model integrates THP-1 monocytes, Jurkat T lymphocytes, and lymphoblastoid cell lines (LCLs) cultured in a medium with reduced animal-derived components to improve both human relevance and ethical feasibility. Immunosuppressive effects were assessed by measuring interleukin-8 (IL-8) release following stimulation and chemical exposure, with responses normalised to cellular metabolic activity. The optimised three-component coculture exhibited good biocompatibility and enhanced IL-8 secretion compared with monocultures, indicating functional intercellular communication and improved immune responsiveness.

To evaluate model performance, a panel of 12 known immunosuppressive substances and 5 non-immunosuppressive controls was tested across nano- and micromolar concentration ranges. At 10 μM, the assay demonstrated strong predictive performance, as judged by determined specificity and positive predictive value, alongside with a sensitivity and a Matthews correlation coefficient. The model was found to support reliable discrimination between immunosuppressive and non-immunosuppressive compounds. Furthermore, application of the model to bisphenol S and selected derivatives identified TGSA as an immunosuppressive compound at micromolar concentrations.

Overall, the presented human cell line–based coculture model represents a robust, reproducible, and ethically aligned NAM for the primary screening of immunosuppressive effects of xenobiotics and offers a promising tool for early hazard identification and prioritisation in immunotoxicity assessment.

Razvojna imunotoksičnost

Nataša Petrovič

Nacionalni inštitut za javno zdravje, Slovenija

Razvojna imunotoksikologija (Developmental Immunotoxicity; DIT) je področje, ki se osredotoča na to, kako izpostavljenost okoljskim dejavnikom, kot so ksenobiotiki, kemikalije in farmacevtski izdelki v zgodnjih obdobjih življenja vpliva na razvoj imunskega sistema kar lahko vodi v dolgotrajne motnje v delovanju, vključno z alergijami, astmo, avtoimunskimi boleznimi ter dovzetnostjo za okužbe. Razumevanje DIT je pri tem ključnega pomena vsled večji ranljivosti razvijajočega imunskega sistema v primerjavi z odraslimi, zlasti v kritičnih obdobjih razvoja zarodka. Izpostavljenost lahko vpliva na imunsko toleranco, diferenciacijo in funkcionalno zorenje imunskih celic.

Trenutno testiranje DIT se opira predvsem na živalske modele (*in vivo*), ki so omejeni predvsem zaradi vrstno-specifičnih razlik v imunskem razvoju in relevantnosti za ljudi. Upoštevaje etične vidike in načela 3R (zamenjava, izboljšanje, omejitev) se razvijajo alternativne metode (NAMi) za testiranje DIT. Le-te vključujejo (1) *in vitro* testiranje bioloških sistemov kot so hematopoetske matične celice, timusni organoidi in organi na čipih, ki natančneje posnemajo imunski razvoj človeka v primerjavi z živalskimi modeli; ter (2) in silico modeliranje za napovedovanje imunskih motenj. Poleg priložnosti za nadomestitev testiranja na živalih pa je posebnega pomena zagotoviti a) zanesljivost novih metod z doslednim izpolnjevanjem meril za validacijo, b) ustreznost za zdravje ljudi, c) natančnost napovedi in d) regulativno sprejemljivost.

Glavni izziv pri strategiji NAMov za testiranje DIT je prevajanje podatkov v učinke relevantne za zdravje ljudi, zato gredo raziskave v smer podpore z umetno inteligenco in strojnim učenjem, optimizacije za regulativne namene in razumevanja kritičnih oken imunske ranljivosti za boljšo zaščito zdravja otrok.

Developmental immunotoxicity

Nataša Petrovič

National Institute of Public Health, Slovenia

Developmental Immunotoxicity (DIT) is a field that focuses on how exposure to environmental agents such as xenobiotics, chemicals and pharmaceuticals during early life affects the development of the immune system, which can lead to long-term dysfunctions, including allergies, asthma, autoimmune diseases and susceptibility to infections. Understanding DIT is crucial due to the greater vulnerability of the developing immune system compared to adults, especially during critical periods of embryonic development. Exposure can affect immune tolerance, differentiation and functional maturation of immune cells.

Current DIT testing relies mainly on animal models (*in vivo*), which are limited mainly due to species-specific differences in immune development and relevance to humans. Due to ethical considerations and the 3R principles (Replace, Refine, Reduce), alternative methods (NAMs) for DIT testing are being developed. These include (1) *in vitro* testing of biological systems such as hematopoietic stem cells, thymic organoids, and organs on chips that more closely mimic human immune development compared to animal models; and (2) in silico modeling for predicting immune disorders. In addition to the opportunity to replace animal testing, it is of particular importance to ensure a) the reliability of new methods by consistently meeting validation criteria, b) relevance to human health, c) accuracy of predictions, and d) regulatory acceptability.

A key challenge in the NAMs' strategy for DIT testing is translating data into effects relevant to human health, and research is moving in the direction of supporting artificial intelligence and machine learning, optimizing for regulatory purposes, and understanding critical windows of immune vulnerability to better protect children's health.

POVZETKI VABLJENIH PREDAVANJ

Dejavniki, ki vplivajo na toksičnost snovi/*Factors affecting the toxicity of substances*

Vpliv spola in starosti na imunski sistem

Tijana Markovič[1], Nina Franko[1], Anja Kodila[1], Irena Mlinarič-Raščan[1], Marija Sollner Dolenc[1]

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Biološki spol in starost predstavljata vir interindividualne variabilnosti v imunskem odzivu ter vplivata na dovzetnost za okužbe, učinkovitost cepljenja, avtoimunost in imunotoksičnost ksenobiotikov. Razlike med spoloma v imunskem odzivu izvirajo iz sestave spolnih kromosomov ter steroidnih hormonov, ki prek svojih receptorjev uravnavajo prirojen in pridobljen imunski odziv. Geni kot sta TLR7 in TLR8 se lahko izognejo inaktivaciji kromosoma X, kar lahko skupaj s signalizacijo gonadnih steroidnih hormonov prispeva k razlikam med spoloma v imunskem odzivu, vključno z močnejšim odzivom žensk na nekatere povzročitelje okužb in cepiva, ter tudi z večjo dovzetnostjo za določene avtoimunske bolezni. Na imunski odziv vplivajo tudi ravni steroidnih hormonov, ki se tekom življenja spreminjajo. Staranje preoblikuje imunski odziv z imunosenescenco in kroničnim nizko-intenzivnim vnetjem, kar povečuje dovzetnost za okužbe, maligne in vnetne bolezni ter znižuje učinkovitost cepljenja. Vključevanje spola in starosti kot ključnih bioloških spremenljivk lahko tako izboljša biološko relevantnost vrednotenja imunotoksičnosti spojin.

Z namenom preučevanja vpliva spola na imunotoksičnost bisfenolov smo ovrednotili citotoksične in imunomodulatorne učinke bisfenola A (BPA) ter analogov BPE, BPAF, BPAP, BPPH, TMBPF in TCBPA v limfoblastoidnih celičnih linijah (LCL) desetih darovalcev. Višje povprečne vrednosti IC_{50} smo določili pri vseh spojinah v celicah LCL moških darovalcev (N= 5), pri BPE pa je bila razlika statistično značilna (ženske: 140,2 μM; moški: 173,5 μM; p < 0,05). BPA, BPAF, BPPH in TMBPF (10 μM) so značilno znižali z PMA/ionomicinom aktivirano izločanje IL-2, IL-6, IL-10 in TNF-α; najizrazitejši imunosupresivni učinek je imel BPPH. BPA in TMBPF sta statistično značilno znižala izločanje IL-2 v celicah LCL moških darovalcev v primerjavi z ženskimi, medtem ko večina drugih razlik med spoloma pa ni bila statistično značilna. Imunotoksičnost bisfenolov v celicah LCL je bila odvisna od spojine, koncentracije, darovalca in spola.

Vključevanje spolno uravnoteženih in starostno stratificiranih *in vitro* modelov lahko izboljša biološko relevantnost in interpretacijo imunotoksikoloških raziskav.

Influence of sex and age on the immune system

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Biological sex and age are major determinants of interindividual variability in immune responses, influencing susceptibility to infection, vaccine efficacy, autoimmunity, and responses to xenobiotics. Sex differences in immunity arise from the sex chromosome complement, which affects X-linked gene expression, and from gonadal steroids, which regulate innate and adaptive immune cells through their receptors. Escape of immune genes such as TLR7 and TLR8 from X-chromosome inactivation, together with gonadal steroid signalling, may contribute to sex differences in immune responsiveness, including stronger responses to some pathogens and vaccines in females but also greater susceptibility to certain autoimmune and inflammatory diseases. These differences change throughout life as gonadal hormone levels vary. Ageing further remodels immunity through immunosenescence and inflammageing, increasing vulnerability to infection, malignancy, and inflammatory disorders while reducing vaccine effectiveness. Considering sex and age may therefore improve immunotoxicity assessment.

To investigate the influence of sex on bisphenol immunotoxicity, we evaluated the cytotoxic and immunomodulatory effects of bisphenol A (BPA) and its analogues BPE, BPAF, BPAP, BPPH, TMBPF, and TCBPA in lymphoblastoid cell lines (LCLs) from ten unrelated donors (five female and five male). Higher mean IC_{50} values for all compounds were determined in male-derived LCLs, with a statistically significant difference for BPE (female: 140.2 µM; male: 173.5 µM; p < 0.05). At 10 µM, BPA, BPAF, BPPH, and TMBPF significantly reduced PMA/ionomycin-induced secretion of IL-2, IL-6, IL-10, and TNF-α, with BPPH showing the strongest immunosuppressive profile. BPA and TMBPF reduced IL-2 secretion significantly more in male-derived than female-derived LCLs, whereas most other sex differences were not significant. These findings demonstrate compound-, concentration-, donor-, and sex-dependent variability in bisphenol immunotoxicity.

In conclusion, incorporating sex-balanced and age-stratified *in vitro* models may improve the biological relevance and interpretation of immunotoxicity studies.

Vpliv endokrino delujočih snovi na imunski sistem

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Delovanje imunskega sistema je tesno povezano z endokrinimi signali, ki določajo intenziteto in kakovost prirojenih ter pridobljenih imunskih odzivov. Steroidni hormoni vplivajo na biologijo imunskih celic, vključno s tvorbo citokinov, proliferacijo, diferenciacijo in vnetnim signaliziranjem.

Estrogeni uravnavajo imunske odzive prek signalizacije receptorjev ERα, ERβ in GPER ter s tem vplivajo na tvorbo citokinov in aktivnost vnetnih poti, kot so NF-κB, STAT in TNF. Nasprotno glukokortikoidi zavirajo aktivacijo imunskega sistema preko GR-posredovanega zaviranja vnetne signalizacije[2]. Ščitnični hormoni in mineralokortikoidi dodatno oblikujejo prirojeno in pridobljeno imunost preko signalnih poti tiroidnih (TR) in mineralokortikoidnih receptorjev (MR), medtem ko progesteron in androgeni spodbujajo imunsko toleranco z omejevanjem aktivacije limfocitov T ter zmanjševanjem tvorbe protivnetnih citokinov.

Endokrino delujoče snovi (EDS), kot so bisfenoli, PFAS in parabeni, predstavljajo vse večjo skrb, saj lahko posnemajo ali zavirajo endogeno hormonsko signaliziranje. Mnogi EDS se vežejo na več jedrnih receptorjev, vključno z ER, AR, GR, TR, MR in PPAR, s čimer motijo endokrino-imunski preplet ter uravnavajo vnetne odzive, sproščanje citokinov in delovanje imunskih celic. Naraščajoče število raziskav nakazuje, da ti mehanizmi pomembno prispevajo k imunotoksičnosti in motnjam imunskega ravnovesja, povezanim z vnetnimi in avtoimunskimi boleznimi.

Influence of endocrine-active substances on immune system

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Immune function is tightly regulated by endocrine signals that shape the magnitude and quality of innate and adaptive immune responses. Steroid hormones influence multiple aspects of immune-cell biology, including cytokine production, proliferation, differentiation, and inflammatory signalling. Estrogens regulate immune responses through ERα, ERβ and GPER signalling, modulating cytokine production and inflammatory pathways such as NF-κB, STAT and TNF. In contrast, glucocorticoids suppress immune activation via GR-mediated inhibition of inflammatory signalling. Thyroid hormones and mineralocorticoids further shape innate and adaptive immunity through TR- and MR-dependent regulation of inflammatory responses, while progesterone and androgens promote immune tolerance by limiting T-cell activation and proinflammatory cytokine production. Environmental endocrine-disrupting chemicals (EDCs) such as bisphenols, PFAS and parabens are emerging concerns because they can mimic or antagonise endogenous hormonal signalling. Many EDCs bind multiple nuclear receptors, including ER, AR, GR, TR, MR and PPARs, thereby perturbing endocrine-immune crosstalk and modulating inflammatory responses, cytokine release and immune-cell function. Accumulating evidence suggests that these mechanisms may contribute to immunotoxicity and altered susceptibility to inflammatory and autoimmune diseases.

Imunoproteasom in inflamasom kot tarča imunoaktivnih snovi

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Imunski sistem je pomembna tarča številnih zdravil, okoljskih onesnaževal in drugih eksogenih bioaktivnih snovi. Izpostavljenost tem dejavnikom lahko povzroči različne oblike imunomodulacije, od zaviranja oziroma oslabitve imunskega odziva do čezmerne aktivacije vnetnih poti in porušenja imunskega ravnovesja. Razumevanje molekularnih mehanizmov, ki uravnavajo te odzive, je zato ključnega pomena za opredeljevanje imunotoksičnega potenciala kemijskih snovi. Med pomembnimi regulatorji imunskega odziva sta imunoproteasom in inflamasomi, večproteinska kompleksa, ki imata ključno vlogo pri povezovanju zaznavanja celičnega stresa in nevarnostnih signalov z učinkovitim prirojenim in pridobljenim imunskim odzivom. Imunoproteasom je specializirana oblika proteasoma, katerega aktivnost je tesno povezana z uravnavanjem vnetnih procesov, procesiranjem antigenov in nastankom peptidov, ki sodelujejo pri predstavitvi antigenov. Inflamasomi pa delujejo kot znotrajcelični senzorski in signalni kompleksi, ki zaznavajo različne vzorce nevarnosti in celične poškodbe ter prek posledično spodbujajo zorenje vnetnih citokinov, predvsem IL-1β in IL-18, ter lahko sprožijo piroptozo.

Vse več raziskav kaže, da lahko zdravila, okoljska onesnaževala in druge kemijske snovi neposredno ali posredno vplivajo na aktivnost teh kompleksov. Takšne spremembe lahko pomembno preoblikujejo intenzivnost, trajanje in usmerjenost imunskega odziva ter prispevajo k nastanku oziroma napredovanju vnetnih in drugih patofizioloških procesov. Razumevanje vloge imunoproteasoma in inflamasomov pri odzivu na kemične in druge okoljske dejavnike predstavlja pomemben vidik sodobnega preučevanja imunotoksičnosti. Čeprav število raziskav na tem področju narašča, so podatki o vplivu različnih ksenobiotikov na te komplekse še vedno heterogeni in razpršeni. Nadaljnje raziskovanje teh mehanizmov je zato pomembno za boljše razumevanje zgodnjih molekularnih sprememb in celovitejše vrednotenje imunotoksičnih učinkov ksenobiotikov.

Immunoproteasome and inflammasome as targets of immunoactive substances

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The immune system is an important target of numerous pharmaceuticals, environmental pollutants, and other exogenous bioactive substances. Exposure to these factors can induce various forms of immunomodulation, ranging from suppression or impairment of immune responses to excessive activation of inflammatory pathways and disruption of immune homeostasis. Understanding the molecular mechanisms underlying these responses is therefore essential for characterizing the immunotoxic potential of chemical substances. Among the important regulators of immune responses are the immunoproteasome and inflammasomes, multiprotein complexes that play key roles in linking the sensing of cellular stress and danger signals to effective innate and adaptive immune responses. The immunoproteasome is a specialized form of the proteasome whose activity is closely associated with the regulation of inflammatory processes, antigen processing, and the generation of peptides involved in antigen presentation. Inflammasomes, in turn, function as intracellular sensing and signaling complexes that detect various danger signals and cellular damage, thereby promoting the maturation of proinflammatory cytokines, particularly IL-1β and IL-18, and potentially triggering pyroptosis.

An increasing body of evidence indicates that pharmaceuticals, environmental pollutants, and other chemical substances can directly or indirectly modulate the activity of these complexes. Such alterations may substantially influence the magnitude, duration, and nature of immune responses and contribute to the development or progression of inflammatory and other pathophysiological processes. Understanding the role of the immunoproteasome and inflammasomes in responses to chemical and other environmental factors is therefore an important aspect of contemporary immunotoxicity research. Although research in this field is expanding, evidence on the effects of xenobiotics on these complexes remains heterogeneous and fragmented. Further investigation is therefore needed to better understand early molecular responses to exposure and improve the assessment of immunotoxic effects.

POVZETKI VABLJENIH PREDAVANJ

Primeri imunotoksičnosti izbranih onesnaževal in zdravil/*Examples of immunotoxicity of selected pollutants and drugs*

Neželeni učinki zdravil in imunotoksičnost

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Imunotoksičnost zdravil zajema širok spekter neželenih učinkov, ki so po definiciji nenamerni in škodljivi ter izhajajo iz vpliva zdravilnih učinkovin na imunski sistem. Ločiti jo je treba od imunoterapije, kjer je imunski sistem tarča želenega učinka zdravila. Neželeni odzivi imunskega sistema predstavljajo pomemben vidik pri spremljanju varnosti zdravil. Glede na mehanizem lahko imunotoksičnost razdelimo na: imunosupresijo, pretirano imunostimulacijo, preobčutljivostne reakcije ter nespecifične motnje imunskega odziva. Za lažje razumevanje mehanizmov lahko glede na značilnosti in njihovo pojavnost uporabimo tudi razvrstitev neželenih učinkov na tipe A–E. Imunotoksični neželeni učinki so pogosto razvrščeni v tip B, pri katerem neželeni učinki niso povezani s primarnim farmakološkim delovanjem zdravila. Klinična preskušanja sicer vključujejo oceno in spremljanje imunoloških tveganj, vendar so ta lahko kljub temu nepredvidljiva in se pojavljajo na majhnem deležu populacije, tako da jih zaznamo šele v obdobju trženja zdravila, ko zdravilo prejema večje število pacientov.

Imunotoksična tveganja v obdobju trženja zdravila se spremljajo v okviru rednih farmakovigilančnih aktivnosti, pri čemer se lahko zdravilo uvrsti tudi na seznam zdravil, za katera se zahteva dodatno spremljanje varnosti. S specifičnimi »follow up« vprašalniki za določeno zdravilo lahko pridobivamo tudi ciljane informacije o določenem neželenem učinku. Analiza tveganj je tudi vključena v redna varnostna poročila v okviru kliničnih preskušanj in v obdobju trženja zdravila. Pri pomembnih ugotovljenih in potencialnih imunotoksičnih tveganjih se lahko uvedejo specifični ukrepi za zmanjševanje tveganj, ki vključujejo okrepljeno spremljanje in sledljivost, omejitve dostopa do zdravila ter obveščanje.

Za natančnejšo opredelitev tveganj se lahko zahteva izvedba dodatnih študij tudi v času trženja. Pozitivno razmerje med koristjo in tveganjem za določeno indikacijo mora biti zagotovljeno skozi celoten življenjski cikel zdravila. Če ugodnega razmerja ni več mogoče zagotoviti niti z ukrepi za obvladovanje tveganj, se klinično preskušanje lahko ustavi oziroma zdravilo umakne s trga.

Adverse drug reactions and immunotoxicity

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Drug immunotoxicity represents a broad range of adverse reactions, which are defined as unintended and harmful effects of medicinal products on the immune system. It should be distinguished from immunotherapy, where the immune system represents the therapeutic target. Based on the mechanism, immunotoxicity can be classified into immunosuppression, excessive immune stimulation, hypersensitivity, and non-specific immune reactions. For a better understanding of the underlying mechanisms, adverse drug reactions can also be classified into types A-E according to their characteristics. Immunotoxic adverse effects are frequently classified as type B adverse drug reactions which are not directly related to the primary pharmacological action of the medicinal product. Although clinical trials include assessments of immunological risks, such reactions may remain unpredictable as they often occur in a small proportion of patients. Consequently, they are often detected after marketing authorization, with larger population exposure. Medicines with known or potential immunotoxicity are subject to targeted safety monitoring during clinical development.

Immunotoxic adverse reactions occurring during the post-marketing period are monitored through routine pharmacovigilance activities. Medicinal products may also be included in the list of medicines subject to additional safety monitoring. Targeted follow-up questionnaires can also be used to collect detailed information on specific adverse reactions. Risk assessment represents integral part of periodic safety evaluations during both clinical development and the post-marketing period. When important potential or confirmed immunotoxic risks are identified, specific risk minimization measures may be implemented, including enhanced monitoring and traceability, restricted access, and risk communication activities. Additional studies may be required to characterize risks.

Ensuring a positive benefit-risk balance for approved indications is fundamental throughout the entire lifecycle of a medicinal product. If an acceptable benefit-risk profile can no longer be ensured, even after appropriate risk management measures have been implemented, a clinical trial may be terminated or the medicinal product withdrawn from the market.

Imunostoksičnost PFAS

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Per- in polifluoroalkilne snovi (PFAS) so velika skupina umetnih kemikalij, ki se uporabljajo za gašenje požarov, pri predelavi hrane, v gradbeništvu, elektroniki, tekstilu, gospodinjskih pripomočkih itd. So zelo obstojne. Najdemo jih v okolju, hrani in živih bitjih. Glavni viri izpostavljenosti pri ljudeh so pitna voda, hrana vključno z materinim mlekom, predmeti splošne rabe, zrak in prah v notranjih prostorih.

Izpostavljenost PFAS lahko vpliva na imunski odgovor, presnovo, in nevro-endokrino uravnavanje. V epidemioloških študijah so našli povezave med izpostavljenostjo PFAS in upadom koncentracije protiteles po cepljenju proti davici, gripi, mumpsu, rdečkam in tetanusu. Študije pri poskusnih živalih so potrdile zmanjšan odziv s specifičnimi protitelesi na antigene pri izpostavljenosti določenim PFAS. Izpostavljenost PFAS je povezana s povečanim tveganjem za okužbe dihalnega in prebavnega trakta pri ljudeh, še posebej pri otrocih izpostavljenih in utero. Izpostavljenost PFAS lahko prispeva k neustrezni imunski aktivaciji, posebej še k poslabšanju obstoječe astme. Spremembe imunskega sistema, ki jih sprožijo PFAS, lahko začnejo ali prispevajo k dogodkom v bolezenskih procesih onkraj imunskega sistema, kot je zaviranje nastajanja vnetnih proteinov, ki so potrebni za ustrezen pretok krvi med posteljico in materinim endometrijem, kar bi lahko imelo vlogo pri razvoju preeklampsije. Po drugi strani pa je bila opažena povezava med prenatalnimi koncentracijami perfluorooktanojske kisline (PFOA) in provnetnim interlevkinom-1beta ter večjim obsegom pasu pri izpostavljenih otrocih. To nakazuje, da je povečano vnetje prispevalo k neugodnemu metabolnemu profilu. Natančni mehanizmi imunotoksičnosti PFAS še niso pojasnjeni.

Na podlagi imunosupresije pri ljudeh je Evropska agencija za varno hrano določila prag dopustnega tedenskega vnosa za skupino PFOA, perfluorooktan sulfonata (PFOS), perfluorononanske kisline (PFNA) in perfluoroheksansulfonske kisline (PFHxS) pri 4.4 ng/kg tm na teden; prevedeno v na zdravju temelječo smerno vrednost 6,9 μg/L v krvi. Ti vrednosti se uporabljata pri karakterizaciji tveganja.

Immunotoxicity of PFAS

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Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals that are used in fire-fighting, food processing, construction, electronics, textiles, household products etc. They are very persistent and can be found in the environment, food and living organisms. The main sources of exposure in humans are drinking water, food including breast milk, consumer products, indoor air and dust.

Exposure to PFAS may affect immune response, metabolism, and neuroendocrine regulation. In epidemiological studies associations were found between exposure to PFAS and decline in antibody concentrations following vaccination against diphteria, influenza, mumps, rubella, tetanus. Experimental animal studies also support decreased antigen-specific antibody responses caused by exposure to certain PFAS. PFAS exposure was associated with an increased risk of respiratory tract and gastrointestinal infections in experimental animals and humans, particularly in children with in utero maternal PFAS exposure. PFAS exposure may contribute to inappropriate immune activation, in particular, deterioration of preexisting asthma. PFAS-induced immune system changes may be initiating or contributing events in disease processes beyond the immune system such as suppressing production of inflammatory proteins necessary for establishing proper blood flow between the placenta and maternal endometrium which may play a role in development of preeclampsia. On the other hand, an association between prenatal perfluorooctanoic acid (PFOA) levels and interleukin-1beta, a pro-inflammatory cytokine, was linked with a larger waist circumference in the exposed children; suggesting that increased inflammation contributed to an unfavourable metabolic profile. Precise mechanisms of PFAS immunotoxicity remain to be elucidated.

Based on immunosuppression in humans the European Food Safety Authority set the Tolerable Weekly Intake threshold for a group of PFOA, perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) of 4.4 ng/kg bm per week. This translates into Health Based Guidance Value of 6.9 μg/L in blood. These values are used in risk characterisation.

Cepiva in imunotoksičnost

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Cepiva so biološki pripravki, namenjeni zaščiti posameznikov pred nalezljivimi boleznimi, ki spodbujajo imunski sistem. Sestavljena so iz antigenov (živi oslabljeni/inaktivirani mikroorganizmi, beljakovinske podenote, polisaharidi, toksoidi, mRNK, virusni vektorji), adjuvantov, perservativov, stabilizatorjev, pufrov in sledove snovi uporabljenih med proizvodnjo. Po cepljenju telo razvije imunski spomin, kar omogoča hiter in učinkovit odziv ob morebitni kasnejši izpostavljenosti dejanskemu povzročitelju bolezni. Cepljenje je pomembno zmanjšalo pojavnost nalezljivih bolezni, posledično v zadnjih 50 letih preprečilo vsaj 154 milijonov smrti in velja za enega najuspešnejših javnozdravstvenih ukrepov.

Imunotoksičnost pomeni škodljive učinke kemikalij, zdravil, okoljskih dejavnikov ali bioloških snovi na imunski sistem. Ti učinki se lahko kažejo kot imunosupresija, preobčutljivostne reakcije, avtoimunoske reakcije ali kronično vnetje. Ker so cepiva posebej zasnovana za delovanje na imunski sistem, se pred odobritvijo za uporabo opravijo obsežna predklinična in klinična testiranja za oceno njihove morebitne imunotoksičnosti. Regulativni organi od proizvajalcev zahtevajo celovito oceno varnosti cepiv, vključno z morebitnimi imunološko pogojenimi neželenimi učinki.

Večina odobrenih cepiv ima ugoden varnostni profil, resni imunotoksični učinki pa so redki. Blage reakcije, kot so bolečina na mestu injiciranja, povišana telesna temperatura ali utrujenost, so pričakovani znaki in pomenijo, da se imunski sistem odziva na cepivo. Nasprotno so hude imunsko posredovane reakcije, kot so alergijske reakcije ali avtoimunski zapleti izjemno redki in se neprestano spremljajo v okviru sistemov spremljanja varnosti po uvedbi cepiva na trg. V prispevku se bomo podrobneje posvetili imunskim zapletom po cepljenju proti covid-19 z mRNK cepivi, po cepljenju z rekombinantnim cepivom proti pasovcu, po cepljenju proti oslovskemu kašlju ter po cepljenju proti gripi.

Na splošno koristi cepljenja bistveno presegajo morebitna tveganja za imunotoksičnost. Nenehne raziskave, farmakovigilančno spremljanje in napredek v razvoju cepiv prispevajo k izboljšanju njihove varnosti ter hkrati zagotavljajo učinkovito zaščito pred nalezljivimi boleznimi. Razumevanje imunotoksičnosti omogoča razvoj še varnejših cepiv in krepi zaupanje javnosti v programe cepljenja po vsem svetu.

Vaccines and Immunotoxicity

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The immune system is an important target of numerous pharmaceuticals, environmental pollutants, and other exogenous bioactive substances. Exposure to these factors can induce various forms of immunomodulation, ranging from suppression or impairment of immune responses to excessive activation of inflammatory pathways and disruption of immune homeostasis. Understanding the molecular mechanisms underlying these responses is therefore essential for characterizing the immunotoxic potential of chemical substances. Among the important regulators of immune responses are the immunoproteasome and inflammasomes, multiprotein complexes that play key roles in linking the sensing of cellular stress and danger signals to effective innate and adaptive immune responses. The immunoproteasome is a specialized form of the proteasome whose activity is closely associated with the regulation of inflammatory processes, antigen processing, and the generation of peptides involved in antigen presentation. Inflammasomes, in turn, function as intracellular sensing and signaling complexes that detect various danger signals and cellular damage, thereby promoting the maturation of proinflammatory cytokines, particularly IL-1β and IL-18, and potentially triggering pyroptosis.

An increasing body of evidence indicates that pharmaceuticals, environmental pollutants, and other chemical substances can directly or indirectly modulate the activity of these complexes. Such alterations may substantially influence the magnitude, duration, and nature of immune responses and contribute to the development or progression of inflammatory and other pathophysiological processes. Understanding the role of the immunoproteasome and inflammasomes in responses to chemical and other environmental factors is therefore an important aspect of contemporary immunotoxicity research. Although research in this field is expanding, evidence on the effects of xenobiotics on these complexes remains heterogeneous and fragmented. Further investigation is therefore needed to better understand early molecular responses to exposure and improve the assessment of immunotoxic effects.

Ocena tveganja izpostavljenosti imunotoksičnim snovem in zakonodaja/*Risk assessment of exposure to immunotoxic substances and legislation*

Imunogenost terapevtskih peptidov: regulatorne zahteve

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Peptidna zdravila predstavljajo vse pomembnejši in hitro rastoči segment farmacevtskega trga. Imunogenost je eden ključnih dejavnikov, ki jih je treba upoštevati pri vrednotenju varnosti peptidnih zdravil. Pri originatorskih peptidih se imunogenost praviloma opredeli med kliničnim razvojem z vrednotenjem protiteles proti učinkovini. V prispevku se bomo osredotočili na generična peptidna zdravila, pri katerih klinične študije pogosto niso potrebne. V takšnih primerih ocena varnosti temelji predvsem na obsežnem poznavanju referenčnega zdravila, zato je ključno, da so vse razlike med generičnim in referenčnim izdelkom natančno opredeljene ter ustrezno ovrednotene glede na njihov potencialni vpliv na imunogenost.

Posebna pozornost je namenjena razlikam v profilih nečistot, saj lahko le-te povečajo tveganje za imunogenost generičnega zdravila v primerjavi z referenčnim. Medtem ko lahko nečistote, povezane s proizvodnim procesom, sprožijo aktivacijo prirojenega imunskega odziva, lahko peptidne nečistote vplivajo na pridobljeni imunski odziv zaradi nastanka novih T-celičnih epitopov. Ker za ocenjevanje imunogenega potenciala peptidnih nečistot ni splošno sprejete metodologije, ocena imunogenega tveganja temelji na celoviti presoji vseh razpoložljivih podatkov, vključno z rezultati ustreznih *in silico* ter *in vitro* študij.

Regulatorne zahteve za generične peptide se še naprej razvijajo. FDA je leta 2026 umaknila splošno smernico za določene generične peptide in jo nadomestila s 17 posodobljenimi, za posamezne peptidne proizvode specifičnimi smernicami, kar predstavlja pomemben napredek v znanstveno utemeljenem regulatornem pristopu k ocenjevanju generičnih peptidnih zdravil. Posodobitve med drugim omogočajo višje meje za kvalifikacijo peptidnih nečistot, kar v določenih primerih zmanjšuje potrebo po dodatnih študijah imunogenosti. Tudi EMA ocenjuje, da je tveganje za imunogenost pri sinteznih peptidih na splošno manjše kot pri proteinih, pri čemer poudarja pomen ustrezne karakterizacije in vrednotenja novih nečistot. Ne glede na regulatorni okvir pa ostaja temeljno načelo nespremenjeno: dokazati je treba, da morebitne razlike med generičnim in referenčnim zdravilom nimajo vpliva na varnost, učinkovitost ali imunogenost zdravila.

Immunogenicity of Therapeutic Peptides: Regulatory Requirements

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Peptide drugs represent a growing and increasingly important segment of the pharmaceutical market. A key component of peptide safety evaluation is the assessment of immunogenicity risks. For originator peptides, clinically relevant immunogenicity is typically characterized during clinical development through anti-drug antibody evaluation. This overview focuses on generic peptide drug products, for which clinical studies are often not required. In such cases, the assessment relies largely on the prior knowledge available for the reference listed drug (RLD). Consequently, any product-related differences between the generic product and the RLD must be carefully characterized, and potential immunogenicity risks must be evaluated.

Particular attention is given to differences in impurity profiles, as these may increase the immunogenicity risk of the generic product relative to the RLD. Process-related impurities are generally associated with activation of innate immune pathways, whereas peptide-related impurities may contribute to adaptive immune responses through the generation of novel T-cell epitopes. As no universally accepted methodology exists for evaluating the immunogenicity risk of peptide impurities, such assessments are typically performed on a case-by-case basis using appropriate *in silico* and *in vitro* tools.

Regulatory expectations for generic peptides continue to evolve. In 2026, FDA withdrew its general ANDA guidance for certain synthetic peptides and published 17 revised draft product-specific guidances for peptide products, marking a significant advancement in the agency's scientific and regulatory approach to generic peptide drug assessment. Key changes include higher impurity qualification thresholds and reduced requirements for nonclinical in vitro immunogenicity studies. Similarly, EMA considers the immunogenicity risk of synthetic peptides to be generally lower than that of proteins, while emphasizing the need to adequately control and assess novel impurities. Across both regulatory frameworks, the fundamental principle remains unchanged: any differences between the proposed generic product and the RLD must be demonstrated not to adversely affect safety, efficacy, or immunogenicity.

Preobčutljivost na sestavine v hrani

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Neželeni odzivi na sestavine živil so pomemben vidik prehranske obravnave bolnikov. Preobčutljivost na hrano zajema heterogen spekter netoksičnih neželenih odzivov, ki jih delimo na imunološko posredovane alergije na hrano in neimunološke intolerance na hrano. Klasične alergije na hrano, ki jih sprožijo prehranske beljakovine prek IgE- ali ne-IgE-posredovanih mehanizmov, pogosto zahtevajo strogo izogibanje alergenu. Nasprotno pa so odzivi na posamezne aditive, vključno z umetnimi barvili, konzervansi, ojačevalci okusa ter nekaterimi nekaloričnimi sladili, pretežno neimunski ter povezani s farmakološkimi, presnovnimi ali drugimi, slabo pojasnjenimi (idiopatskimi) mehanizmi.

Klinične manifestacije se lahko izrazito razlikujejo in segajo od dermatoloških (urtikarija, angioedem, poslabšanje ekcema), gastrointestinalnih (bolečina v trebuhu, napenjanje) in respiratornih simptomov do hudih sistemskih reakcij pri dovzetnih posameznikih. Poleg simptomatske obremenitve lahko neprepoznana ali slabo vodena preobčutljivost vodi v prekomerno omejevanje prehrane, kar negativno vpliva na vnos hranil in predstavlja resno tveganje za razvoj prehranskih primanjkljajev, zlasti pri ranljivih skupinah prebivalstva (nosečnice, doječe matere, otroci in starejši odrasli).

Dolgoročna obravnava bolnikov s prehranskimi omejitvami temelji na personaliziranem pristopu. Praktične veščine branja deklaracij, strategije za preprečevanje navzkrižne kontaminacije ter izobraževanje bolnikov in njihovih svojcev so ključne za dosledno in varno izvajanje ustrezne diete. Redne kontrole pri kliničnem dietetiku omogočajo spremljanje rasti in prehranskega stanja otrok, pri čemer se prehranske omejitve sproščajo, ko se razvije toleranca, saj lahko otroci določene oblike preobčutljivosti sčasoma prerastejo. Prav tako so redne kontrole smiselne pri odraslih in starejših, saj lahko klinični dietetik sproti optimizira prehrano glede na specifične omejitve in preference posameznika, s čemer dolgoročno ohranja optimalen prehranski status in izboljšuje kakovost življenja.

Hypersensitivity to Food Ingredients

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Adverse reactions to food ingredients are an important consideration in the nutritional management of patients. Food hypersensitivity encompasses a heterogeneous spectrum of non-toxic adverse responses, broadly classified into immunologically mediated food allergies and non-immunological food intolerances. Classical food allergies, triggered by dietary proteins through IgE- or non-IgE-mediated mechanisms, often require strict avoidance of the causative allergen. Conversely, reactions to specific food additives, including artificial colorants, preservatives, flavor enhancers, and certain non-caloric sweeteners, are predominantly non-immune and associated with pharmacological, metabolic, or other poorly understood (idiopathic) mechanisms.

Clinical manifestations vary markedly, ranging from dermatological (urticaria, angioedema, eczema exacerbation), gastrointestinal (abdominal pain, bloating), and respiratory symptoms to severe systemic reactions in susceptible individuals. Beyond the symptom burden, unrecognized or poorly managed hypersensitivity can lead to excessive dietary restrictions, negatively impacting nutrient intake and posing a serious risk for nutritional deficiencies, particularly in vulnerable populations (pregnant and lactating women, children, and older adults).

Long-term management of patients with dietary restrictions relies on a personalized approach. Practical label-reading skills, strategies to prevent cross-contamination, and education of patients and their families are essential for the consistent and safe implementation of an appropriate diet. Regular follow-ups with a clinical dietitian enable the monitoring of growth and nutritional status in children, with dietary restrictions being relaxed as tolerance develops, given that children may outgrow certain forms of hypersensitivity over time. Similarly, regular check-ups are valuable for adults and older individuals, allowing the clinical dietitian to continually optimize nutrition based on individual constraints and preferences, thereby maintaining optimal nutritional status and improving quality of life over the long term.

Regulatorni vidiki določanja imunotoksičnosti pri pripravi poročila o varnosti kozmetičnega izdelka

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Pri pripravi poročila o varnosti kozmetičnega izdelka (CPSR) se imunotoksičnost v praksi najpogosteje obravnava skozi oceno kožne preobčutljivosti, alergijskega kontaktnega dermatitisa in neželenih imunskih odzivov na sestavine kozmetičnih izdelkov. Uredba (ES) št. 1223/2009 zahteva, da je kozmetični izdelek varen za zdravje ljudi, hkrati pa prepoveduje testiranje kozmetičnih izdelkov in njihovih sestavin na živalih za kozmetične namene. Posledično nastaja pomemben razkorak med zakonodajno zahtevo po znanstveno utemeljeni oceni varnosti in praktično razpoložljivostjo toksikoloških podatkov.

Ocenjevalci varnosti kozmetičnega izdelka se pri oceni imunotoksičnosti pogosto opirajo na nepopolne podatke iz varnostnih listov, literaturo, razvrstitve po CLP, podatke iz REACH dosjejev, mnenja SCCS, podatke o znanih alergenih in informacije dobaviteljev. Obstoječi podatki iz testiranj na živalih, kot so LLNA in starejši testi na morskih prašičkih, se lahko uporabijo kot del zgodovinskega dokaznega gradiva, kadar so znanstveno relevantni in zakonito dostopni. Posebno vprašanje predstavlja HRIPT, ki daje neposredno človeške podatke, vendar je etično sporen, saj lahko pri zdravih prostovoljcih povzroči indukcijo preobčutljivosti.

Zaradi navedenih omejitev imajo vse večji pomen novi pristopi k oceni varnosti oziroma novi pristopi k oceni varnosti brez uporabe živali. Pri oceni kožne preobčutljivosti se uporabljajo predvsem metode, ki pokrivajo ključne dogodke v mehanizmu nastanka alergijskega odziva: in chemico metode, kot je DPRA za oceno vezave na proteine, *in vitro* metode, kot sta KeratinoSens in h-CLAT za oceno celične aktivacije, ter in silico metode, kot so QSAR modeli, strukturni opozorilni znaki in read-across. Posebej pomembni so definirani pristopi po OECD TG 497, ki rezultate več metod združujejo v vnaprej določen interpretacijski okvir. Ti pristopi ne odpravljajo vseh negotovosti, vendar omogočajo bolj sistematično, pregledno in regulatorno sprejemljivejšo integrirano oceno dokazov brez novih testiranj na živalih.

Regulatory Aspects of Immunotoxicity Assessment in the Preparation of a Cosmetic Product Safety Report

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In the preparation of a Cosmetic Product Safety Report (CPSR), immunotoxicity is most commonly addressed in practice through the assessment of skin sensitisation, allergic contact dermatitis and adverse immune responses to cosmetic product ingredients. Regulation (EC) No 1223/2009 requires cosmetic products to be safe for human health, while at the same time prohibiting the testing of cosmetic products and their ingredients on animals for cosmetic purposes. Consequently, there is a significant gap between the legal requirement for a scientifically justified safety assessment and the practical availability of toxicological data.

Cosmetic product safety assessors often rely on incomplete information from safety data sheets, published literature, CLP classifications, data from REACH dossiers, SCCS opinions, information on known allergens and supplier data when assessing immunotoxicity. Existing animal testing data, such as LLNA and older guinea pig studies, may be used as part of the historical body of evidence, provided that they are scientifically relevant and legally accessible. HRIPT represents a particular issue, as it provides direct human data but is ethically controversial, since it may induce sensitisation in healthy volunteers.

Due to these limitations, increasing importance is being placed on new approaches to safety assessment, namely New Approach Methodologies (NAM), which enable safety assessment without the use of animals. In the assessment of skin sensitisation, methods are primarily used that cover key events in the mechanism leading to an allergic response: in chemico methods, such as the Direct Peptide Reactivity Assay (DPRA), for assessing protein binding; *in vitro* methods, such as KeratinoSens and h-CLAT, for assessing cellular activation; and in silico methods, such as QSAR models, structural alerts and read-across. Defined approaches under OECD TG 497 are particularly important, as they combine the results of several methods within a predefined interpretation framework. These approaches do not eliminate all uncertainties, but they enable a more systematic, transparent and regulatorily acceptable integrated assessment of evidence without new animal testing.

POVZETKI POSTERJEV/POSTER ABSTRACTS

Advancing use of in silico approaches in Novartis' skin sensitization assessment strategy for workplace applications

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Reliable identification of skin sensitizers is essential for protecting workers handling pharmaceutical compounds. In silico approaches are increasingly considered to support skin sensitization (SS) assessment and reduce reliance on animal testing. However, their use for pharmaceutical compounds remains challenging due to structural complexity and limited validation datasets. This project aims to support the refinement of Novartis' SS assessment strategy by evaluating the performance of two in silico models using a large proprietary dataset with available *in vivo* data.

A total of 288 Local Lymph Node Assay (LLNA) and 88 Guinea Pig Maximization Test (GPMT) reports were systematically reviewed. Test compounds were classified as sensitizers or non-sensitizers. In addition, statistical significance, dose-response, variability in control groups, consistency between related parameters, and potential for confounding factors (toxicity and irritation), were considered to categorize conclusions as either reliable or questionable.

The same compounds were assessed for skin sensitization alerts using expert rule based, Derek Nexus (DN), and statistical based, CASE Ultra (CU), in silico systems. Model performance (individual and combined approaches) was evaluated by comparing in vivo classifications with the predictions. Prediction coverage was assessed separately as the proportion of compounds for which a conclusive prediction was obtained.

Preliminary analyses demonstrated limited predictive performance of the individual models for this pharmaceutical dataset. A combined approach showed improved senstivity and prediction coverage. These findings suggest that combining two complementary in silico systems may support potential use within a broader weight-of-evidence assessment strategy. The project is ongoing, focusing on optimization of the combined approach and evaluation of *in vivo* data consistency. Large pharmaceutical datasets remain essential for improving and validating in silico models for SS assessment. In the longer term, such approaches may support more informed hazard assessment and wider use of alternative methods in the pharmaceutical and chemical sectors.

Inflammatory effects of benzo[g,h,i]perylene and benzo[b]fluoranthene in a human lung co-culture model at the pseudo-air–liquid interface

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Indoor air pollution is a major public health concern and has been associated with millions of premature deaths annually. Polycyclic aromatic hydrocarbons (PAHs) are among the most hazardous airborne pollutants, with benzo[g,h,i]perylene (BGP) and benzo[b]fluoranthene (BBF) being widely present in both indoor and outdoor air. Despite their prevalence, information on their respiratory inflammatory effects remains limited. In this study, we investigated the inflammatory responses induced by BGP and BBF using a human lung coculture model consisting of alveolar epithelial cells (A549) and differentiated macrophage-like cells (dTHP-1) maintained at a pseudo-air–liquid interface. Cells were exposed for 24 h to noncytotoxic concentrations of BGP (≤18.1 μM) and BBF (≤39.6 μM). Inflammatory endpoints were evaluated using flow cytometry, ELISA, and gene expression analyses. Both PAHs triggered inflammatory responses in the co-culture system. BGP significantly increased IL-8 secretion and elevated the proportion of cells expressing TNF-α, IL-6, and IL-1β. BBF induced a milder but detectable response, increasing the proportion of cells expressing TNF-α, IL-6, and IFN-γ. Transcriptomic profiling further confirmed the pro-inflammatory activity of both compounds, revealing upregulation of multiple inflammation-related genes, including TNF-α, IL-6, IL-8, IL-1β, IL-10, and IL-17d. Notably, BGP generally elicited stronger inflammatory responses than BBF. These findings demonstrate that BGP and BBF can activate inflammatory signalling pathways in human alveolar cells even at non-cytotoxic concentrations. The observed effects suggest that prolonged exposure to these PAHs can trigger pronounced inflammatory signalling. Our results highlight the importance of considering inflammatory endpoints when assessing the health risks of airborne PAHs and demonstrate the value of physiological relevant lung co-culture models for assessing inhalation exposure risks of airborne PAHs.

Supported by HEU CutCancer (101079113), HEU EDIAQI (101057497), ARIS (P1-0245).

Ocena izpostavljenosti ljudi *N*-nitrozaminom preko različnih virov vnosa na evropskem območju

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N-nitrozamini so nečistote, ki izkazujejo genotoksične, mutagene, rakotvorne ali imunotoksične učinke. Nekateri, kot je *N*-nitrozodimetilamin, lahko zavirajo humoralni in celično posredovani imunski odziv ter predstavljajo pomembno, vendar še vedno premalo raziskano tveganje za zdravje ljudi. Raziskava na podlagi sistematičnega pregleda literature podaja celovito oceno izpostavljenosti *N*-nitrozaminom preko različnih virov na evropskem območju od leta 2000. Na podlagi podatkov o njihovi vsebnosti v različnih izdelkih ter njihove povprečne in bolj konservativne (95. percentil) uporabe smo ocenili, da skupna dnevna izpostavljenost pri povprečni uporabi znaša 164,0–176,9 ng/kg tm/dan, pri konservativni uporabi pa 373,8–406,2 ng/kg tm/dan. Tobačni izdelki predstavljajo glavni vir vnosa (68–70 %), sledijo živila, ki prispevajo 21–23 % skupne dnevne izpostavljenosti. Ostale skupine izdelkov prispevajo skupaj manj kot 10 %, pri čemer kozmetični izdelki predstavljajo 5 %, zdravila 1,8–3,2 %, prehranska dopolnila 0,9–1,4 % ter pitna voda 0,1 %. V primerjavi z začetnim obdobjem zaznav *N*-nitrozaminov v zdravilih je njihov današnji prispevek značilno nižji (0,2–0,4 %), kar je posledica redkejše pojavnosti in bistveno nižjih koncentracij zaradi vzpostavljene strožje regulative. Ker nekadilci predstavljajo kar 77 % evropske populacije, smo ocenili tudi dnevno izpostavljenost brez upoštevanja tobačnih izdelkov. Ta se je znatno zmanjšala pri povprečni (48,5–60,9 ng/kg tm/dan) in konservativni (121,5–152,9 ng/kg tm/dan) uporabi. Glavni vir predstavljajo živila (70,3–71,8 %), sledijo kozmetični izdelki (15,3–18,5 %), medtem ko ostali viri skupaj prispevajo približno 10 %. Ob upoštevanju genotoksičnega potenciala posameznih *N*-nitrozaminov ostane doprinos posameznih skupin izdelkov primerljiv, delež izpostavljenosti preko kozmetičnih izdelkov pa se približno podvoji.

Na podlagi implementacije regulative na področju zdravil, ki se odraža v zmanjšani izpostavljenosti *N*-nitrozaminov v zdravilih, raziskava nakazuje smiselnost uvedbe ustreznih regulativnih zahtev tudi na drugih področjih, ki pomembno prispevajo k dnevni izpostavljenosti, predvsem za tobačne izdelke in živila.

Assessment of human exposure to *N*-nitrosamines from multiple sources in the European Region

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N-nitrosamines are contaminants that exhibit genotoxic, mutagenic, carcinogenic or immunotoxic effects. Certain *N*-nitrosamines, such as *N*-nitrosodimethylamine, can suppress humoral and cell-mediated immune response and represent a significant, still insufficiently investigated, risk to human health. The present study provides a comprehensive assessment of *N*-nitrosamine exposure from various sources in the European region since 2000, based on a systematic literature review. Considering data on *N*-nitrosamine contents in different products and their mean and conservative (95^{th} percentile) consumption patterns, total daily exposure was estimated at 164.0–176.9 ng/kg bw/day and 373.8–406.2 ng/kg bw/day, respectively. Tobacco products represented the major source of *N*-nitrosamine exposure (68–70%), followed by food, contributing 21–23% of total daily exposure. Other sources collectively accounted for <10%, mainly from cosmetic products (5%), followed by drug products, food supplements, and drinking water (1.8–3.2%, 0.9–1.4%, and 0.1%, respectively). Compared with the early stages of *N*-nitrosamine detection in drug products, their current contribution is substantially lower (0.2–0.4%), reflecting the lower prevalence and concentrations resulting from stricter regulatory frameworks. Since non-smokers account for nearly 77% of the European population, daily exposure was re-evaluated excluding tobacco products. Significantly reduced exposure (48.5–60.9 ng/kg bw/day and 121.5–152.9 ng/kg bw/day based on mean and conservative consumption patterns, respectively) was observed. Food became the predominant source of exposure (70.3–71.8%), followed by cosmetic products (15.3–18.5%), while the remaining sources collectively accounted for approximately 10%. Considering the genotoxic potential of individual *N*-nitrosamines, the relative contribution of the different sources remained comparable, while the contribution of cosmetic products increased approximately twofold.

As established pharmaceutical regulatory frameworks resulted in significantly reduced *N*-nitrosamine levels in drug products and daily exposure, these findings highlight the rationale for implementing similar regulatory frameworks in other fields targeting major contributors (tobacco products, food).

Vključitev Kupfferjevih celic v 3D model HepG2 razkriva imunsko modulirane odzive na izpostavljenost karcinogenom

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Jetrni *in vitro* celični modeli predstavljajo temelj genetske toksikologije, vendar mnogi modeli, ki temeljijo izključno na hepatocitih, ne uspejo ustrezno posnemati kompleksnosti in vivo jetrnega tkiva, kar omejuje njihov odziv na imunsko modulirano toksičnost. Vrzel je mogoče premostiti z vključitvijo neparehimskih jetrnih celic, zlasti Kupfferjevih celic (KC), rezidenčnih makrofagov jeter, v jetrne *in vitro* modele. V tej študiji smo primerjali 3D modela monokulture HepG2 in kokulture HepG2 s KC, izpostavljenima dvema karcinogenoma z različnim mehanizmom delovanja, kadmijevemu kloridu (CdCl2) in griseofulvinu (GF) za 24h. Analizirali smo, kako CdCl2 in GF vplivata na živost celic (test LDH), izločanje jetrnospecifičnih proteinov (izločanje albumina), genotoksičnost (test komet), izločanje vnetnih citokinov (ELISA) in na izražanje genov, povezanih s celičnimi procesi vpetimi v kancerogenezo. Citotoksičnost in izločanje albumina sta bila med modeloma primerljiva, kar kaže na ohranjeno funkcijo hepatocitov v obeh testnih sistemih. Obe kemikaliji sta v obeh modelih povzročili koncentracijsko-odvisne genotoksične odzive, pri čemer je CdCl2 izkazoval večji genotoksičen potencial, skladno z uveljavljenim, od oksidativnega stresa odvisnim mehanizmom genotoksičnosti, medtem ko je za GF bolj značilen aneugen mehanizem genotoksičnega delovanja z motnjami delitvenega vretena. Najizrazitejše razlike med modeloma so bile opažene pri izločanju vnetnih kemokinov in genskem izražanju po izpostavitvi CdCl2, kjer je kokultura HepG2/KC pokazala znatno povišano izhodiščno izločanje IL8 in selektiven odziv genskega izražanja z zvišano regulacijo CCL2 in IL1B, ki v monokulturi HepG2 nista bila zaznana. Vnetni podpis je bil odsoten pri pro-vnetnih citokinih IL6 in TNF, kar je vzorec, ki ga pojasnjuje sočasna aktivacija poti Nrf2 in njeno zaviranje signalizacije NF-κB. Te ugotovitve dokazujejo, da lahko že ciljno dodajanje enega samega tipa imunskih celic v jetrni 3D model omogoči biološko pomembne vnetne odzive, ki jih v modelih zgolj s hepatociti ni mogoče zaznati, kar zagotavlja izboljšano fiziološko ustreznost.

Raziskavo so financirali ARIS (P1-0245), HEU CutCancer (101079113) in HEU PARC (101057014).

Incorporating Kupffer cells into a 3D HepG2 spheroid model reveals immunemodulated responses to carcinogen exposure

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Hepatic *in vitro* cell models serve as a cornerstone of genetic toxicology, yet many traditional hepatocyte-only models inadequately recapitulate the complexity of in vivo multicellular liver, limiting their capacity to respond to immune-mediated toxicity. The incorporation of nonparenchymal hepatic cells, particularly Kupffer cells (KCs), the resident macrophages of the liver, into hepatic *in vitro* models represents a strategy to bridge the gap with introduction of immunologically competent cells. In the present study, we compared HepG2 monoculture and HepG2/KC coculture 3D models exposed for 24 h to two mechanistically distinct carcinogens, cadmium chloride (CdCl2) and griseofulvin (GF), using a multi-endpoint approach including cytotoxicity (LDH assay), liver-specific functionality (albumin secretion), genotoxicity (alkaline comet assay), inflammatory cytokine secretion (ELISA), and targeted transcriptomic profiling of genes associated with cellular processes comprised in hallmarks of cancer. Cytotoxicity and albumin secretion were broadly comparable between models, indicating at preserved hepatocyte function in both test systems. Both chemicals induced concentrationdependent genotoxic responses in both models, with CdCl2 being more potent, consistent with its indirect oxidative stress-driven genotoxic mechanism, while GF acted through aneugenic spindle disruption. The most pronounced model-specific differences were observed in inflammatory chemokine secretion and gene expression after exposure to CdCl2, where HepG2/KC coculture exhibited significantly elevated baseline IL8 secretion and a selective gene expression response with upregulation of CCL2 and IL1B, which were not in the HepG2 monoculture. The inflammatory signature was absent for pro-inflammatory cytokines IL6 and TNF, a pattern explained by concurrent Nrf2-mediated pathway activation and its suppression of NF-κB signalling. These findings demonstrate that even targeted addition of a single immune cell type to a hepatic 3D model can enable biologically meaningful inflammatory responses, that are not detectable in hepatocyte-only cell models, providing an improved physiological relevance.

The study was funded by ARIS (P1-0245), HEU CutCancer (101079113) and HEU PARC (101057014).

Računalniške NAM-metode za oceno imunotoksičnosti: trenutno stanje, izzivi in priložnosti

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Ocena imunotoksičnosti spojin je velik izziv zaradi kompleksnosti delovanja imunskega sistema, raznolikosti možnih škodljivih učinkov in omejene razpoložljivosti standardiziranih preskusnih metod. Računalniške nove metodologije pristopov (New Approach Methodologies, NAM) lahko podpirajo presejanje kemikalij, zgodnje prepoznavanje nevarnosti in mehanistično razlago učinkov, ter tako hkrati prispevajo k zmanjšanju potrebe po preskusih na živalih. Namen te študije je bil identificirati računalniške NAM-e, ki so uporabni za oceno imunotoksičnosti, ter ovrednotiti njihovo trenutno uporabo, prednosti in omejitve. Obravnavani pristopi so vključevali modele kvantitativnega razmerja med strukturo in aktivnostjo (QSAR) ter poti neželenega izida (Adverse Outcome Pathways, AOP). Ocenili smo jih glede na obravnavane končne točke imunotoksičnosti, področje uporabe modelov, status validacije in poročano napovedno uspešnostjo. Računalniški NAM-i izkazujejo velik potencial za prepoznavanje opozorilnih znakov imunotoksičnosti, prednostno razvrščanje kemikalij za nadaljnje testiranje ter podporo mehanistični razlagi imunsko posredovanih učinkov. Vendar pa je njihova širša uporaba trenutno omejena zaradi pomanjkanja kvalitetnih eksperimentalnih podatkov, heterogenosti tarč, in nezadostne zunanje validacije modelov. Trenutna prizadevanja za razvoj harmoniziranih *in vitro* testov za imunotoksičnost in izboljšanje preglednega ter standardiziranega poročanja eksperimentalnih rezultatov bodo v prihodnosti omogočila pridobivanje kakovostnejših podatkov za razvoj in validacijo računalniških modelov. Posledično bodo računalniški NAM-i lahko postali pomemben del naslednje generacije pristopov za oceno imunotoksičnosti.

Computational NAMs for Immunotoxicity Assessment: Current Landscape, Challenges and Opportunities

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Immunotoxicity assessment remains challenging due to the complexity of the immune system, the diversity of potential adverse outcomes, and the limited availability of standardized, human-relevant testing methods. Computational new approach methodologies (NAMs) may support chemical screening, hazard identification, and mechanistic interpretation while contributing to the reduction of animal testing. This study aimed to identify computational NAMs applicable to immunotoxicity assessment and to evaluate their current applications, strengths, and limitations. The approaches examined included quantitative structure–activity relationship models and adverse outcome pathway frameworks. They were evaluated with respect to the immunotoxicity endpoints addressed, model interpretability, applicability domain, validation status, and reported predictive performance. Computational NAMs show considerable potential for identifying immunotoxicity alerts, prioritizing chemicals for further testing, and supporting the mechanistic interpretation of immune-mediated effects. However, their broader application is currently limited by the scarcity of curated immunotoxicity datasets, heterogeneous endpoint definitions, and insufficient external validation. Ongoing efforts to develop harmonized *in vitro* immunotoxicity assays and improve the transparent and standardized reporting of experimental results are expected to generate higher-quality data for computational model development and validation. Consequently, computational NAMs could become an important component of next-generation immunotoxicity assessment.

Getting on Board - Promoting Effective Transport Through Healthy Bus Drivers

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The public transport sector plays a crucial role in sustainable mobility but currently faces a severe shortage of bus drivers (BDs). The PROMETHEUS project aims to enhance the preventive value of statutory occupational health surveillance by integrating biomonitoring with targeted health education for BDs. Over a two-year follow-up period, the project will assess the feasibility and impact of this approach on drivers' health and wellbeing. PROMETHEUS adopts an innovative interdisciplinary framework that combines epidemiological, biological, toxicological and medical expertise with occupational health promotion strategies. This approach integrates work-related hazard prevention with broader health promotion and disease prevention measures to improve BDs' wellbeing. The project is organised into five work packages (WPs). WP1 adapts Swiss expertise to assess exposure of Romanian and Croatian BDs to physical, chemical, and ergonomic hazards, generating inventories and Bus-Exposure Matrices. WP2 establishes harmonised BD cohorts across three countries to enable pooled analyses of health outcomes while ensuring contextual relevance through collaboration with local stakeholders. WP3 pilots expanded occupational medical examinations incorporating biomarkers of exposure and effect to evaluate the role of biomonitoring in occupational health practice. WP4 develops guidance, training, and educational materials to strengthen communication between occupational health providers and drivers. WP5 oversees project coordination and dissemination of results. PROMETHEUS will provide robust scientific evidence on occupational exposures in the public transport sector and generate new knowledge on the occupational risks faced by bus drivers. These findings will support policymakers in the EU and Switzerland in developing more effective regulations and preventive measures to protect the health and wellbeing of this essential workforce.

This study was funded within the framework of the Multilateral Academic Projects (MAPS) by the Swiss National Science Foundation, Croatian Science Foundation, and Executive Agency for Higher Education, Research, Development and Innovation Funding (Grant no. IZ11Z0_230941).

Leukocyte telomere length as a biomarker of cumulative biological damage in children with respiratory conditions

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Telomeres, repetitive DNA at chromosome ends, protect genomic stability and progressively shorten with cell division. Because of their sensitivity to oxidative damage and limited repair capacity, they are considered a biomarker of cumulative biological damage, reflecting longer-term systemic effects rather than transient changes. Their accelerated shortening has been linked to chronic inflammation, oxidative stress, and increased immune cell turnover—key features of chronic respiratory diseases in children, including asthma and allergic rhinitis.

This study included 180 children aged 3–20 years: 123 with asthma and/or allergic rhinitis and 57 healthy controls. Relative telomere length was assessed in peripheral blood leukocytes using qPCR. T/S values were $\log_2$-transformed, and general linear models were adjusted for age, sex and qPCR run. Adjusted leukocyte telomere length was numerically lower in children with asthma and/or allergic rhinitis than in healthy controls, although the difference was not statistically significant ($p = 0.382$). Across clinical groups, adjusted mean telomere values followed the numerical pattern control > asthma > rhinitis > both, with no significant overall effect of clinical condition ($p = 0.794$). Increased primary DNA damage and micronucleus frequency previously observed within the broader EDIAQI cohort indicate greater genomic instability in affected children. Taken together, these findings suggest an increased cumulative biological burden associated with childhood asthma and allergic rhinitis. Longitudinal studies are needed to determine whether persistent inflammation and genomic instability are associated with accelerated telomere attrition.

Supported by the HEU EDIAQI (#101057497) and EU BioMolTox (#533-03-23-0006)

Health-related biomarkers comparison in people with different dietary habits (UZDRAVLJe)

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Diet is a modifiable component of the human exposome and a key determinant of health and well-being. Our previous results showed that vegetarians and/or vegans exhibited significantly higher levels of genome damage compared to omnivores. Furthermore, a positive association was identified between DNA damage and the duration of adherence to vegan dietary patterns. Low-grade chronic inflammation is recognised as one of the drivers of genome damage. Although the health benefits of different dietary patterns have been extensively investigated, their effects on multiple biological pathways, including inflammatory biomarkers, remain inconclusive. The UZDRAVLJe project aims to address this knowledge gap by comparing different dietary patterns using a comprehensive multi-biomarker approach in human volunteers. The study integrates nutritional and dietary profiling, body composition, DNA damage, microbiome composition, biochemical biomarkers, and epigenetic markers to provide a multidimensional assessment of diet-health relationships. The project will generate evidence of interest to the scientific community, general public, policymakers, and industry, supporting informed decisions on healthy and sustainable dietary choices. It will also strengthen research capacity and provide opportunities for early-career researchers to gain experience in internationally relevant, interdisciplinary research. By investigating how dietary preferences are associated with multiple indicators of human health, including hs-CRP as a biomarker of chronic inflammation, UZDRAVLJe contributes to the development of personalised health approaches, in line with the Smart Specialisation Strategy (S3), while supporting the sustainability objectives of the European Green Deal.

Supported by Croatian Science Foundation (#2823) and BioMolTox

Transporter-Mediated Uptake of Oxime Antidotes: Implications for Treatment Safety in Organophosphorus Poisoning

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Organophosphorus poisoning is associated with severe cholinergic toxicity, while inflammatory and immune-related responses may contribute to its broader toxicological consequences. Oximes are administered to reactivate inhibited acetylcholinesterase, but their cellular disposition remains insufficiently understood. Characterising membrane transport of these antidotes is therefore relevant to assessing treatment safety alongside their therapeutic action.

We examined the permeability and transporter interactions of standard oximes (HI-6 and pralidoxime) and newly synthesised quinuclidinium- and imidazolium-based derivatives. Passive permeability was assessed by parallel artificial membrane permeability assay (PAMPA). Transporter-mediated accumulation and inhibition were investigated in HEK293 cells stably overexpressing organic cation transporters OCT1 or OCT2, using LC-MS/MS quantification and fluorescent ASP+ uptake assays.

Most oximes showed poor passive membrane permeability, whereas the more lipophilic Q5 displayed noticeable diffusion. Direct uptake assays identified transporter-dependent accumulation of the tested compounds other than HI-6. The novel derivatives inhibited OCT-mediated substrate uptake, particularly X and Q5. Kinetic analyses showed differences between transporters, with lower Km values for OCT2 and generally higher OCT1 transport capacity for X and Q5. A preliminary substrate-cocktail experiment also suggested that oxime X can inhibit uptake of several clinically relevant OCT1 substrates.

These findings identify organic cation transporters as potential determinants of oxime exposure and drug interaction risk during antidotal therapy. Understanding transporter-dependent oxime disposition may also inform future investigations of antidote safety in the context of inflammatory and immune responses following organophosphorus exposure.

Supported by the Croatian Science Foundation (UIP-2017-05-7260); EU NextGenerationEU (Class 643-02/23-01/00016; Reg. 533-03-23-0006). Facilities and equipment were supported by ERDF project KK.01.1.1.02.0007.

ZAHVALA

Zahvaljujemo se Nacionalnemu inštitutu za biologijo v Ljubljani, ki je podprl izvedbo kongresa in omogočil izvedbo v svojih prostorih.