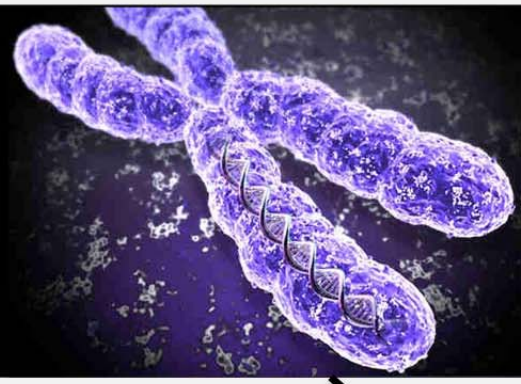
XXI Congreso de la Sociedad Española de Mutagénesis Ambiental

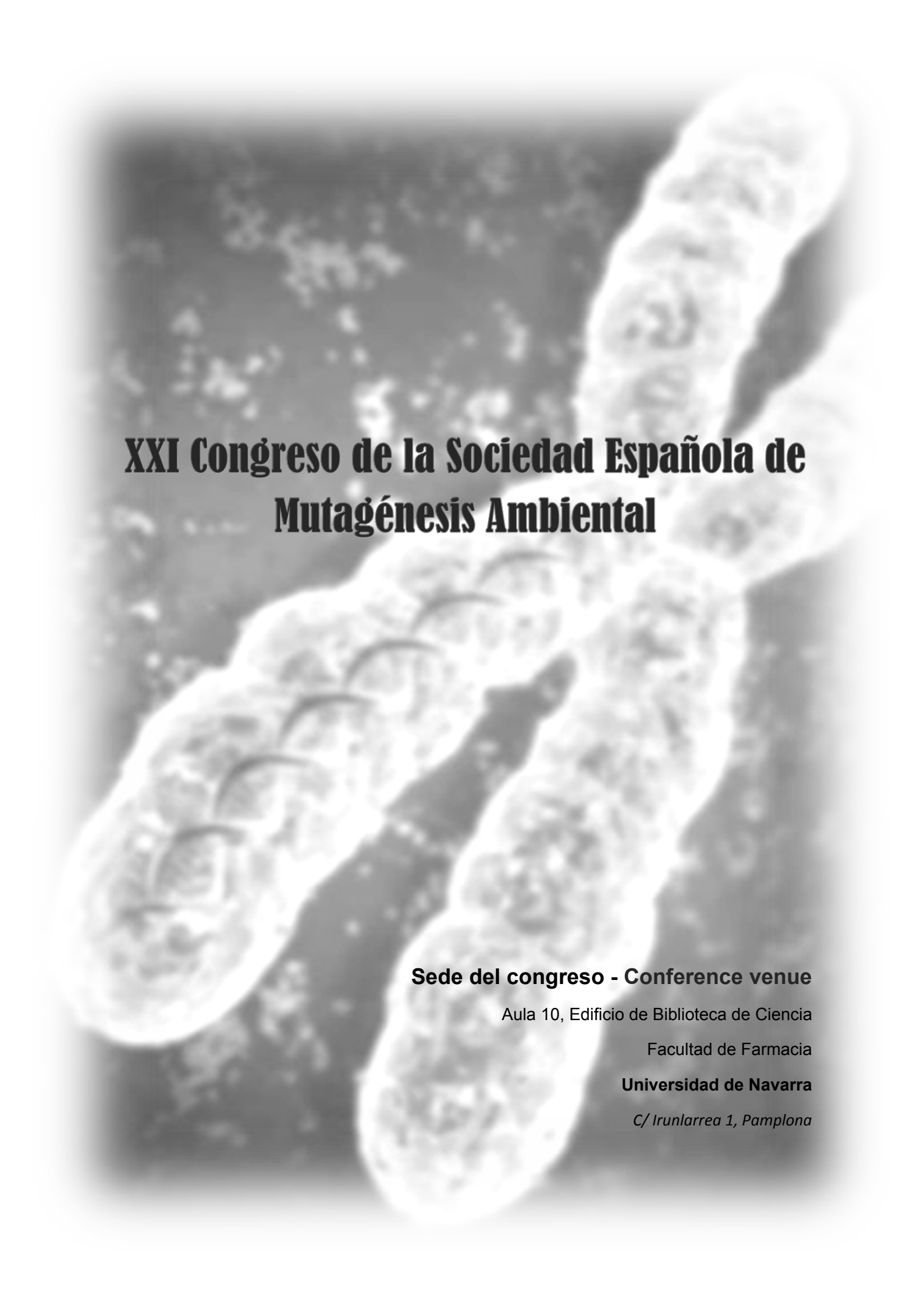


Plaza del Castillo, Pamplona

Universidad de Navarra

18, 19 y 20 Junio 2014
Facultad de Farmacia
Universidad de Navarra
Pamplona

SOCIEDAD ESPAÑOLA DE MUTAGENESIS AMBIENTAL



XXI Congreso de la Sociedad Española de Mutagénesis Ambiental

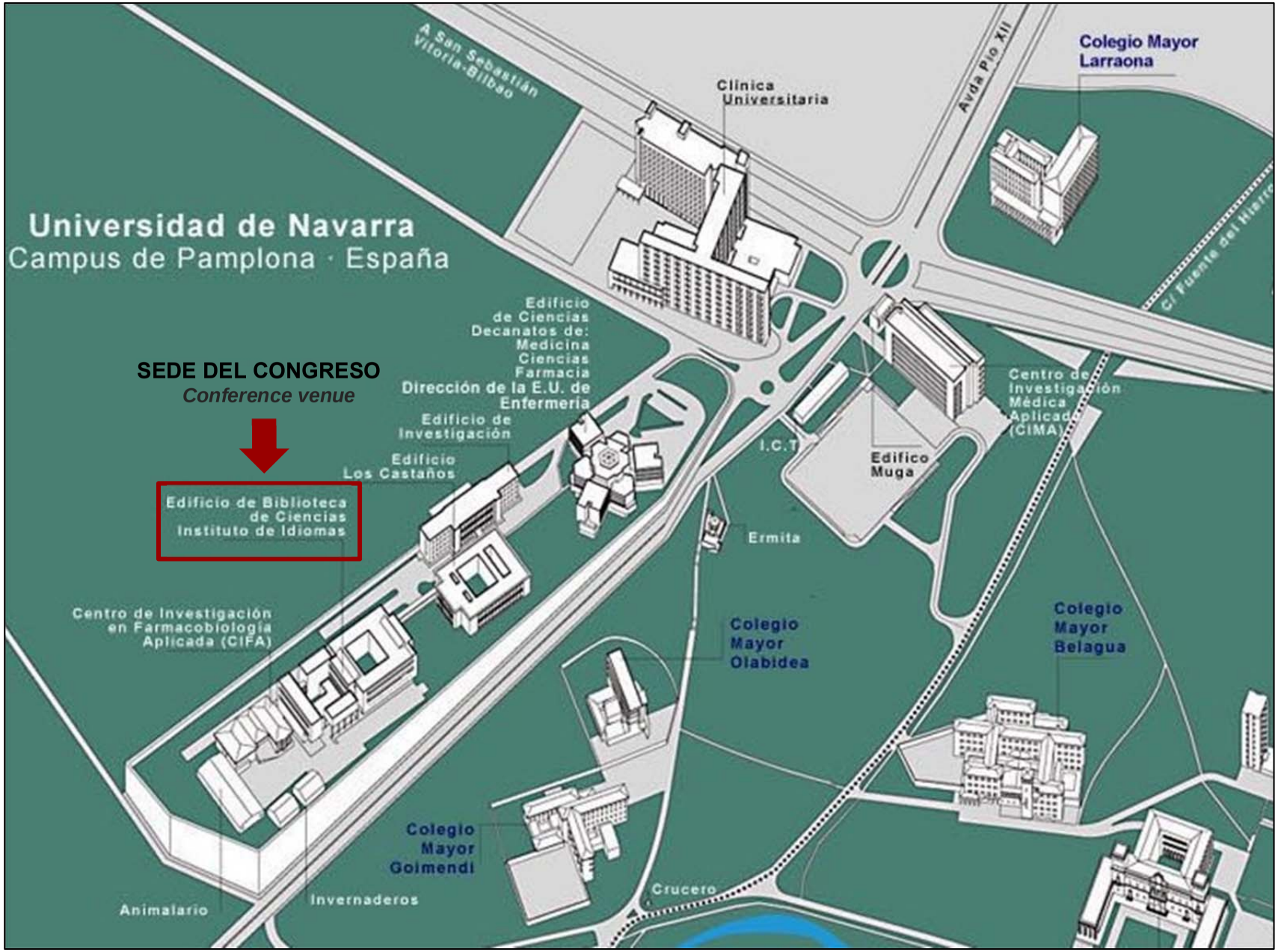
Sede del congreso - Conference venue

Aula 10, Edificio de Biblioteca de Ciencia

Facultad de Farmacia

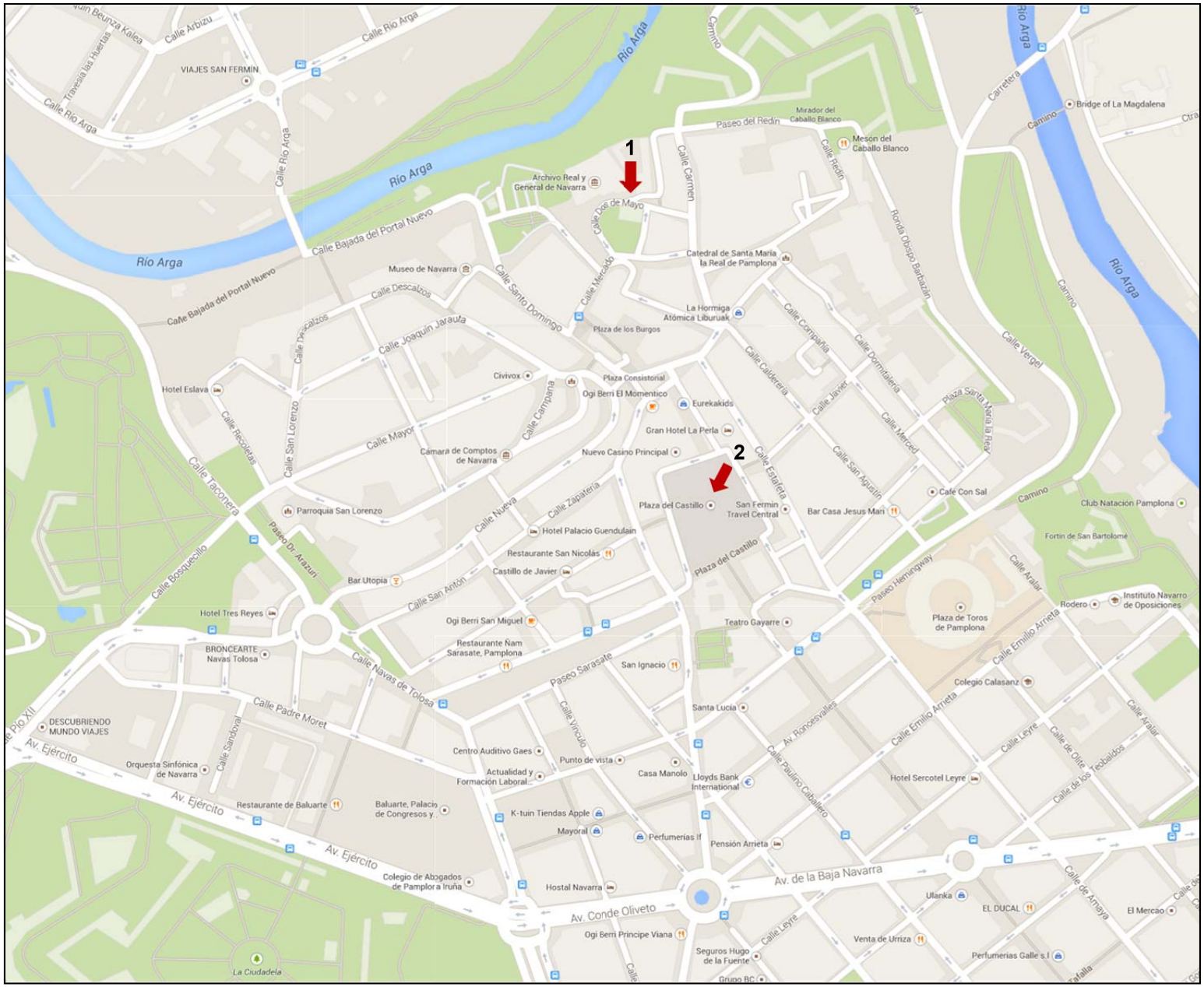
Universidad de Navarra

C/ Irunlarrea 1, Pamplona


A San Sebastián
Vitoria-Bilbao
Clínica
Universitaria
Avda Pío XII
Colegio Mayor
Larraona
Universidad de Navarra
Campus de Pamplona · España
Edificio
de Ciencias
Decanatos de:
Medicina
Ciencias
Farmacia
Dirección de la E.U. de
Enfermería
SEDE DEL CONGRESO
Conference venue
Edificio de
Investigación
Edificio
Los Castaños
Edificio de Biblioteca
de Ciencias
Instituto de Idiomas
I.C.T
Edificio
Muga
Centro de
Investigación
Médica
Aplicada
(CIMA)
Ermita
Centro de Investigación
en Farmacobiología
Aplicada (CIFA)
Colegio
Mayor
Olabidea
Colegio
Mayor
Belagua
Colegio
Mayor
Goimendi
Animalario
Invernaderos
Crucero

1. Hotel Puerta del Camino
Cena de gala. C/ Dos de Mayo, Nº4

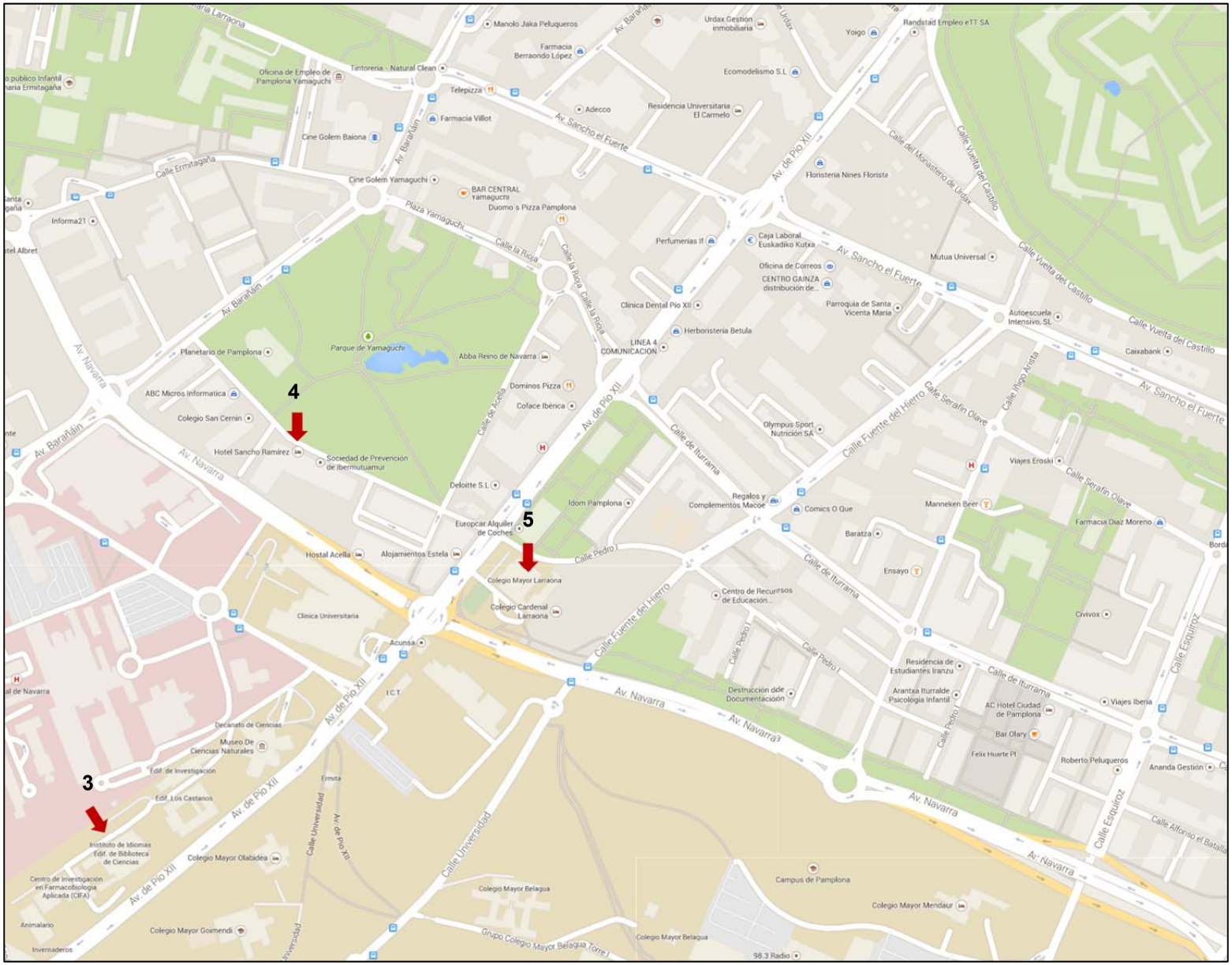
2. Plaza del Castillo
Punto de encuentro.
Visita guiada *"Pamplona Monumental"*



3. Edificio de Biblioteca de Ciencias. Universidad de Navarra
Lugar de celebración del XXI Congreso de la SEMA. Aula 10. C/ Irunlarrea, Nº 1.

4. Hotel Sancho Ramírez
Calle Sancho Ramírez, Nº 11

5. Colegio Mayor Larraona
Av. Pío XII, Nº 45



COMITÉ CIENTÍFICO – SCIENTIFIC COMMITTEE

Leire Arbillaga Lacunza

Amaya Azqueta Oscoz

Amadeu Creus Capdevila

Eduardo de la Peña de Torres

Adela López de Cerain Salsamendi

Ricard Marcos Dauder

Santiago Mateos Cordero

Carmen Pueyo de la Cuesta

Mª Teresa Roldán Arjona

Luisa María Sierra Zapico

Ariane Vettorazzi Armental

COMITÉ ORGANIZADOR – ORGANISING COMMITTEE

Presidenta - President:	Amaya Azqueta Oscoz
Secretaria - Secretary:	Leire Arbillaga Lacunza
Tesorera - Treasurer:	Ariane Vettorazzi Armental
Vocales - Assistants:	Adela López de Cerain Salsamendi
	Laura Pastor Castro
	Tamara Iglesias Alonso
	José Manuel Enciso Gadea

ÍNDICE – CONTENT

PROGRAMA CIENTÍFICO – SCIENTIFIC PROGRAMME

MIÉRCOLES 18 DE JUNIO - WEDNESDAY JUNE 18

15:00 - 15:30 Registro y entrega de documentación - Registration

15:30 - 16:00 Acto de Inauguración Oficial de la Reunión - Opening Ceremony

Congresos de la SEMA desde Talavera de la Reina-2007 a Pamplona-2014.

Eduardo De la Peña (*Consejo Superior de Investigaciones Científicas-CSIC)*

16:00 - 18:40 Sesión I: Inestabilidad Genómica, Mutagénesis y Carcinogénesis - Session I: Genomic instability, mutagenesis and carcinogenesis
Moderador - Chair
Ricard Marcos (Universidad Autónoma de Barcelona)

16:00 **Comunicación invitada - Invited Communication**

Presente y futuro de los marcadores genéticos en las neoplasias hematológicas.

María José Calasanz (Universidad de Navarra)

16:30 *Genomic damage as a biomarker of chronic kidney disease status*
Zuray Corredor (Universidad Autónoma de Barcelona)

16:50 *Sex-dependent gene expression of kidney transporters after ochratoxin A exposure in F344 rats*

Laura Pastor (Universidad de Navarra)

17:10 - 17:40 Café - Coffee

17:40 *Oxidative DNA damage enhances the carcinogenic potential of chronic arsenic exposures*

Jordi Bach (Universidad Autónoma de Barcelona)

18:00 *Distribution of PON1Q192R susceptibility genotypes to organophosphate pesticides in agricultural workers from Elqui and Limarí valleys of IV Region, Chile*

Liliana Zúñiga (Universidad Católica del Norte)

20:00 - 21:30 Visita guiada a pie por el Casco Antiguo de Pamplona - City tour (by foot) trough Pamplona´s old town

JUEVES 19 DE JUNIO – THURSDAY, JUNE 19

9:00 - 12:20 Sesión II: Daño genotóxico, protección y reparación - Session II: DNA damage, DNA protection and repair

Moderador - Chair

Eduardo De la Peña (Consejo Superior de Investigaciones Científicas-CSIC)

9:00 **Comunicación invitada - Invited Communication**

Individual differences in DNA damage and repair: what do they mean?

Andrew Collins (University of Oslo)

9:30 *Does time of lysis really affect the in vitro alkaline comet assay results/outcome*?

José Manuel Enciso (Universidad de Navarra)

9:50 *Mutagenic and genotoxic effects induced by carvacrol, thymol and their mixture in Caco-2 cells*

Maria Llana-Ruiz-Cabello (Universidad de Sevilla)

10:10 *Determination of genotoxicity in oral mucosa cells with fixed orthodontic and miniscrew appliances using the comet assay*

María Puerto (Universidad de Sevilla)

10:30 *Antimutagenic activity of coffee brew and spent coffee*

Carmen Monente (Universidad de Navarra)

10:50 - 11:20 Café - Coffee

11:20 *Cisplatin resistance analysis: insights on the combination of DNA adducts and DNA strand breaks detection in human cells*

Marta Espina (Universidad de Oviedo)

11:40 *The comet assay in Drosophila: analysis of in vivo and in vitro repair approaches over MMS induced DNA damage*

Luisa María Sierra (Universidad de Oviedo)

12:00 *Arsenic exposure disrupts the normal function of the FA/BRCA repair pathway*

Jana Peremartí (Universidad Autónoma de Barcelona)

12:30 - 13:30 Mesa redonda - Round table

Moderador – Chair

Adela López de Cerain y Leire Arbillaga (Universidad de Navarra)

Pharmaceutical genotoxicity testing: regulatory testing and strategies for early candidate selection

Antonio Guzmán / Antonio Rodríguez (Esteve)

14:00 - 15:30 Comida - Lunch (Hotel Sancho Ramírez; C/ Sancho Ramírez 11)

16:00 - 18:20 Sesión III: Ecogenotoxicología - Session III: Ecogenotoxicology

Moderador - Chair

Amadeu Creus (Universidad Autónoma de Barcelona)

16:00 **Comunicación invitada - Invited Communication**

Genotoxicity assessment in environmental toxicology - somewhere between the "cult of the imperfect" and the "perfect solution"

Mario Pacheco (University of Aveiro)

16:30 *The transience of DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish (Anguilla Anguilla) upon cessation of exposure*

Sofia Guilherme (University of Aveiro)

16:50 *Genotoxicity and DNA repair induced by a glyphosate-based herbicide in fish upon exposure and post-exposure periods*

Ana Marques (University of Aveiro)

17:10 - 17:40 Café - Coffee

17:40 *Identification of genes associated with DDE-induced toxicity in Mus spretus by PCR array focused on stress response.*

Noelia Morales-Prieto (Universidad de Córdoba)

18:00 *Genotoxicidad inducida por antiinflamatorios no esteroideos en organismos acuáticos*

Nadia C. Neri (Universidad Autónoma del Estado de México)

18:20 - 19:10 Asamblea SEMA - SEMA Assembly

21:00 Cena - Dinner

Hotel Puerta del Camino: C/ Dos de Mayo Nº 4

VIERNES 20 DE JUNIO – FRIDAY, JUNE 20

9:30 - 12:30 Sesión IV: Nanogenotoxicología - Session IV: Nanogenotoxicology
Moderador – Chair
Luisa María Sierra (Universidad de Oviedo)

9:30 **Comunicación invitada - Invited Communication**

Genotoxicity assesment of nanoparticles

Miquel Borrás (Universidad de Barcelona)

10:00 *Inter-comparison of the biological properties of nanomaterials just after dispersion and after its freezing (liquid N2 / -80 ºC)*

Laura Vila (Universidad Autónoma de Barcelona)

10:20 *Evaluation of the genotoxic effects of polymeric nanoparticles designed to cross biological barriers*

Tamara Iglesias (Universidad de Navarra)

10:40 *Long-term exposures to low doses of cobalt nanoparticles induce cell-transformation enhanced by oxidative damage*

Balasubramanyam Annangi (Universidad Autónoma de Barcelona)

11:00 - 11:30 Café - Coffee

11:30 *Antioxidant properties of cerium oxide nanoparticles in BEAS-2B cells*

Laura Rubio (Universidad Autónoma de Barcelona)

11:50 *Biological effects of cerium nanoparticles using Drosophila as a model organism*

Mohamed Alaraby (Universidad Autónoma de Barcelona)

12:10 *Titanium dioxide and zinc oxide nanoparticles are not mutagenic in the mouse lymphoma assay but modulate the effect of UVC-light post-treatment*

Esref Demir (Akdeniz University y Universidad Autónoma de Barcelona)

12:30 Clausura - Closing Ceremony

Congresos de la SEMA desde Talavera de la Reina-2007 a Pamplona-2014

Eduardo De la Peña (Consejo Superior de Investigaciones Científicas)

Esta es la tercera vez que hago una revisión gráfica de los Congresos y Reuniones celebrados antes y después de la creación de la Sociedad Española de Mutagénesis Ambiental SEMA (1988); en Murcia presenté y en su programa quedan reflejadas las reuniones desde 1984 a 1998 realizadas previas a la creación de SEMA, Madrid - 1984, Córdoba - 1985, Majadahonda - 1987 y Madrid - 1988; en este mismo año en agosto se constituyó y se aprobaron los Estatutos de la Sociedad Española de Mutagénesis Ambiental, desde entonces hemos celebrado reuniones en Barcelona - 1989, Córdoba-1989 (EEMS), Oviedo - 1990, Pamplona - 1991, Zaragoza - 1992, Barcelona - 1993 (23 *Annual Meeting of the EEMS)*, Córdoba - 1994, Alcobendas (Madrid) - 1995, Sevilla - 1996, en el que solo pude participar con un cartel, enviado desde la Universidad de California Riverside, al coincidir con mi año sabático en USA (1995-1996); Oviedo - 1997, y Murcia - 1998, denominada *MRCIA98* (*Proceding* SEMA Murcia, 1998); de todas estas reuniones se reseñan y citan a los organizadores y las personas invitadas como Ponentes; deseo también destacar la edición del libro titulado "Evaluación Mutagénica y Genotóxica", donde se recopilan los trabajos de los diferentes grupos que regularmente asisten a las reuniones de SEMA y que contribuyeron en el citado libro, editado como parte de las actividades de la Reunión MRCIA98 (de la Peña *et al*. 1999).

En 1998 celebramos una mesa redonda en el II Congreso de la Sociedad Española de Genética en La Coruña - 1999, Barcelona - 2000 (mi primera ausencia a una reunión de SEMA por causa mayor), Bilbao - 2002, Santiago de Compostela - 2003, Segovia - 2004, Oviedo - 2005, Córdoba - 2006, y Talavera de la Reina - 2007, donde publicamos la segunda revisión gráfica de programas, organizadores y ponentes (*Proceding* SEMA Talavera de la Reina, 2007).

Desde 2007 a la actualidad, hemos celebrado reuniones en Barcelona - 2009, La Coruña - 2010, que empezamos ya a denominar Congreso, Córdoba - 2012, a la que mi tiempo de espera para un trasplante hepático me impidió asistir, pero la información que me llegaron hicieron patente que se desarrolló con total éxito, como lo han sido todos los Congresos y Reuniones celebradas por SEMA.

La amabilidad de los actuales organizadores (Amaya Azqueta, Leire Arbillaga, Ariane Vettorazzi, Adela López de Cerain, Laura Pastor, Tamara Iglesias, José Manuel Enciso) me permite presentar la tercera revisión gráfica de los programas, los organizadores e invitados de todos ellos, con alguna imagen interesante de los asistentes.

Deseo terminar con el deseo de un éxito del presente XXI Congreso de la SEMA, en Pamplona-2014. Ya en el congreso de la EEMS de Maastricht - 2004, hablamos de su potencial organización Adela, Amaya, Leire y Ricardo; y en el Congreso de AETOX de Salamanca-2013, acordamos entre Amaya, Ariane, Adela y Ana Gloria, ponerlo en marcha y organizarlo en Pamplona, por ello y porque lo celebramos en la Universidad de Navarra, mi Universidad, me siento particularmente contento, y deseo que sea un fructífero y agradable Congreso, como han venido siendo nuestras Reuniones y Congresos de la SEMA, celebrados con anterioridad y que se pueden consultar y ver sus correspondientes programas, organizadores y ponentes en la *web* de nuestra Sociedad Española de Mutagénesis Ambiental – SEMA.

(http://mutagenesisambiental.com/wp-content/uploads/varios/Reuniones%20SEMA%201984-2014.pdf)

Deseo destacar la importancia de la valoración mutagénica, pieza clave y fundamental en la evaluación predictiva del riesgo cancerígeno de las sustancias y mezclas para el hombre.

SESIÓN I: INESTABILIDAD GENÓMICA, MUTAGÉNESIS Y CARCINOGÉNESIS – SESSION I: GENOMIC INSTABILITY, MUTAGENICITY AND CARCINOGENICITY

PRESENTE Y FUTURO DE LOS MARCADORES GENETICOS EN NEOPLASIAS HEMATOLOGICAS

María José Calasanz

Departamento de Bioquímica y Genética, Facultad de Ciencias, Universidad de Navarra, Pamplona.

En leucemias y linfomas, el análisis genético constituye una herramienta esencial no sólo a nivel diagnóstico, ya que existen marcadores genéticos específicos, sino también a nivel pronóstico, lo que permite estratificar a los pacientes en grupos de riesgo y adaptar el tratamiento a ese nivel de riesgo. Además, existen terapias dirigidas a una diana genética, por lo que un análisis genético permite discernir que pacientes son susceptibles de beneficiarse de ese tratamiento, evitando los tratamientos oncológicos convencionales de quimioterapia y radioterapia. Estas terapias moleculares son las que han mejorado de forma espectacular la tasa de curación. Así por ejemplo, determinadas leucemias, antes mortales, ahora tienen una respuesta positiva al tratamiento y pueden curar definitivamente o mantener al paciente con vida durante muchos.

La descripción y utilización de marcadores genéticos crece de manera exponencial, debido al enorme impulso que ha supuesto la publicación del genoma humano y al desarrollo imparable de nuevas técnicas de análisis genético a gran escala, como la secuenciación masiva. Por ello, los laboratorios de análisis genético se ven constantemente obligados a implementar técnicas que supongan un claro beneficio para los pacientes.

En la ponencia se explicarán las distintas técnicas de diagnóstico genético, así como los marcadores genéticos específicos de los distintos tipos de leucemias y linfomas, y se verán ejemplos de cómo, a través del diagnóstico genético, podemos dirigir el tratamiento y controlar el éxito del mismo.

Notas - Notes

Genomic Damage as a Biomarker of Chronic Kidney Disease Status

Zuray Corredor[1], Elitsa Stoyanova[1], Lara Rodríguez-Ribera[1], Elisabet Coll[2], Irene Silva[2], Juan Manuel Diaz[2], José Ballarin[2], Ricard Marcos[1,3], Susana Pastor[1,3]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]Fundació Puigvert, 08025 Barcelona, Spain. [3]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Chronic kidney disease (CKD) is characterized by a progressive loss of kidney function. Thus, CKD patients are defined as those showing either kidney damage or a glomerular filtration rate (GFR) < 60 mL/min per 1.73 m^2 for three or more months. Consequently, a five-stage classification system is used to define the severity of the pathology in adults. As renal disease worsens, from stage 1 to 5, kidney functions deteriorate and at the end-stage of renal failure, kidney replacement or dialysis therapies are required.

It has been reported that patients suffering from CKD exhibit a high incidence of cancer and cardiovascular diseases, as well as high levels of genomic damage. To confirm the association of CKD and genomic damage we have carried out the largest study ever performed using a total of 602 subjects (187 controls, 206 pre-dialysis CKD patients and 209 CKD patients in hemodialysis). We also studied the effect of vitamin E, as antioxidant, in a group of 15 end-stage renal disease patients, submitted to hemodialysis with a membrane with vitamin E during 6 months. Genomic and DNA oxidative damage was measured in all individuals, using the comet assay.

Our results indicate that CKD patients present significantly higher levels of DNA damage, in comparison to controls, but no significant differences were observed between pre-dialysis and dialysis (HD) patients. When oxidative damage was measured, no differences were observed between patients and controls, although HD patients showed significantly high levels of oxidative damage than pre-dialysis patients. No differences neither in the levels of genomic nor oxidative DNA damage were observed after 6 months of hemodialysis with vitamin E (preliminary results). In addition, a good relationship was demonstrated between genomic damage and all-cause mortality.

Our study confirms that the levels of genomic damage can be considered a good indicator measuring the status of CKD patients, and that the presence of high levels of DNA damage may indicate a poor prognosis in HD patients. Individuals with high levels of basal DNA damage have increased risk for chronic renal failure, in addition to other outcomes such as cancer.

Notas - Notes

Sex-dependent gene expression of kidney transporters after ochratoxin A exposure in F344 rats

Laura Pastor, Ariane Vettorazzi, Adela López de Cerain

Department of Pharmacology and Toxicology, School of Pharmacy, University of Navarra, Pamplona, Spain.

Ochratoxin A (OTA) is a mycotoxin produced by fungi of the genera *Aspergillus* and *Penicilium*. It contaminates several food commodities such as cereals, nuts and spices. It is considered as a potent renal carcinogen in rodents but its mechanism of action is still not understood. It is well known that rats are considerably more sensitive than mice. Moreover, a high susceptibility of male rats to tumour formation has been demonstrated. It has been hypothesized that sex- and species-differences in OTA-mediated toxicity might be due to variations in transport mechanisms in kidney cells. According to this, some renal transporter families, which can be responsible for OTA transport, have demonstrated sex differences and also variations in their expression levels after OTA exposure. Therefore, the aim of this study was to analyze, by RT-qPCR, renal transporters expression in 15-week-old male (M) and female (F) F344 rats at basal level and after single oral OTA administration (0.50 mg/kg bw). Temporal profiles (24h, 48h, 72h, 96h, 1 and 2 months) were studied per sex and transporter.

Oatp1 (M>F), Oat2 and Pept2 (F>M) sex-differences were confirmed at basal level. Moreover, a high Bcrp expression was observed in males. After OTA exposure, females showed a general decrease in all transporters studied, mainly after 48 hours. In males, expression changes were observed after 24 hours and mainly in apical proteins; besides, at 48 hours, an increase in Oat2 expression (reabsorption transporter) and a decrease of Mrp2 and Bcrp (exclusion transporters) were also observed.

While similar time-profiles were determined for Abc, Oatp (Slco1) and Pept (Slc15) families, the highest sex differences involved Oat (Slc22) transporters. Oat2, Oat3 and Oat5 expression showed a significant increase in males while Oat1, Oat2 and Oat5 level decreased considerably in females. These molecular changes might be implicated in the highest male susceptibility to OTA renal carcinogenesis.

Notas - Notes

Oxidative DNA damage enhances the carcinogenic potential of chronic arsenic exposures

Jordi Bach[1], Jana Peremartí[1], Balasubramanyam Annangi[1], Ricard Marcos[1,2], Alba Hernández[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Chronic arsenic exposures are known to increase the incidence of several cancers in humans. Our previous work demonstrated that environmentally relevant arsenic exposures generate a more rapid accumulation of pre-carcinogen 8-OH-dG DNA lesions in MEF *Ogg1*-deficient cells; nevertheless, it remains unsolved whether this observed arsenic induced oxidative DNA damage (ODD) is certainly important in terms of cancer acquire.

In this study isogenic MEF *wild-type* and *Ogg1*$^{-/-}$ cells were exposed to sub-toxic doses of sodium arsenite. The selected doses were 0.5, 1 and 2 μM and the exposure last for 40 weeks. Hallmarks of cell transformation such as matrix metalloproteinase (MMP) activities measured by zymography, colony formation and promotion of cancer progression evaluated by soft agar assay, and cellular invasiveness measured by transwell assay were assessed. On the other hand intrinsic cancer-like phenotypic changes such as alterations in cellular morphology growth and differentiation status -analysed by real time RT-PCR- were included as complementary measures of transformation.

Results shown that MEF *Ogg1*$^{-/-}$ cells showed a cancer-associated phenotype after 30 weeks of exposure, as indicated by morphological changes, increased proliferation, de-regulated differentiation status, increased MMPs secretion, anchorage independent cell growth and enhancement of tumour growth and invasiveness. Conversely, MEF *wild-type* cells did not present changes in morphology or proliferation, exhibited a milder degree of gene de-regulation and needed 10 weeks of additional exposure to the highest arsenite doses to show tumour enhancing effects.

To sum up our results demonstrate that *Ogg1* genetic background and arsenic-induced 8-OH-dG appear relevant for arsenic-mediated carcinogenic effects. This is the first study directly linking ODD with arsenic carcinogenesis. Implications in human exposed populations need to be explored since individuals carrying variant *OGG1* alleles are expected to be at higher risk.

Notas - Notes

Distribution of PON1Q192R susceptibility genotypes to organophosphate pesticides in agricultural workers from Elqui and Limarí valleys of IV Region, Chile

Zúñiga-Venegas L. & Pancetti-Vaccari F

Laboratory of Environmental Neurotoxicology. Department of Biomedical Sciences. Faculty of Medicine. Universidad Católica del Norte. Coquimbo-Chile.

In recent years Chile has experienced an enormous increase in agricultural activity, which is reflected in the increased use of pesticides; indeed, the sales of these compounds have increased by more than 400% between 2001 and 2010, exposing local population to new and high levels of contaminants. Although the benefits to agricultural productivity are undeniable, their use is associated to complex human health problems.

Several studies have demonstrated the capability of human plasma to metabolize toxic organophosphate compounds (OP's) and its strong correlation with a serum protein: the Paraoxonase-1 (PON1), but PON1 activity shows a great variability in the population (individuals with low, medium, and high activity level) as a result of a substitution at position 192: glutamine (Q) or arginine (R). Having this polymorphic allele and its relation with metabolic activity, we measured plasma hydrolytic activity in two groups of volunteers (85 exposed to OP's and 103 control) using paraoxon [paraoxonase (PONase) activity], phenylacetate [arylesterase (AREase) activity], and diazoxon [diazoxonase (DZOase) activity] as substrates with standardized spectrophotometric assay. Thus, we determined the *Status* PON1 which is the combination of PON1Q192R genotype by plotting DZOase *vs* PONase activities and Q192R phenotype using PONase/AREase and PONase/DZOase ratios. Genotypes were verified by qPCR using TaqMan® probes.

Allelic and genotypic frequencies of $PON1_{Q192R}$ polymorphism, as well as their metabolic activities, were established for the first time for a group of agricultural Chilean workers; Q allele was more frequently represented in our exposed and control population (~60%), being this allele less efficient than R allele to metabolize the pesticide *chlorpyrifos*, the most used OP pesticide in this area. Moreover, a great inter-individual variability of PON1 activity was observed suggesting a wide individual susceptibility to these agricultural pesticides, aspect which must be considered in human monitoring studies.

Notas - Notes

SESIÓN II: DAÑO GENOTÓXICO, PROTECCIÓN Y REPARACIÓN – SESSION II: DNA DAMAGE, DNA PROTECTION AND REPAIR

Individual differences in DNA damage and repair: what do they mean?

Andrew R. Collins

Department of Nutrition, University of Oslo, Oslo, Norway.

Human biomonitoring studies make great use of the comet assay to measure DNA damage (strand breaks and altered bases), antioxidant status, and DNA repair activity, generally in lymphocytes isolated from venous blood. The involvement of DNA damage as the initiating event in carcinogenesis is evident, and yet almost all damage is repaired before it can cause mutations; DNA damage is therefore best seen as a marker of exposure to genotoxins. DNA repair is regarded as a marker of susceptibility; a high intrinsic repair rate should protect against mutations and cancer. However, rather little is known of the regulation of repair. Inter-individual variation – in both base BER and NER (base and nucleotide excision repair) activities – is far greater than can be explained by genetic polymorphisms, and environmental and/or intrinsic factors are clearly involved. There is evidence for induction of repair by exposure to certain genotoxins, and dietary factors have been shown to modulate both BER and NER. The mechanism of regulation, which seems to be at a post-transcriptional level, is not yet understood, but we assume that the various factors interact with a common cell signalling pathway.

I will describe some recent and on-going studies. The NewGeneris project aimed to investigate the influence of maternal exposure and nutrition on health of newborns, using a range of biomarkers including the comet assay. In Norway, we are involved in the 'Typisk Norsk' study, in which colorectal cancer patients, post-surgery, are encouraged to adopt a healthy diet, rich in fruits and vegetables. The ComNet project is currently collecting comet assay data from a large number of human studies, in order to carry out a pooled analysis, and to obtain definitive answers to questions such as whether men and women have similar levels of DNA damage, how damage and repair relate to age, the effect of smoking, and the influence of nutritional factors. Ideally, prospective cohort studies are needed, to see whether DNA damage and repair are predictive markers of risk of cancer or other diseases.

As a postscript, I will describe a simple method for retrieval of leukocytes from frozen blood that could greatly simplify the collection and storage of samples from human trials.

Notas - Notes

Does time of lysis really affect the *in vitro* alkaline comet assay results?

José Manuel Enciso, Oscar Sánchez, Adela López de Cerain, Amaya Azqueta

Department of Pharmacology and Toxicology, School of Pharmacy, University of Navarra, Pamplona, Spain.

Single-cell gel electrophoresis (the comet assay) is now the method of choice for measuring several kinds of DNA damage in cells and tissues. It has several applications such as in genotoxicity testing, human biomonitoring and ecogenotoxicology. Although this assay has been in use for almost 30 years, due to its versatility it is still under development. Various organisations and regulatory bodies have an interest in monitoring chemicals for genotoxicity with this method; in fact, a new version of the "*In vivo* mammalian alkaline comet assay" draft OECD guideline for the testing of chemicals was published just a few months ago. However, little effort has been made to standardise the *in vitro* version of the comet assay. Agarose concentration, alkaline unwinding time and electrophoresis conditions have been identified as critical points affecting the alkaline comet assay outcome. Nevertheless, there is no scientific publication reporting the effect that modifying the time of lysis would have. Here we tried 10 different times of lysis in control HeLa cells and HeLa cells treated with different concentrations of either methyl methanesulfonate (MMS) or H_2O_2. We also tested 7 different times of lysis in the alkaline comet assay combined with formamidopyrimidine DNA glycosylase (FPG) in untreated HeLa cells and cells treated with the photosensitiser Ro 19-8022 (Ro) plus light. Leaving the gels on ice before lysis appears to increase the DNA damage detected in MMS-treated cells, but this effect was not observed in H_2O_2 or Ro plus light-treated cells. Besides, it was observed that MMS or H_2O_2-induced DNA damage can be detected in the absence of lysis, with similar results from 0 min to 1 h of lysis. Nevertheless, no enzyme-sensitive sites were detected in the absence of lysis in Ro plus light-treated cells, presumably because the enzyme is not capable of entering intact cells. In this case, a 5-min-lysis step was enough to detect enzyme-sensitive sites. Finally, as longer times of lysis (i.e. more than 1 h) increase the sensitivity of the assay, different times of lysis might be employed under circumstances requiring enhanced sensitivity.

Notas - Notes

MUTAGENIC AND GENOTOXIC EFFECTS INDUCED BY CARVACROL, THYMOL AND THEIR MIXTURE IN CACO-2 CELLS

Llana-Ruiz-Cabello M[1], Puerto M[1], Maisanaba S[1], Prieto AI[1], Pichardo S[1], Jos A[1], Cameán AM[1]

[1]Area of Toxicology. Faculty of Pharmacy. University of Seville. Professor García González n°2, 41012 Seville, Spain.

Oregano essential oil has been selected by food industry as additive to develop new active packaging systems in order to improve shelf life of perishable products. The aim of this study was to evaluate the possible mutagenic and genotoxicity effects of carvacrol and thymol, main components of this essential oil, and their mixture. The mutagenic effects of carvacrol, thymol and their mixture were performed according to the recommendations of Maron and Ames (1983), and following the principles of OCDE guideline 471 (1997). Five *Salmonella typhimurium* histidine-auxotrophic strains were used for the assay. Different concentrations of carvacrol (29, 56, 115, 230 and 460 µM), thymol (15.6, 31.3, 62.5, 125 and 250 µM) and the mixture (29-2.9, 56-5.6, 115-11.5, 230-23, 460-46 µM) were assessed either with or without metabolic activation (S9) in three independent experiments. Moreover, the possible genotoxicity of carvacrol and thymol was evaluated in the intestinal Caco-2 cell line using the comet assay. Cells were exposed to carvacrol (115, 230 and 460 µM), thymol (62.5, 125, 250 µM) or their mixture 10:1 carvacrol/timol (75:7.5; 150:15; 300:30 µM) for 24 or 48 h. The standard comet assay and detection of oxidative DNA damage with enzyme (Formamidopyridine DNA glycosylase, FPG or Endonuclease III) comet assay were used. Regarding Ames test, thymol did not show any mutagenic effect at any concentrations tested, although carvacrol demonstrated a mutagenic effect in all concentrations tested. Similarly, the mixture of carvacrol-thymol showed mutagenic activity at 115-11.5 and 460-46 µM only in presence of S9 metabolic activation system. In the standard comet assay, the results revealed no significant increase in DNA strand breakage for both compounds and the mixture in any concentrations and exposure time. However, the Fpg-modified comet assay revealed a significant increase after 48h of treatment when Caco-2 was exposed to the highest concentration of carvacrol (460µM) and their mixture (300:30 µM). Therefore, considering that carvacrol and the mixture with thymol presented potential mutagenicity and oxidative damage in purine bases at the highest concentration assayed, further safety studies should be carried out before essential oils compounds could be widely used in foods.

Acknowledgements: Authors wish to thank to the Ministerio de Ciencia e Innovación from Spain (AGL2012-38357-C02-01) and Junta de Andalucía (AGR-7252) for funding this work and the service of Biology CITIUS Seville University for technical support.

Notas - Notes

Determination of genotoxicity in oral mucosa cells with fixed orthodontic and miniscrew appliances using the comet assay

Martín-Cameán Ana[1], Puerto María[2], Jos Angeles[2], Azqueta Amaya[3], Iglesias-Linares Alejandro[4], Solano Enrique[1], Cameán Ana M[2]

[1]Stomatology Department, School of Dentistry, University of Seville, Spain; [2]Area of Toxicology, Faculty of Pharmacy, University of Seville, Spain; [3]Department of Pharmacology and Toxicology, School of Pharmacy, University of Navarra, Spain; [4]Stomatology Department, School of Dentistry, University Complutense of Madrid, Spain.

Orthodontic appliances are usually made of stainless steel alloys, which contain metals. The mouth properties (thermal, microbiological and enzymatic) offer an ideal environment for the biodegradation of orthodontic appliances, consequently facilitating the release of metal ions that are related to adverse health effects, such as cellular and genetic toxicity. Although the genotoxic potential of some orthodontic appliances (e.g. brackets) has been investigated, as far as we know, this is the first time to determine the genetic toxicity in patients undergoing orthodontic, and orthodontic and miniscrew treatment, using the comet assay in buccal cells. Oral mucosa cells were obtained from 60 individuals ranging from 19 to 63 years old. These patients were classified into three groups: the orthodontic group (11 men, 9 women), treated with fixed orthodontic appliances in both arches; orthodontic-miniscrew group (10 men, 10 women), with fixed orthodontic and miniscrew treatment; and the control group (8 men, 12 women). The eligibility criteria for control subject selection included nonsmokers and without oral diseases, systemic diseases, oral restorations or prosthetics. The results indicated a significant increase of % DNA in tail and olive moment in the orthodontic and orthodontic-miniscrew groups, compared to the control group, but not between each other. When orthodontic, orthodontic-miniscrew and control groups were compared by gender, similar results were obtained in women. In contrast, no significant differences were observed in orthodontic groups-men compared to the control group-men. In conclusion, fixed orthodontic appliances and orthodontic and miniscrew appliances induced DNA strand breaks in buccal cells, being the % DNA in tail and the olive moment similar in both groups. Further investigations are necessary in order to assess the genotoxic potential of orthodontic fixed appliances associated with miniscrews.

Acknowledgement: The authors wish to thank the Junta de Andalucía (PAIDI CTS-358) for the financial support for the present study. The present results constitute part of the doctoral thesis of Martín-Cameán A at the University of Seville, Spain.

Notas - Notes

Antimutagenic activity of coffee brew and spent coffee

Carmen Monente[1], Jimena Bravo[1], Leire Arbillaga[2], M. Paz De Peña[1], Concepción Cid[1]

[1]Department of Nutrition, Food Science and Physiology, School of Pharmacy, University of Navarra, Pamplona, Spain; [2]Department of Pharmacology and Toxicology, School of Pharmacy, University of Navarra, Pamplona, Spain.

Coffee has proven to be an excellent source of bioactive compounds, mainly phenolic acids, such as chlorogenic acids (CGA). These compounds have been extensively associated with a risk decrease in several chronic and degenerative diseases. The by-product generated after brewing processes, named spent coffee, could have similar characteristics and also might contribute with health related properties. In a recent study of our research group, Bravo et al. (2012, 2013) found that spent coffee has antioxidant capacity measured by chemical based assays and in *in vitro* cell cultures, attributed to a high content of phenolic acids, as well as caffeine and Maillard reaction products. The aim of the present study was to evaluate the ability of coffee brew and spent coffee to protect against mutagens responsible of cell mutation. Spent coffee extracts from filter and espresso brewing process, as well as their respective coffee brews, were analyzed using the Salmonella mutagenicity test (Ames Test). Three non-toxic concentrations of the samples were tested with (S9) and without (-S9) metabolic activation. The data prove that both spent coffees extracts and coffee brews were able to reduce the action of direct (NPD) and indirect (2-AF) acting mutagens. The results showed that spent coffee extracts after no metabolic activation (-S9) had higher antimutagenic activity than their respective coffee brews. The protection percentage against direct mutagens (NPD) was up to 35%, showing no dose-dependent pattern. However, spent coffee extracts and coffee brews had a strong protection activity against indirect acting mutagens (2-AF), with a dose-dependent pattern, up to 92% in Arabica spent coffee after metabolic activation (S9). In conclusion, the high antimutagenic activity of spent coffee found in this work suggests that this by-product could be considered as a potential food ingredient to enhance healthy properties of functional foods. However, further experimental and clinical studies would be needed to claim their functional properties.

This research was funded by the Spanish Ministry of Science and Innovation (AGL2009-12052). We thank the Unión Tostadora S.A. for providing the coffee.

Bravo, J., Juaniz, I., Monente, C., Caemmerer, B., Kroh, L., De Peña, M.P., Cid, C., 2012. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. J. Agric. Food Chem. 60, 12565-12573.

Bravo, J., Arbillaga, L., De Peña, M.P., Cid, C., 2013. Antioxidant and genoprotective effects of spent coffee extracts in human cells. Food Chem. Toxicol. 60, 397-403.

Notas - Notes

Cisplatin resistance analysis: insights on the combination of DNA adducts and DNA strand breaks detection in human cells

Marta Espina[1], Mario Corte[2], María Montes[2], Marta Sierra[3], Elisa Blanco[2], L. María Sierra[1]

[1]Dpto. Biología Funcional, Área de Genética e IUOPA, University of Oviedo; [2]Dpto. Química Física y Analítica, University of Oviedo, Spain; [3]Laboratorio de Oncología Médica- IUOPA, University of Oviedo, Spain.

Despite its wide use as a chemotherapeutic drug, cisplatin presents an important problem: resistance development. Although processes like chemical metabolism, DNA repair activity or cell uptake are known to be involved in this resistance, there is not a method to detect it in advance. In Drosophila the combined study of cisplatin induced adduct levels and their genetic consequences, in terms of DNA strand breaks, seemed a potentially useful tool in the identification of cisplatin resistance, since a significant correlation was found between adduct and DNA strand breaks levels, in different conditions of the nucleotide excision repair (NER) system. To check this possibility in human cells, two pairs of cell lines were chosen: 1) NER deficient (GM04321, *XPA* mutant) and efficient (A549) cell lines; 2) cisplatin sensitive (A2780) and resistant (A2780cis) cell lines. These cells lines were exposed to two different treatments: 3 h and 3 h plus 1 h recovery without cisplatin. DNA strand breaks were measured with the alkaline comet assay. Cisplatin induced adducts were quantified using HPLC, followed by ICP-MS. Intracellular Pt levels after 3 h treatment were measured by ICP-MS in all cell lines to estimate cellular uptake.

Total intracellular Pt results showed that there were no differences between the first paired cell lines, for any concentration, whereas total Pt in A2780 was 2-3 times higher than in those and 10 times higher than in A2780cis. Comet assay results, with the first pair of cell lines, showed some differences between them: i) cisplatin induced significant DNA strand breaks in GM04321 but not in A549; ii) after 1 h recovery, GM04312 showed two different responses: (A) double strand breaks were induced by cisplatin, and (B) treatment decreases the levels of spontaneous DNA breaks; iii) no differences between 3 h and 3 h plus 1 h recovery treatments were found for A549, whereas differences were found in the case of the B response for GM04321. For the second pair of cell lines, comet results showed no differences between them for 3 h treatment, although A2780cis showed two different responses. In the case of 3h plus 1 h treatment, a decrease of the spontaneous strand breaks was detected for the resistant cell line. Finally, the analyses of relationships between DNA adducts and strand breaks showed a statistically significant correlation for the first pair of cell lines, with 3 h plus 1 h treatment, considering the repair status.

Notas - Notes

The comet assay in Drosophila: analysis of *in vivo* and *in vitro* repair approaches over MMS induced DNA damage

Rubén Rodríguez[1]#, Isabel Gaivão[2], Leticia Aguado[1], Marta Espina[1], Jorge García-Martínez[1]‡, L. María Sierra[1]*

[1]Dpto. Biología Funcional e IUOPA, Área de Genética, Universidad de Oviedo, Spain. [2]Dpt. of Genetics and Biotechnology, Universidade de Trás-os-Montes e Alto Douro, Portugal. # Present address: Institute of Functional Biology and Genomics (IBFG), University of Salamanca-CSIC, Spain. ‡ Present address: Laboratory of Genomic Instability in Development and Disease, ERIBA, University Medical Center Groningen, The Netherlands.

The comet assay in Drosophila was used to study DNA repair of MMS induced DNA damage in somatic cells, specifically the roles of nucleotide excision repair (NER) system and Mus308 (dmPOLQ) protein, checking the value of the *in vivo* and *in vitro* comet repair approaches. We have used the *mus201*, *mus308* and *mus201;mus308* mutant strains and the wild-type efficient repair *OK* strain in these analyses. For both approaches, third instar larvae were treated *in vivo*, and the comet assays were performed with neuroblast cells from brain ganglia. For the *in vivo* approach, larvae from the different strains were treated with increasing MMS concentrations, and the solvent. In the *in vitro* approach larvae from the wild-type *OK* strain were treated with 1 mM MMS, and the solvent, and comet nucleoids were incubated with cell free protein extracts from the different strains. Results demonstrated that whereas both approaches allowed the detection of NER and Mus308 repair activities on MMS induced damage, only the *in vitro* one permitted the quantification of these activities, comparing them with that of the *OK* strain: considering the Tail DNA comet parameter, the repair activity of *mus201* strain on MMS induced damage was 47% of the *OK* strain activity, whereas that of the *mus308* strain was 63%. These results demonstrate that the *in vitro* comet repair assay is applicable in Drosophila and may be a useful tool to study DNA repair.

Notas - Notes

Arsenic exposure disrupts the normal function of the FA/BRCA repair pathway

Jana Peremartí[1], Facundo Ramos[1], Ricard Marcos[1,2], Alba Hernández[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Chronic arsenic exposure is known to enhance the genotoxicity/carcinogenicity of other DNA-damaging agents by inhibiting DNA repair activities. Interferences with nucleotide excision repair (NER) and base excision repair (BER) are well documented, but interactions with other DNA repair pathways are poorly explored so far. The Fanconi anemia FA/BRCA pathway is a DNA repair mechanism necessary for eliminating interstrand crosslinks (ICLs) from DNA, and it is required for maintaining genomic stability and preventing cancer.

We have explore possible interactions between arsenic compounds and the FA/BRCA pathway by using isogenic *FANCD2*$^{-/-}$ (FA/BRCA-deficient) and *FANCD2*$^{+/+}$ (FA/BRCA-corrected) human fibroblasts. To study whether arsenic compounds generate DNA lesions susceptible to be repaired by the FA/BRCA mechanism, both cell lines were exposed to different concentrations of As^{III}, As^{V}, MMA^{III}, MMA^{V} and ATO. The activation of the FA/BRCA pathway was then evaluated, along with the characteristic hipersensitivity and enhanced G2/M arrest of FA/BRCA-deficient cells. To study whether arsenic disrupts the normal FA/BRCA function, *FANCD2*$^{+/+}$ cells were pre-exposed to sub-toxic doses of the above mentioned arsenic compounds for 2 weeks. The cellular response to mitomycin-C (MMC), a typical ICL inducer, was then evaluated and compared to that of *FANCD2*$^{-/-}$ cells.

Our results show that exposures to pentavalent arsenicals As^{V} and MMA^{V} activate the FA/BRCA pathway and produce an effect in FA/BRCA-deficient cells slightly similar to that of MMC, whereas pre-exposure of FA/BRCA-corrected cells to the trivalent arsenic compounds MMA^{III} and ATO induce a cellular FA/BRCA-deficient phenotype, with increased sensitivity to ICLs.

Overall, our data indicate that the FA/BRCA pathway participates in the arsenic-mediated cellular DNA damage response, and demonstrate that chronic arsenic exposure disrupts the normal function of the FA/BRCA activity, therefore supporting a novel source of arsenic co- and carcinogenic effects. Derived implications in arsenic-associated cancer therapy are of interest, as treatment combining ATO with ICL-agents are expected to retrieve a better outcome.

Notas - Notes

SESIÓN III: ECOGENOTOXICOLOGÍA – SESSION III: ECOGENOTOXICOLOGY

Genotoxicity assessment in environmental toxicology - somewhere between the “cult of the imperfect” and the “perfect solution”

Mário Pacheco

Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

Genotoxicity is one of the first events occurring in organisms exposed to pollutants and stands for the most adverse impact on wildlife. Therefore, the evaluation of the risk to genomic integrity is an unavoidable but also challenging task in the framework of environment health assessment. The difficulty of this pursuit results from three levels of complexity viz. the pollution scenarios, biological and ecological organization, and the net of sub-cellular processes involved in the pro- and anti-genotoxic pathways.

Environmental genotoxicology has accompanied the evolution of other subareas of environmental toxicology and, besides the pollutants traditionally evaluated, showed to be aware to emergent genotoxicants such as pharmaceutical substances, nanomaterials, biotoxins, or radionuclides. A set of diagnostic tools has been applied in a wide diversity of approaches, involving *in vitro*, *ex vivo*, *in vivo* and *in situ* exposures, as well as surveying of wild native specimens. The genotoxicity endpoints commonly used include the detection of DNA adducts, comet assay, induction of chromosomal aberrations (CA) and sister chromatid exchanges (SCE), micronuclei (MN) and erythrocytic nuclear abnormalities (ENA) tests. The comet and ENA/MN are the most adopted assays and their combined use has been recommended since they reflect different levels of genetic impairment. The wide variety of species addressed, tissues sampled and experimental approaches adopted have led to a profusion of adaptations on the protocols. A standardization of procedures for environmental studies is strongly recommended.

The knowledge of tissue- and species-specific temporal patterns of genotoxic responses (e.g. the progression of DNA damage in long-term exposures as well as after removal of genotoxic pressure) is still scarce, which can represent an obstacle on results interpretation.

Some authors state that the ultimate goal of ecogenotoxicoloy should be linking genetic damage at individual level to deleterious effects on higher organizational levels, mainly at population level (e.g. abundance and reproduction impairments). This is a hardly achievable goal for the majority of studies.

Though the “cult of the imperfect” should be avoided and a continuous effort should be carried out by environmental genotoxicologists towards more reliable genotoxicity indicators and the definition of suitable strategies, the “perfect solution” was not yet found, probably because it does not exist.

“If you never miss a plane, you're spending too much time at the airport".

“The best is the enemy of the good”.

"Democracy is the worst form of government, except for all those other forms that have been tried from time to time" (from a House of Commons speech on Nov. 11, 1947).

The perfect solution fallacy is an informal fallacy that occurs when an argument assumes that a perfect solution exists and/or that a solution should be rejected because some part of the problem would still exist after it were implemented.

Notas - Notes

The transience of DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish (*Anguilla Anguilla*) upon cessation of exposure

S. Guilherme[1], F. Venturini[1], M.A. Santos[1], I. Gaivão[2], M. Pacheco[1]

[1] Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal. [2] CECAV and Department of Genetics and Biotechnology, Trás-os-Montes and Alto Douro University, Vila Real, Portugal.

The huge increase of pesticides use in agricultural fields is considered a major problem worldwide. Due to crops seasonality, periodic terrestrial applications imply typically intermittent inputs of these agrochemicals into the aquatic systems. Consequently, fish exposure to this type of contaminants can be short and followed by a period of permanence in non-contaminated areas. Bearing this in mind, the assessment of genotoxic endpoints in fish after removal of the contamination source appears as a crucial step to improve the knowledge on the dynamics of herbicide induced genotoxicity, as well as to determine the actual magnitude of risk posed by these agrochemicals to fish. Therefore, the present study intended to shed light on fish ability to recover from the DNA damage induced by short-term exposures to the herbicide formulations Roundup® (glyphosate-based) and Garlon® (triclopyr-based) upon the exposure cessation. European eel (*Anguilla anguilla*) was exposed to the previous commercial formulations, for 3 days, and allowed to recover for 1, 7 and 14 days (post-exposure period). The comet assay was used to identify the DNA damage in blood cells during both exposure and post-exposure periods. As an attempt to clarify the DNA damaging mechanisms involved, an extra-step including the incubation of the nucleotides with DNA lesion-specific repair enzymes (Endonuclease III - EndoIII and formamidopyrimidine DNA glycosylase - FPG) was added to the standard comet protocol. The genotoxic potential of both herbicides was confirmed, concerning the exposure period. In addition, the involvement of oxidative DNA damage on the action of Roundup® (pointed out as pyrimidine bases oxidation) was demonstrated, while for Garlon® this damaging mechanism was less evident. Fish exposed to Garlon®, though presenting some evidences towards a recovery tendency, didn't achieve a complete restoration of DNA integrity. In what concerns to Roundup®, a recovery was evident when considering non-specific DNA damage, on day 14 post-exposure. In addition, this herbicide was able to induce a late oxidative DNA damage (day 14). It was also recognized that blood cells of *A. anguilla* exposed to Roundup® appeared to be more successful in repairing damage with a non-specific cause, than that associated to bases oxidation. Overall, the present findings highlighted the genetic hazard to fish associated to the addressed agrochemicals, reinforcing the hypothesis of long-lasting damage.

Notas - Notes

Genotoxicity and DNA repair induced by a glyphosate-based herbicide in fish upon exposure and post-exposure periods

Ana Marques[1], Sofia Guilherme[1], Isabel Gaivão[2], Maria Ana Santos[1], Mário Pacheco[1]

[1] Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal; [2] CECAV and Department of Genetics and Biotechnology, Trás-os-Montes and Alto Douro University, 5001-801 Vila Real, Portugal.

The widespread use of herbicides, including the glyphosate-based formulation Roundup®, represents a risk to non-target organisms. These biocides, applied to both agricultural and non-agricultural purposes, can easily reach the water systems, endangering aquatic organisms, namely fish. Among the effects described on fish, genotoxicity has been pointed out as one of the most hazardous. However, the genotoxic mechanisms of Roundup® are not entirely understood as well as the involvement of the DNA damage repair system. Hence, this work aimed to improve the knowledge on the progression of DNA damage upon short-term exposure (3 days) and post-exposure (1 - 14 days) periods in association with DNA repair processes in *Anguilla anguilla* exposed to Roundup® (58 and 116 μgL^{-1}). DNA damage in hepatic cells was evaluated by the comet assay improved with the DNA-lesion specific endonucleases FPG and EndoIII. In order to evaluate the oxidative DNA damage repair ability, an *in vitro* base excision repair (BER) assay was performed, testing hepatic subcellular extracts. Besides the confirmation of the genotoxic potential of this herbicide, oxidative damage was implicit as an important mechanism of genetic damage, which showed to be transient, since DNA integrity returned to the control levels on the first day after cessation of exposure. An increased capacity to repair oxidative DNA damage emerging in the post-exposure period revealed to be a crucial pathway for the *A. anguilla* recovery; nevertheless, DNA repair machinery showed to be susceptible to inhibitory actions during the exposure period, disclosing another facet of the risk associated to the tested agrochemical.

Notas - Notes

Identification of genes associated with DDE-induced toxicity in *Mus spretus* by PCR array focused on stress response.

Noelia Morales-Prieto, Nieves Abril, Carmen Pueyo

Department of Biochemistry and Molecular Biology, Agrifood Campus of International Excellence ceiA3, University of Córdoba, Córdoba, Spain.

Chronic exposure to pollutants is a matter of serious concern because of their potential of causing deleterious effects on human and environmental health. Efficient tools for environmental risk assessment, mainly linked to biological responses, are hence necessary. *Omic* methodologies together to conventional biomarkers provide great potential in this regard, as reported in our previous studies using the free living Algerian mouse (*Mus spretus*) as bioindicator of environmental pollution in Doñana National Park (SW, Spain). However, the complexity of the ecosystems and the synergism/antagonism processes among contaminants make quite difficult to establish a clear link between one contaminant and the specific biological responses by it elicited. For this reason it is necessary to carry out laboratory experiments of exposition to a particular contaminant under controlled condition.

This study considers a semi-omic approach for the evaluation of *M. spretus* mice response to a model contaminants, DDE under controlled experimental conditions. DDE (dichlorodiphenyl-dichloroethylene) is the most prevalent breakdown product of DDT (dichlorodiphenyl-dichloroethane). This organochloride pesticide persists in the environment, concentrates up the food chain and is stored in fatty tissues of animals, fish, and humans. We have used PCR-Arrays to analyze the effect of DDE at the transcriptional level in *M. spretus* mice raised in captivity. PCR-Arrays allow quantitative real-time reverse transcriptase PCR in a more high-throughput way, as it quantifies up to 96 different transcripts, usually involved in one metabolic pathway, on one single plate. The rational of using PCR-Arrays designed for *M. musculus* with *M. spretus* is the phylogenetic proximity between both species. By using a PCR-Array focused on stress response, 21 genes were identified in *M. spretus* liver as responsive to DDE treatment. As PCR-Array generate semi-quantitative data, the results have been verified by absolute quantification by qRT-PCR of the transcripts amounts of a group of selected with primers pairs specific for *M. spretus* sequences. The functional analysis showed deregulation of genes involved in the oxidative stress, the immune response and apoptosis (**CTM2012-38720-C03-02, BIO1657**).

Notas - Notes

GENOTOXICIDAD INDUCIDA POR ANTIINFLAMATORIOS NO ESTEROIDEOS EN ORGANISMOS ACUÁTICOS

Neri C. N[1], Gómez O. LM[1]*, Galar M. M[2], García M. S[2], Dublán G. O[1], López M. LX[1]

[1]Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México. [2]Laboratorio de Toxicología Acuática, Sección de Graduados e Investigación, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional.*Paseo Colón intersección Paseo Tollocan s/n. Col. Residencial Colón, 50120 Toluca, Estado de México, México, Teléfono: (722) 2173890, e-mail:nadianeri85@gmail.com.

Introducción: El agua es un líquido vital para la existencia de todos los organismos vivos. Sin embargo, este recurso se encuentra amenazado por la contaminación, ocasionando problemas en el medio ambiente acuático por la presencia de diferentes xenobióticos tales como metales pesados y compuestos orgánicos, dentro de los que se pueden incluir a los hidrocarburos y plaguicidas. Actualmente los medicamentos de consumo humano y veterinario se han convertido en fuente importante de contaminación, denominados contaminantes de preocupación emergente, dado que diversos estudios han demostrado un incrementado notablemente de su concentración en los mantos acuíferos a nivel mundial. Muchos de estos compuestos son considerados tóxicos para los organismos acuáticos, debido a que dependiendo de su naturaleza y concentración, pueden causar daños letales o crónicos en los mismos. Estos compuestos muestran una gran persistencia en ambientes acuáticos, ya que están diseñados para ser resistentes a la biodegradación.

Existen diversos estudios que refieren que los AINES como el IBP, DCF, NPX y AAS a bajas concentraciones son capaces de producir estrés oxidativo y daño a las macromoléculas (Gómez-Oliván *et al*., 2014, Islas-Flores *et al*., 2014). Sin embargo, la toxicidad en los ecosistemas naturales por lo general no resulta de la exposición a una sola sustancia, sino que es resultado de la exposición a mezclas de sustancias tóxicas y son pocos los estudios que evalúan la toxicidad de estas

Objetivo: el presente trabajo evalua la toxicidad producida por el IBP en *Cyprinus carpio*, NPX en *Hyalella azteca* y DCF en *Daphnia magna* a través de biomarcadores de genotoxicidad.

Metodología: Se determinó la concentración letal media (CL_{50}) del IBP, NPX y DCF por separado, para cada uno de los boindicadores, estos valores sirvieron para determinar la concentración equivalente al LOAEL de cada fármaco las cuales se utilizaron en el estudio subletal en el que se realizó la evaluación del daño genotóxico a través de las pruebas de micronúcleos *(Countrymam y Heddle, 1976)* para *Cyprinus carpio*, electroforesis celular o ensayo cometa *(Tice et al, 2000; Lankoff et al., 2006)*

para *Hyalella azteca* y ensayo cometa modificado, empleando la FPG para identificar las bases púricas oxidadas y la ENDO III para las pirimídicas en *Daphnia magna*.

Resultados y discusión: *Determinación de micronúcleos:* se observó que el IBP produce incremento significativo del número de micronúcleos a las 72 h con respecto al grupo control ($p < 0.05$).También se determinó el daño al material genético por medio del ensayo cometa, encontrándose que el NPX incrementó el índice de daño al DNA en las células de la *H. azteca*, respecto al testigo ($p<0.05$). Por otra parte en el caso de DCF con *Daphnia magna* a fin de determinar si el daño al DNA, se debía a oxidación, se realizó un ensayo cometa modificado, empleando las enzimas FPG y ENDO III, que permiten detectar bases púricas y pirimídicas oxidadas. Observándose un incremento significativo en el daño oxidativo con respecto al testigo en las bases pirimídicas y púricas.

El daño al DNA tiene consecuencias serias, tales como mutaciones y trasformaciones carcinogénicas, llegando incluso a provocar la muerte celular (Medeiros, 2008). En este sentido, los biomarcadores son una herramienta muy útil ya que permiten detectar rápidamente el daño en etapas tempranas, a fin de contrarrestar los efectos de estos compuestos. En el caso particular de los AINEs, así como de otros contaminantes emergentes, el uso de los biomarcadores de daño temprano en especies acuáticas es muy importante debido al uso indiscriminado de estos agentes por parte de la población en general, que conlleva a su amplia distribución en los ecosistemas acuáticos.

Conclusiones: Los datos permiten concluir que el IBP, NPX y DCF inducen genotoxicidad en los organismos bioindicadores esto evidenciado con los biomarcadores utilizados.

Notas - Notes

SESIÓN IV: NANOGENOTOXICOLOGÍA – SESSION IV: NANOGENOTOXICOLOGY

Genotoxicity assessment of nanoparticles

Miquel Borrás

Unit of Experimental Toxicology and Ecotoxicology / CERETOX, Parc Cientific de Barcelona, Barcelona, Spain

The current expansion of nanotechnology and the steady broadening of the range of its practical applications allow to predict, for the coming years, a very substantial increase in the levels of exposure (accidental or intentional) to nanoparticles of anthropogenic origin for humans (including especially sensitive population groups) and for the environment. It is, therefore, necessary to put the basis to be able to perform a risk assessment, preferably integrated, in both areas. This includes having in each case the relevant data of both exposure and toxicity (hazard).

Particles smaller than 100 nm have certain characteristics which distinguish its toxic potential compared to the material (of the same chemical composition) from which they originate. These small particles, among other features, have an increased absorption, especially by unusual ways such as inhalation or skin, are capable of crossing barriers impassable in other conditions (such as blood-brain or retinal), penetrate in organs or tissues hard to reach for other compounds (such as the prostate), or interact with subcellular organelles in special and poorly characterized conditions.

On the other hand, the same considerations apply when nanotechnology is used to vectorize and deliver drugs, despite these being, often, previously known and duly characterized products. The presentation of the same compound in a nano-scale can modulate its toxicity in a way that we cannot predict a priori. While it is expected that nanotechnology will enable us to achieve lower equipotent doses, and therefore obtain lower toxicity levels (as well as a more accurate vectorization should also help to reduce the deleterious effects), we cannot ignore the fact that the drug may reach new tissular, cellular or molecular targets, and it will do so in conditions that make their behaviour difficult to predict.

There is a relatively abundant literature on nanotoxicology. However, certain aspects are identified that still require significant efforts of reflection and experimentation, both in regard to methodological and theoretical considerations or to specific aspects of the toxicological evaluation which have been unsatisfactory or insufficiently addressed.

Among the latter are for example, the need for standardization of procedures in vitro, the lack of repeated dose toxicological data in vivo, and of genotoxicity or teratogenicity data. Because of the limited knowledge we have about the behaviour of these materials, the application of broad spectrum techniques such as toxicogenomics would be also of special interest. Equally important is research to adapt the ecotoxicity tests, especially in regard to forms of exposure. It appears also as especially relevant issue the study of the distribution of nanoparticles in the body and the dynamics of internalization into cells.

We will present our experiences in genotoxicity assessment of an array of nanoparticles, going from gold with or without coating to cobalt ferrite, magnetite, gadolinium/prussian blue complexes, ceria particles, zirconium oxide or graphene oxide. Specially worth of mention is the case of genotoxicity produced by eliciting red-ox reactions by particles sequestered within the lysosomes, ie., without reaching the nucleus.

Notas - Notes

Inter-comparison of the biological properties of nanomaterials just after dispersion and after its freezing (liquid N_2 / -80 ºC)

Laura Vila[1], Laura Rubio[1], Balasubramanyam Annangi[1], Alba Hernández[1,2], Ricard Marcos[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

To test the biological properties of nanomaterials they must be used just after its dispersion to avoid possible precipitations or agglomerations. This supposes to repeat this process any time a new experiment is running on. Thus, slight differences in the repeated procedure can suppose differences in the level of the achieved dispersion. This is particularly important when long-term or chronic exposures at sub-toxic doses are used; in these studies, lasting for months, the removal of the culture media (with the nanomaterial) every 3 days is required. This supposes the existence of a potential substantial variation between every process of dispersion. To avoid these problems we propose to freeze at -80 ºC different replicates of an initial dispersion that are thawed under request.

To demonstrate the efficiency of the proposed alternative we have compared the physico-chemical and biological properties of fresh and frozen nanomaterials. ZnO, TiO_2 and CeO_2 nanoparticles (NPs) were used. NPs characterization (size and morphology) was carried out by transmission electron microscopy (TEM). Furthermore, characterization of hydrodynamic size and zeta potential by dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) methodologies, respectively, was performed. Cell viability was determined by the Beckman counter method and cell uptake and ROS production was assessed by transmission electron microscopy (TEM) and flow cytometry (FC). Short-term exposures (24 h) to several doses and the BEAS-2B cell line (human bronchial epithelium cells) were used.

The obtained results show no significant differences between frozen and fresh NPs both in their physico-chemical characteristics and their biological effects. Furthermore, the results show that nano-ZnO and nano-TiO_2 are toxic for BEAS-2B cells, the first one being more toxic than the second one. Moreover, our results show the internalization of nano-TiO_2, nano-CeO_2 and nano-ZnO, although the last one (ZnO) in a minor extent, and that only nano-ZnO were capable to generate significant increase in the intracellular ROS production at the highest concentration.

This study would indicate that there are not differences between using fresh and frozen NPs and that using -80 ºC NPs in short and long-term exposure could minimize the variability between samples.

Notas - Notes

Evaluation of the genotoxic effects of polymeric nanoparticles designed to cross biological barriers

Tamara Iglesias[1], Amaya Azqueta[1], Nekane Martín[2], Laura Inchaurraga[2], Adela López de Cerain[1]

[1]Pharmacology and Toxicology Department, School of Pharmacy, University of Navarra, Pamplona, Spain. [2]Pharmacy and Pharmaceutical Technology Department, School of Pharmacy, University of Navarra, Pamplona, Spain.

Orally administrated biotechnological drugs have to deal with numerous problems including crossing biological barriers in order to reach the epithelium at therapeutic concentrations without being toxic for the organism. In order to reach penetration through these biological barriers, biodegradable polyanhydride nanoparticles (NPs) have been synthesized with different types of ligand making varying physicochemical properties.

The purpose of this work was to evaluate the genotoxicity of different biodegradable polyanhydride NPs by the comet assay in combination with the enzyme formamidopypiridine DNA-glycosylase (FPG). Furthermore, the mitogen capacity of the NPs was evaluated by the proliferation assay.

NPs were tested at different concentrations (0, 0.5, 1 and 2 mg/mL) in Caco-2 cells with a treatment period of 3 h. The comet assay was performed immediately after the treatment and cell proliferation was assessed by counting the cells after their incubation at 37 °C for 48h. Caco-2 cells treated with 1 μM of the photosensitiser Ro 19-8022 plus 5 min of light were included as positive control in all the experiments.

The NPs studied did not result in any increase in the frequency of strand breaks or alkali-labile sites in Caco-2 cells but they induced a slight concentration-dependent increase in net FPG sensitive sites (oxidized and/or alkylated bases). Treated cells showed similar levels of proliferation (100% of control levels) with all evaluated NPs.

Acknowledgments: We acknowledge EC support for this research through the FP7-2011-NMP-280761 “ALEXANDER” project.

Notas - Notes

Long-term exposures to low doses of cobalt nanoparticles induce cell-transformation enhanced by oxidative damage

Balasubramanyam Annangi[1], Jordi Bach[1], Gerard Vales[1], Laura Rubio[1], Ricard Marcos[1,2], Alba Hernández[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

So far, *in vitro* studies of nanoparticles (NPs) on the toxic, genotoxic and carcinogenic potential mainly focused on acute-exposure and high-dose conditions. This makes difficult to extrapolate to human beings in real scenarios. This means that there is a serious lack of information on the genotoxic/carcinogenic potential of NPs at low doses and prolonged exposures.

To overcome this point, we have evaluated the cell-transforming ability of cobalt nanoparticles (CoNPs) after long-term exposures (12 weeks) to sub-toxic doses (0.05 and 0.1 μg/mL). To get further information on whether CoNPs-induced oxidative DNA damage is relevant for CoNPs carcinogenesis, the cell lines selected for the study were the *wild-type* mouse embryonic fibroblast (MEF *Ogg1*$^{+/+}$) and its isogenic *Ogg1 knockout* partner (MEF *Ogg1*$^{-/-}$), unable to properly eliminate the 8-OH-dG lesions from DNA. Differences between cell lines offer relevant information on the role of oxidative damage on the studied biomarker.

Our initial short-term exposure experiments demonstrate that low doses of CoNPs are able to induce reactive oxygen species (ROS) and that MEF *Ogg1*$^{-/-}$ cells are more sensitive to CoNPs-induced acute toxicity and oxidative DNA damage. On the other hand, long-term exposures of MEF cells to sub-toxic doses of CoNPs were able to induce cell transformation, as indicated by the observed morphological cell changes, significant increases in the secretion of metalloproteinases (MMPs) and anchorage-independent cell growth ability, all cancer-like phenotypic hallmarks. Interestingly, such changes were significantly dependent on the cell line used, the *Ogg1*$^{-/-}$ cells being particularly sensitive.

Altogether, the data presented here confirms the potential carcinogenic risk of CoNPs and points out the relevance of ROS and *Ogg1* genetic background on CoNPs-associated effects.

Notas - Notes

Antioxidant properties of cerium oxide nanoparticles in BEAS-2B cells

Laura Rubio[1], Laura Vila[1], Balasubramanyam Annangi[1], Alba Hernández[1,2], Ricard Marcos[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Nanotechnology is an emerging interdisciplinary science with very promising future expectations. Working with particles comparable in size with intracellular molecules gives many possibilities translatable to an increasing list of applications. Among the different nanomaterials, cerium oxide nanoparticles (Ce-NPs) a rare-earth element, is becoming a very used element due to its interesting properties: this nanomaterial presents two different oxidation states that could result in an autoregenerative redox cycle. Thus, a potential application of Ce-NPs to quench reactive oxygen species (ROS) in biological systems is currently being investigated. In this manner Ce-NPs may represent a novel agent to protect cells and tissues against damage by its regenerative free radical scavenging property.

Several lines of evidence suggest that both oxidative stress and inflammatory responses play an essential role in the biological processes of different diseases, as those arising in the lungs from cigarette smoking. In this study we have used an epithelial lung cell line, BEAS-2B, as a model to study the possible antioxidant effect of Ce-NPs in the pulmonary-like system.

We have assessed the protective effect of the 24 h pre-treatment of Ce-NPs in front of a well defined agent inducing oxidative stress ($KBrO_3$), through different endpoints like: toxicity, intracellular reactive oxygen species (ROS) induction, genotoxicity and DNA oxidative damage (comet assay) and gene expression alterations.

The obtained results confirmed the antioxidant properties of Ce-NPs. Thus, its pre-treatment significantly reduced the intracellular production of ROS induced by $KBrO_3$. Similarly a reduction in the levels of DNA oxidative damage, as measured with the comet assay complemented with FPG enzyme, were also observed. Similarly, pre-treatment of BEAS-2B cells with Ce-NPs slightly increased the viability of cells treated with $KBrO_3$ as well as smoothly down regulated the expression of genes involved in the oxidative NRF2 pathway. As indicated, the confirmation of antioxidant properties of Ce-NPs over a lung cell line (lung-model), would suggest and interesting potential of these NP for the treatment of smoking-related diseases.

Notas - Notes

Biological effects of cerium nanoparticles using *Drosophila* as a model organism

Mohamed Alaraby[1], Alba Hernández[1,2], Balasubramanyam Annangi[1], Esref Demir[1], Laura Rubio[1,2], Amadeu Creus[1,2], Ricard Marcos[1,2]

[1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain; [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Due to the massive and continuous production of nanomaterials they inevitably reach the environment and, as consequence, human beings are certainly exposed. The different physicochemical properties of nanomaterials may suppose new biological properties that can interfere with crucial biological processes affecting human health. This means that a deep knowledge on the potential biological effects of nanomaterials is strongly needed. Although *in vitro* approaches are easily performed, *in vivo* studies lead to more relevant information. In this context, we promote the use of *Drosophila* as a suitable *in vivo* model to characterize the potential risks associated to nanomaterials exposure. *Drosophila* is the forefront organism in genetic studies and it is a strong candidate to detect the effect of nanoparticles due to its unique characteristics, especially that it share 60-70% homology to human genes and the counterparts of several genes responsible for more than 700 different human diseases are found in *Drosophila*.

Our aim was to determine the different biological effects of cerium nanoparticles (Ce-NPs) by using a wide battery of experimental approaches. Thus, egg-adult viability, particles uptake, gene expression, intracellular reactive oxygen species (ROS) production, genotoxicity and antigenotoxicity were the endpoints evaluated. Genotoxicity and antigenotoxicity were measured with the wing-spot assay. Ce-NPs >25nm were used in this study.

In spite of TEM images showing the internalization of CeNPs in intestinal and haemocyte cells, together with the significant expression of *Hsp* genes at the highest dose tested (10 mM), neither toxicity nor genotoxicity related to the both forms of Ce tested was observed. It is noteworthy that Ce-NPs significantly reduced both the genotoxic effect of potassium dichromate and the intracellular ROS production. No morphological malformations were detected after larvae treatment but, on the contrary, a reduction in the frequency of scutellum bristles duplication of the adults was found.

All results indicate that the Ce-NPs have neither toxic nor genotoxic effects, but contrarily show anti-genotoxicity and anti-oxidative stress properties. This study reflects the importance of *Drosophila* in studying different biological effects of nanoparticulated materials and, in addition, had developed a model to study the effects of nanomaterials on the gastrointestinal barrier.

Notas - Notes

Titanium dioxide and zinc oxide nanoparticles are not mutagenic in the mouse lymphoma assay but modulate the effect of UVC-light post-treatment

Eşref Demir[1,2], Amadeu Creus[2,3], Ricard Marcos[2,3]

[1]Akdeniz University, Faculty of Sciences, Department of Biology, 07058-Campus, Antalya, Turkey; [2]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Campus de Bellaterra, 08193 Cerdanyola del Vallès, Spain; [3]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Nanogenotoxicity is an emergent field, relevant for estimating the potential genotoxic risk of nanomaterials. Among nanomaterials, titanium dioxide nanoparticles (TiO_2 NPs) and zinc oxide nanoparticles (ZnO NPs) are widely used in industrial products including cosmetics, sunscreens, UV-light blockers, paints and medical materials. Since conflicting data exist on their possible risk for humans, we have selected two different NP sizes (between 1-50 nm and 50-100 nm) of TiO_2 (1, 10 and 100 µg/mL) and ZnO NPs (1, 10 and 100 µg/mL) and their microparticulated forms to determine their ability to induce mutagenicity in mammalian cells. There is an important lack of mutagenicity data on mammalian cells for such products, which represents an important gap for any risk-assessment estimation. We have used the mouse lymphoma assay (MLA) to determine the mutagenic potential of these NPs. The MLA assay detects a broad spectrum of mutational events, from point mutations to chromosome alterations. In addition, interactions with UVC-light (0.004 J/cm^2) have also been analysed. UVC-light exposures ranging from 0.0001 to 0.004 J/cm^2 produce a direct dose-response relationship with statistical significance after exposures equal or higher than 0.002 J/cm^2. Neither the selected NPs nor their microparticulated forms are mutagenic in the MLA assay. Nevertheless these NPs reduce the mutagenic effect of UVC-light, in a direct dose-effect relationship. These *in vitro* results, obtained with the L5178Y/Tk$^{+/-}$-3.7.2C mouse lymphoma cell line, contribute to increase the current database on the TiO_2 and ZnO NPs mutagenic effects as well as to the open the discussion about the possible risk associated with their use in photoprotection sunscreens.

Notas - Notes

MESA REDONDA – ROUND TABLE

Pharmaceutical genotoxicity testing: regulatory testing and strategies for early candidate selection

Antonio Guzmán

Toxicology Department, Esteve. Parc Cientific de Barcelona. Baldiri i Reixac, 4. 08028 Barcelona, Spain.

Toxicology testing is a pivotal component of the drug discovery and development process, and allows to characterize the toxicological properties of the drug candidate and assess its potential to produce adverse effects in humans. Testing for genotoxicity aims at identifying the potential to cause DNA damage, which is considered essential for the induction of carcinogenesis and hereditary defects. Compounds that are positive for genotoxicity are considered to be potentially human carcinogens and/or mutagens. Because of its relevance for human safety, genotoxicity testing of pharmaceutical drug candidates is regulated by an extent of national and international guidelines. The ICH Guideline S2(R1) plays a central role in regulatory genotoxicity testing, as it establishes the requirement that pharmaceuticals are assessed for genotoxic potential in a "standard battery" of *in vitro* and *in vivo* assays, there being two options. Option 1, requires the conduct of (1) an *in vitro* test for gene mutation in bacteria (usually the Ames test), (2) an *in vitro* mammalian cell genotoxicity test (either a cytogenetic test for chromosomal damage or a mouse lymphoma *Tk* gene mutation assay, and (3) an *in vivo* test, generally for chromosomal damage in rodent hematopoietic cells. In Option 2, the *in vitro* mammalian cell assay is replaced by a second *in vivo* assay, or by including a second endpoint for assessment in the rodent micronucleus assay. ICH S2(R1) further establishes the minimum requirements for these studies to be acceptable to support the conduct of clinical studies and marketing authorization application. Genetic toxicology studies are conducted early in the safety evaluation program, in a generally accepted tiered approach, with *in vitro* studies preceding *in vivo* studies, and with ICH M3(R2) establishing when, in the context of the proposed clinical development programme, the different studies should be conducted. Within this battery, *in vivo* assays are considered to play a relevant role in the overall assessment of genotoxic potential, as they take fully into account the biological processes of absorption, distribution, metabolism and excretion of the test article. Recently, *in silico* prediction models have become an integral part of regulatory genotoxicity testing, with the computational prediction of the outcome of bacterial mutagenicity assay being used in the qualification process of potential genotoxic impurities. This aspect is reflected in the current draft ICH guideline M7, which is expected to be implemented within the first half of the current year 2014.

Notas - Notes

Pharmaceutical genotoxicity testing: regulatory testing and strategies for early candidate selection

Antonio Rodríguez

Toxicology Department, Esteve. Parc Cientific de Barcelona. Baldiri i Reixac, 4. 08028 Barcelona, Spain.

Formal GLP compliant genotoxicity studies are required by the Regulatory Authorities (such as EMA or FDA) to demonstrate the lack of gentoxic properties of a drug candidate during its safety assessment in the preclinical development process. Guidelines ICH S2(R1) and ICH M3(R2) recommend which package of studies should be performed and when they have to be conducted to support clinical trial administration. Due to the impact that these studies have in the *go/no go* decision of the compound research development, a general practice among the pharmaceutical companies is that the whole battery of GLP compliant genotoxicity studies is carried out as soon as possible, preferably in advance of the first administration in humans (phase I). However, before to initiate these formal regulatory studies and to avoid any potential undesired positive gentox result in already advanced phases of the preclinical drug development, pharmaceutical companies have implemented internal *in silico*, *in vitro* and *in vivo* High Throughput Screening assays (commonly known as HTS assays) to anticipate the identification of potential gentoxic properties of the research molecules. This approach is based on the philosophy: “Fail early, Fail cheap”. In other words: the faster the potential genotoxic properties of the research compound could be detected, the better (as this early identification implies a remarkable savings in resources and time). These screening assays are performed since the very initial steps of the drug discovery process using small quantities of compound in comparison with the full GLP studies obtaining limited, but at the same time, valuable information that could help to screening out those molecules showing undesired genotoxic properties. In this direction, the session will deal with the fundamentals of the different well know Medium-High Throughput Screening tools currently available (as DEREK, SOS-*umu*, Greenscreen, reduced versions of Ames, *in vitro* and *in vivo* Micronuclei assays, Comet assay, among others) and that the pharmaceutical industry employs to have a quick insight about the potential genotoxic characteristics of the molecules aimed to become future marketed drugs.

Notas - Notes

ÍNDICE DE AUTORES – AUTHORS INDEX

LISTADO DE PARTICIPANTES – LIST OF PARTICIPANTS

Nombre - Name	**Afiliación - Affiliation**	**Correo electrónico - e-mail**
Abril, Nieves	Universidad de Córdoba	bb1abdim@uco.es
Alaraby, Mohamed	Universidad Autónoma de Barcelona	ma_abdalaziz24@yahoo.com
Annangi, Balasubramanyam	Universidad Autónoma de Barcelona	basucall@gmail.com
Arbillaga, Leire	Universidad de Navarra	larbillaga@unav.es
Azqueta, Amaya	Universidad de Navarra	amazqueta@unav.es
Bach, Jordi	Universidad Autónoma de Barcelona	incubusboyd@hotmail.com
Borrás, Miquel	CERETOX, Parc Cientific de Barcelona	mborras@pcb.ub.es
Calasanz, María José	Universidad de Navarra	mjcal@unav.es
Collins, Andrew R	University of Oslo	a.r.collins@medisin.uio.no
Corredor, Zuray	Universidad Autónoma de Barcelona	zurayfer@gmail.com
Creus, Amadeu	Universidad Autónoma de Barcelona	amadeu.creus@uab.es
De la Peña, Eduardo	Consejo Superior de Investigaciones Científicas	epena@ica.csic.es
De Lapuente, Joaquim	CERETOX, Parc Cientific de Barcelona	jlapuente@pcb.ub.es
Demir, Eşref	Akdeniz University, Turquía Universidad Autónoma de Barcelona	esrefdemir@akdeniz.edu.tr
Enciso, José Manuel	Universidad de Navarra	jenciso@alumni.unav.es
Espina, Marta	Universidad de Oviedo	espinamarta@uniovi.es
Gaivão, Isabel	Universidade de Trás-os-Montes e Alto Douro, Portugal	igaivao@utad.pt
Guilherme, Sofía	Universidade de Aveiro, Portugal	sofia.g.guilherme@ua.pt
Guzmán, Antonio	Esteve	aguzman@esteve.es

Nombre - Name	**Afiliación - Affiliation**	**Correo electrónico - e-mail**
Iglesias, Tamara	Universidad de Navarra	tiglesias@alumni.unav.es
Llana-Ruiz-Cabello, María	Universidad de Sevilla	mllana@us.es
López de Cerain, Adela	Universidad de Navarra	acerain@unav.es
Marcos, Ricard	Universidad Autónoma de Barcelona	ricard.marcos@uab.es
Marqués, Ana	Universidade de Aveiro, Portugal	anammarques@ua.pt
Monente, Carmen	Universidad de Navarra	cmonente@alumni.unav.es
Morales-Prieto, Noelia	Universidad de Córdoba	moralespnoelia@gmail.com
Neri, Nadia	Universidad Autónoma del Estado de México, México	nadianeri85@gmail.com
Pacheco, Mário	Universidade de Aveiro, Portugal	mpacheco@ua.pt
Pastor, Laura	Universidad de Navarra	lpcastro@alumni.unav.es
Peremartí, Jana	Universidad Autónoma de Barcelona	Jana.Peremarti@uab.cat
Puerto, María	Universidad de Sevilla	mariapuerto@us.es
Rodríguez, Antonio	Esteve	arodriguezf@esteve.es
Rubio, Laura	Universidad Autónoma de Barcelona	Laura.Rubio@uab.cat
Sierra, Luisa María	Universidad de Oviedo	lmsierra@uniovi.es
Vettorazzi, Ariane	Universidad de Navarra	avettora@unav.es
Vila, Laura	Universidad Autónoma de Barcelona	laura.vila@uab.es
Zúñiga-Venegas, Liliana A	Universidad Católica del Norte, Chile	lzuniga@ucn.cl

Red Iberoamericana de Toxicología y Seguridad Química en la Mutagénesis Ambiental

A.Pillco, O. Herrero, F. Cavieres, G. Font, E. de la Peña

Http://ritsq.org epena@ica.csic.es cartel nº 56

SEMA 2014 PAMPLONA 18-20 junio

http://aetox.es

En marzo de 2008 se puso en marcha la página web de la RITSQ - Red Iberoamericana de Toxicología y Seguridad Química como una actividad de la AETOX y desde entonces se han registrado en la Red **904** personas de **41** países y contabilizado **63.806 visitas** distribuidas por diferentes países: **6.556** de México, **4.393** de Colombia, **3.717** de Argentina, **3.493** de Perú, **3.011** de Venezuela, **2.183** de USA, **2.120** de Chile, **1.043** de Puerto Rico, **956** de Ecuador y **28.907** de España.

La ***RITSQ*** tiene los siguientes **Objetivos**:

- **Coordinar** la participación de los diferentes grupos existentes en universidades y organismos de investigación de Iberoamérica, implicados en estudios relacionados con la Toxicología.
- **Fortalecer** la colaboración y el intercambio académico entre los programas de Doctorado y Maestría de diferentes países iberoamericanos que tengan como objeto el estudio y la investigación en Toxicología o áreas relacionadas.
- **Favorecer** la realización de proyectos de investigación conjuntos entre docentes e investigadores de Iberoamérica, pasantías estudiantiles y eventos académicos.
- **Profundizar** en el estudio de métodos de ensayo de corta y larga duración utilizados en la evaluación de la carcinogenicidad, la mutagenicidad y la toxicidad para la reproducción de sustancias y productos químicos.
- **Desarrollar y estandarizar** métodos analíticos para la identificación y determinación de biomarcadores de exposición, efecto y susceptibilidad para sustancias y productos químicos en el hombre y el medio ambiente.
- **Aplicar** métodos de evaluación del riesgo para la salud humana y el medio ambiente de sustancias y productos químicos.
- **Fomentar** el intercambio científico de profesionales interesados en Toxicología Ambiental, Clínica, Forense, Analítica y Seguridad alimentaria.
- **Propiciar** el uso de métodos alternativos a la experimentación animal (***http//remanet.net***).

Las Visitas totales por año son las siguientes: **9.609 /2009 ; 14.340 /2010 ; 29.246 / 2011; 39.056 /2012 ; 49.726 / 2013 ; 64.408/2014 .**

La RITSQ desea ser el nexo de unión entre España, Portugal e Iberoamérica

Sema 2014

Organiza:

Universidad de Navarra

Colabora:

Now part of Instem
PERCEPTIVE INSTRUMENTS

Litron Laboratories