In vitro Skin Sensitisation, Photo-DPRA

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Dr. Rahul Date

A Global Contract Research Organisation

Skin Sensitisers

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- Substances which elicit an allergic response following contact with the skin

- Process termed as allergic contact dermatitis (ACD) in humans

ACD day to day examples

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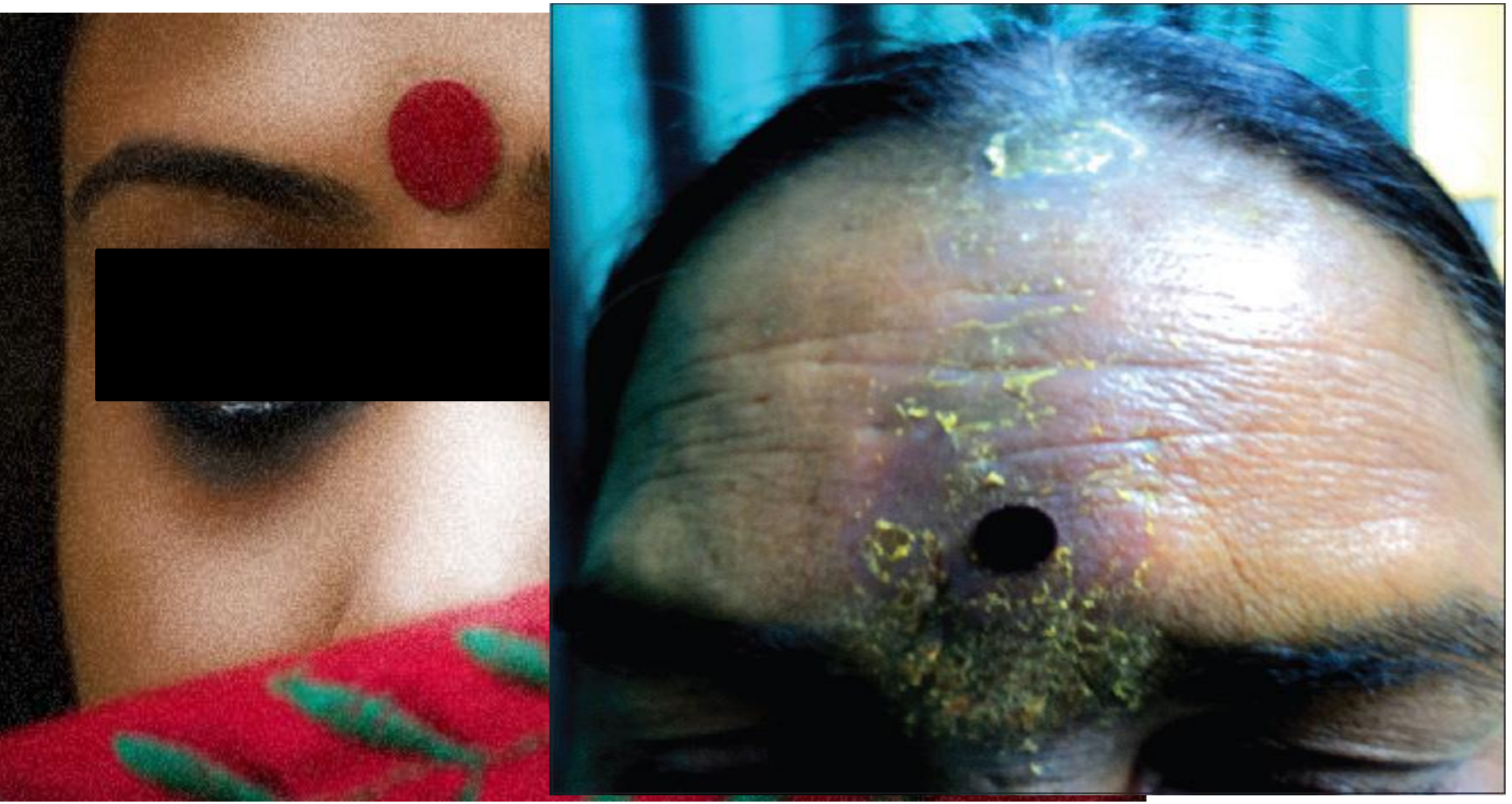

ACD in cannabinoid receptor knock-out mice

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$Cnr1^{-/-}/Cnr2^{-/-}$ mice

Normal ears

Allergic ear response

A

B

- Severe ulcerations in the head and neck region of 30% of the $Cnr1^{-/-}/Cnr2^{-/-}$ mice

Karsak et al. Science 2007

Why ?

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Allergic contact dermatitis (ACD)

- An important occupational and environmental disease

  Cause - topical exposure to chemical allergens

- More than 4,000 chemicals identified as skin sensitisers

- As per epidemiological studies approximately 20% adults allergic to one or more skin sensitisers

Source : WHO report 2016

Immune responses in cutaneous hypersensitivity

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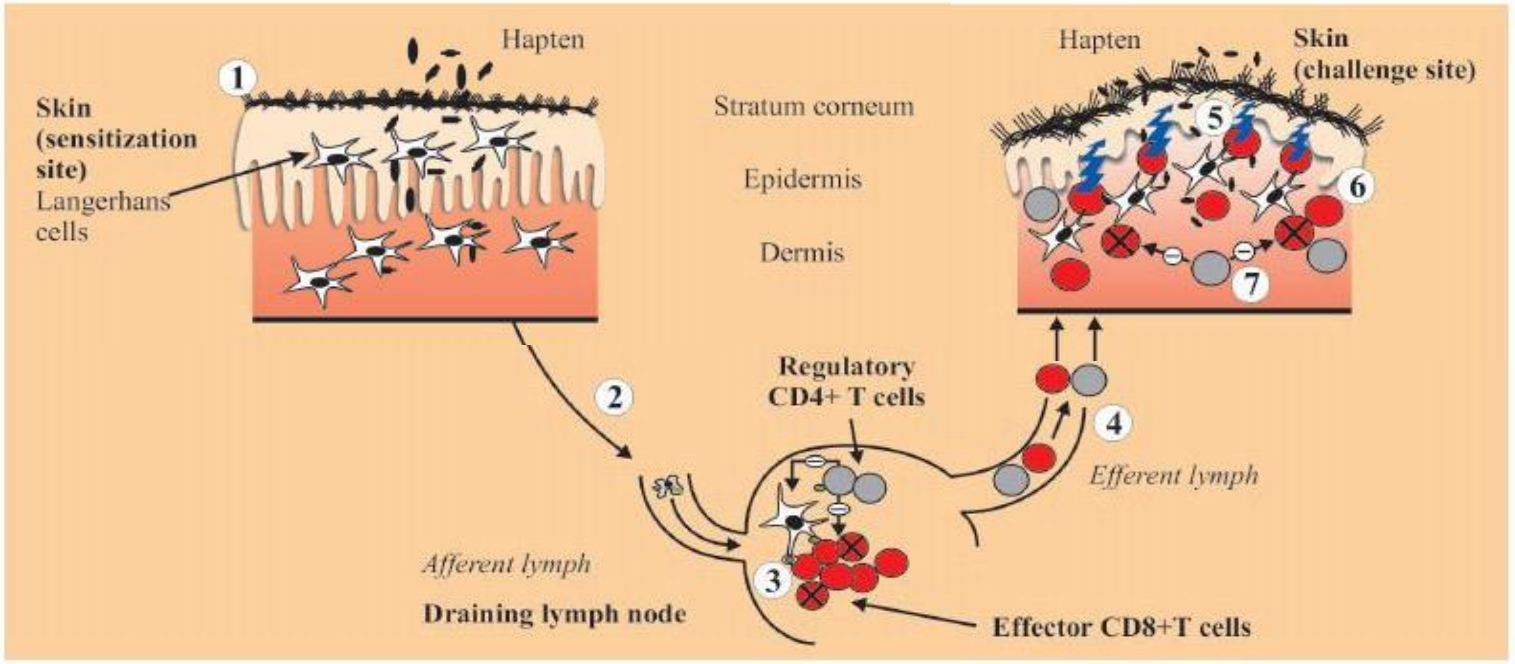
DNFB treatment **Repeated DNFB treatment**



Adapted from Anais Brasileiros de Dermatologia, 2005, vol.80, n. 4

- DNFB = 2,4-dinitrofluorobenzene, causes allergic reaction or sensitisation

EU Legislation

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- Phasing out of animal testing for cosmetics started in 2004
- A complete marketing ban on animal-tested new cosmetic ingredients ------ since March 2013
- EU Regulation on Chemicals (REACH) requires use of alternative methods wherever possible

1. *In vitro* methods
2. *In silico* analysis

At present no single formally validated and regulatory adopted alternative method

The Adverse Outcome Pathway (AOP) for Skin Sensitisation

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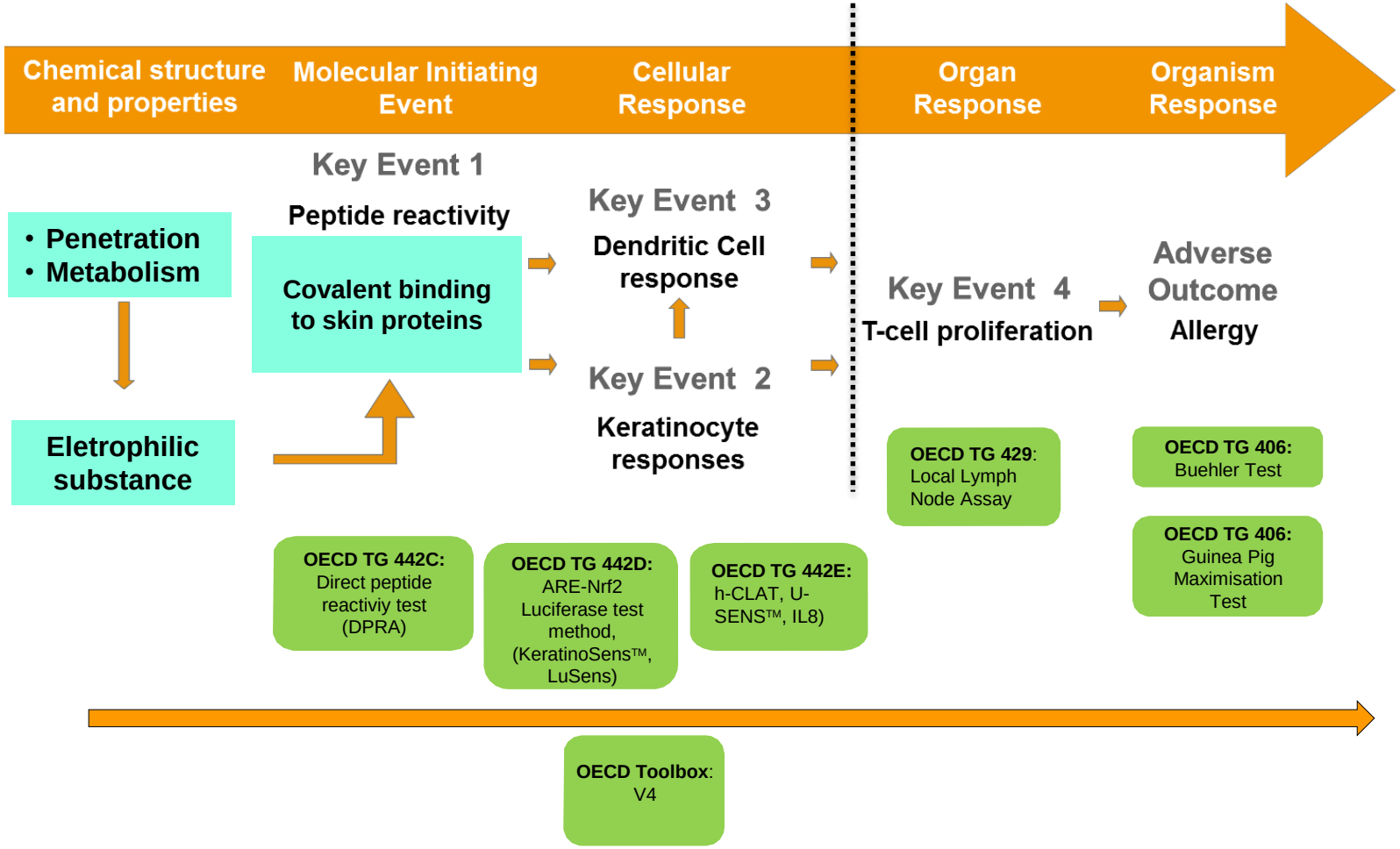

Drug induced ACD

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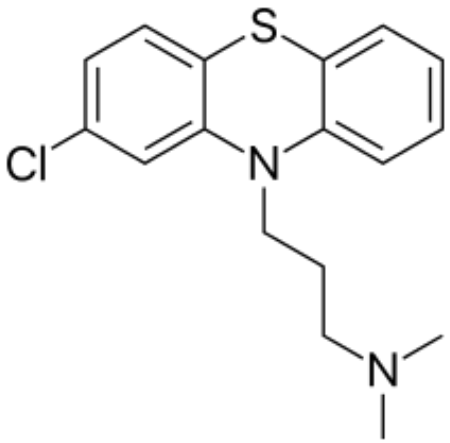
Chlorpromazine



(Thorazine, Largactil, Megatil,Serectil)


a
c
b
d

Mechanism of CPZ

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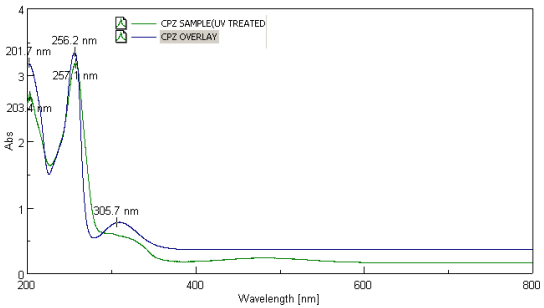

Section of Dermatology

President P D Samman MD

Meeting 21 February 1974

Short Papers

Cellular Mechanisms of Chlorpromazine Photosensitivity

by B E Johnson BSC PHD
(*Department of Dermatology, University of Dundee, Dudhope Terrace, Dundee*)

as rose bengal and eosin, which are adsorbed on to the membrane but do not penetrate the cell, and those such as anthracene and the porphyrins which are concentrated in lysosomes and on exposure to the appropriate radiation lead to the release of hydrolytic enzymes from these intracellular organelles with subsequent autolysis.

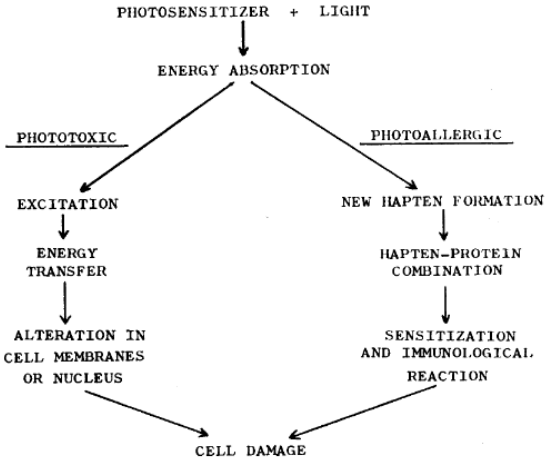

Fig 1 *Mechanism of photosensitivity. Adapted from Harber & Baer (1969)*

Direct Peptide Reactivity Assay (DPRA) Method

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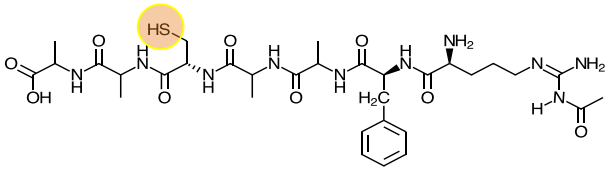
Screening method for evaluation skin sensitization potential (haptens)

- The reactivity is quantified based on the percentage of peptide depletion (HPLC/LCMS)

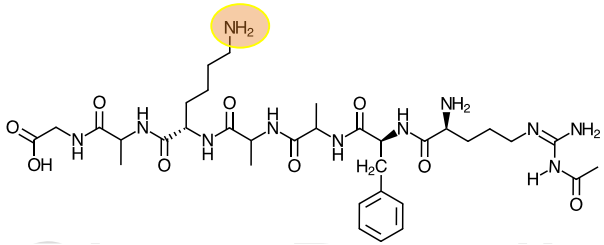
Synthetic model peptides in buffer



Test chemical in solvent

Cysteine (Ac-RFAACAA-COOH)

1:10 at pH 7.4



Lysine (Ac-RFAAKAA-COOH)

1:50 at pH 10.2

Incubation for 24 h, 25° C (dark)

HPLC analysis

Gerberick, *et al.* (2004) *Tox. Sci.* 81, 332-343

In chemico DPRA

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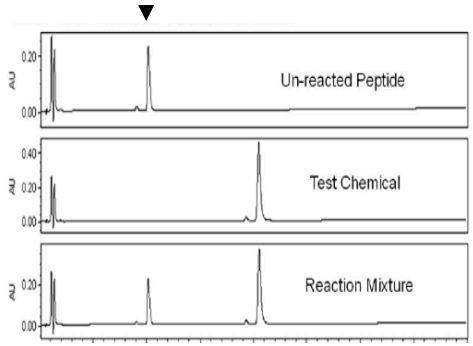
Peptide depletion



- Published dataset of DPRA includes 145 chemicals
  - 30 Extreme/Strong
  - 39 Moderate
  - 33 Weak
  - 43 Non-sensitizers
- Sensitivity = 82%; Specificity = 74%; and Accuracy = 80%

Natsch, *et al.* (2013). Journal ofApplied Toxicology 33: 1337-1352

EURL/ECVAM Results for 157 Chemicals:

Sensitivity = 80%; Specificity = 77%; and Accuracy = 80%

Gerberick *et al*. (2007). *Tox. Sci.*, 97, 417-427

Prediction criteria

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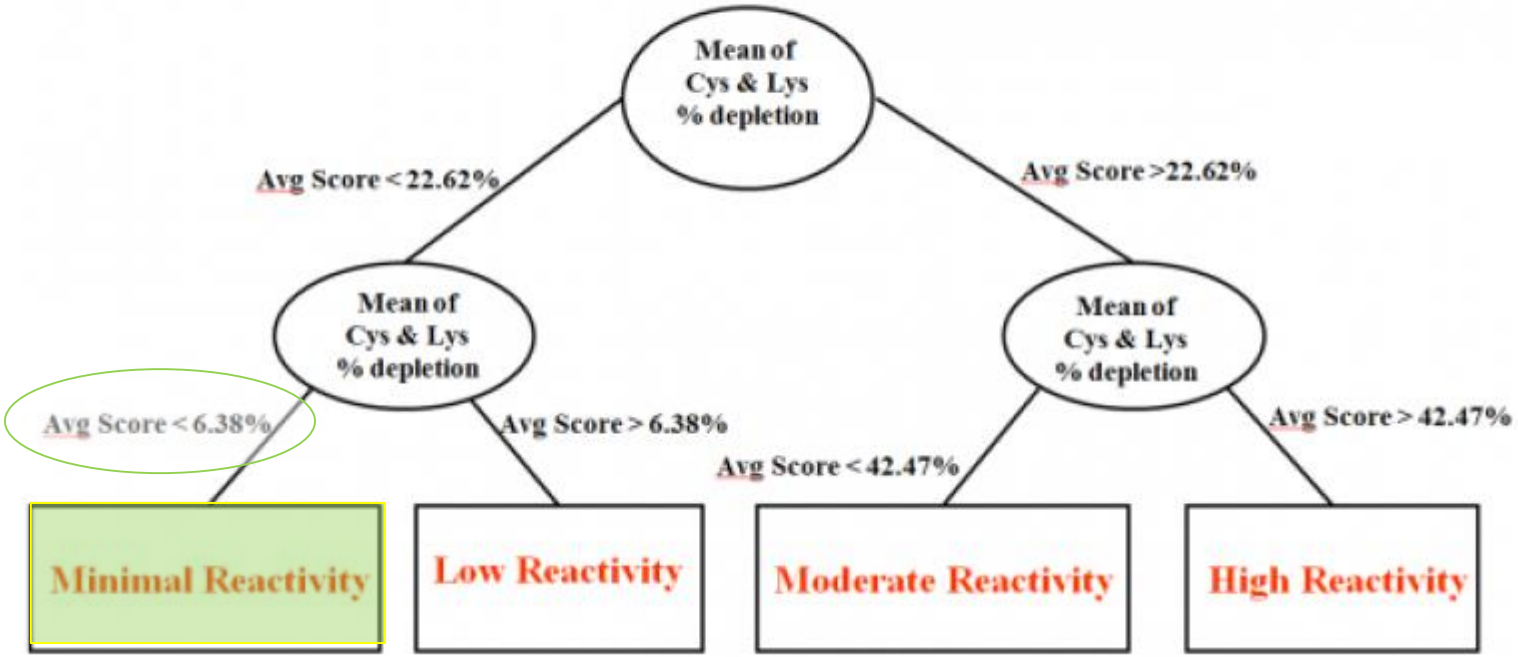
Cysteine 1:10/Lysine 1:50 Prediction model



EURL ECVAM DB-ALM Protocol 154

Modified Direct Peptide Reactivity Assay
(Photo-DPRA)

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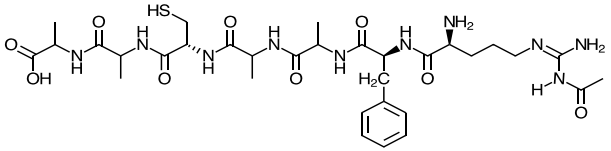
Additional step : Irradiation

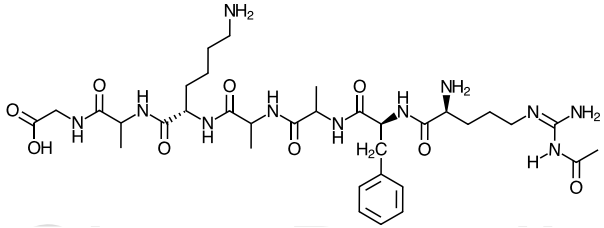
Test chemical in solvent

Synthetic model peptides in buffer



Cysteine (Ac-RFAACAA-COOH)

1:10 at pH 7.4



Lysine (Ac-RFAAKAA-COOH)

1:50 at pH 10.2

UVA irradiation (5 J/cm2)

Incubation for 24 h, 25° C (dark)

HPLC analysis

Characterisation of photo DPRA reaction

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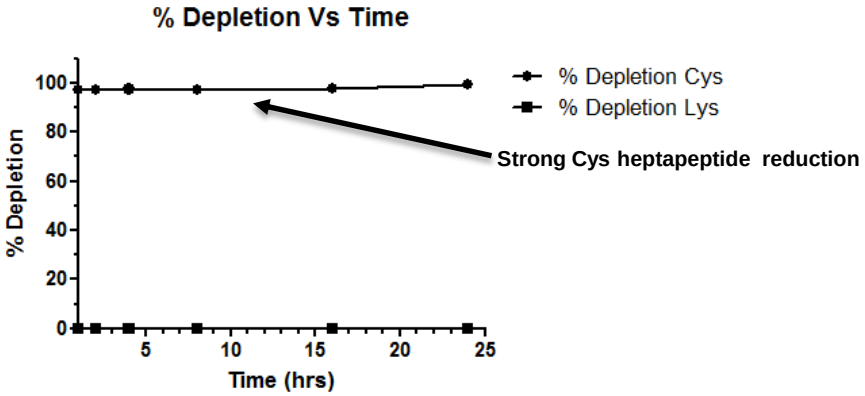

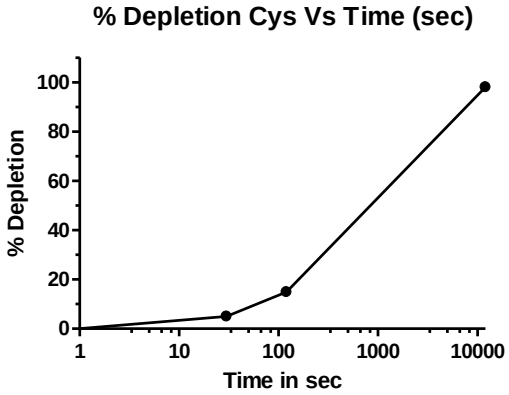

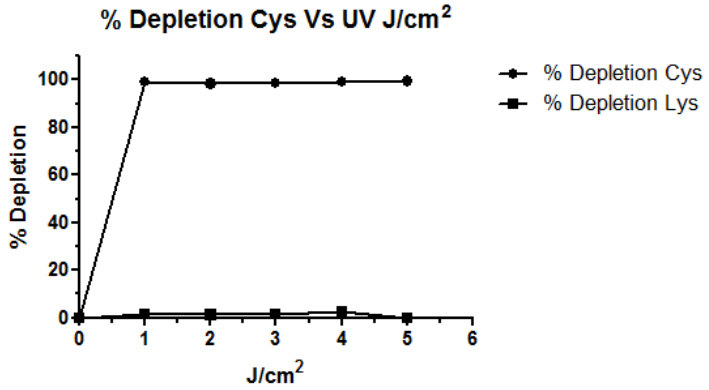

Strong depletion of Cys heptapeptide

Depletion even with 1 J/cm2

Effect of CPZ concentration

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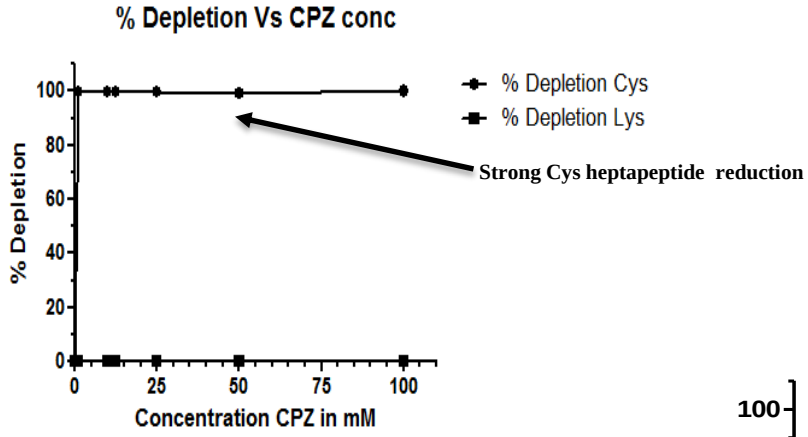

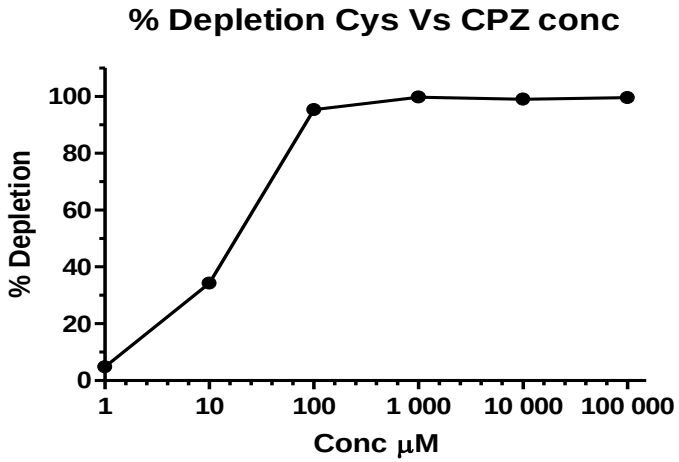

100 µM CPZ causes 100 % depletion

Effect of Cysteine concentration

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CPZ 100 µM @ 5 Joules

% Depletion Cys (mM) Vs Time (hrs)

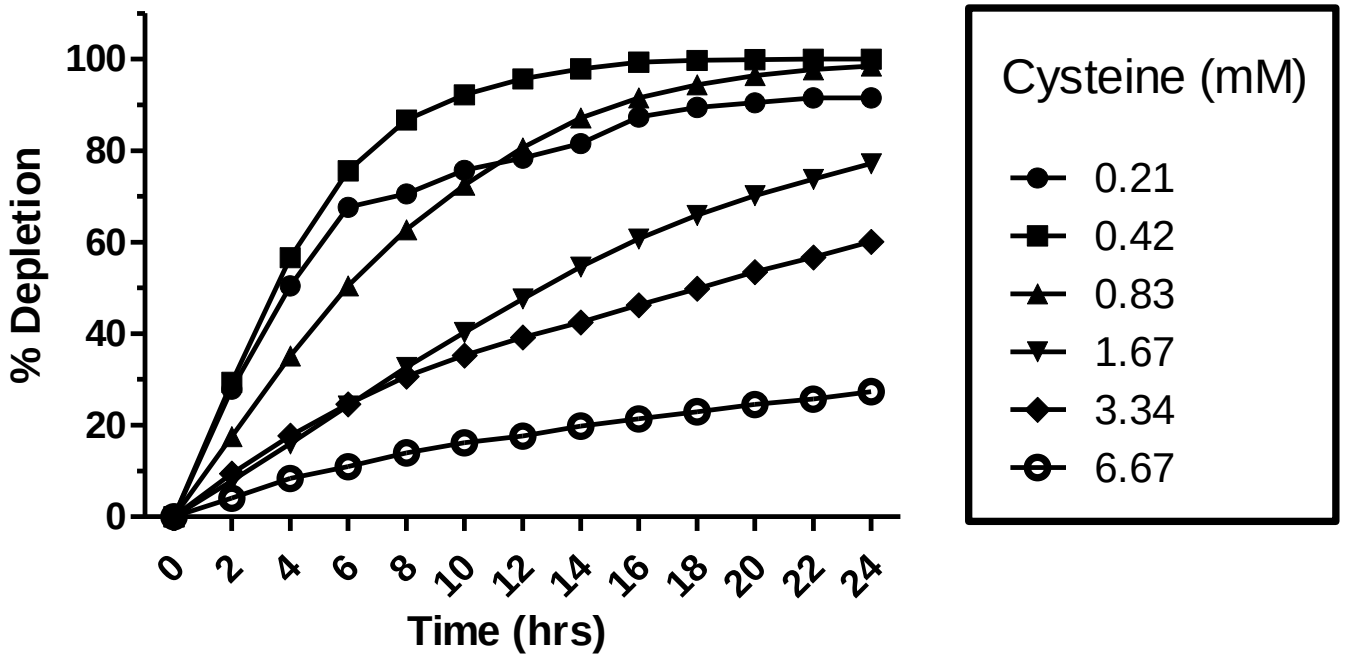

Photo-DPRA analysis

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Proficiency chemicals NRU-3T3 photo toxicity OECD 432

Test Item (100 mM)	% Depletion				
	5 J UV treated		Untreated		
	Mean (Cys+Lys)	DPRA Reactivity	Mean (Cys+Lys)	DPRA Reactivity	Δ
Phototoxic					
Chloropromazine	54.32	High	1.17	No or Minimal	**53.16**
Amiodarone HCl	49.86	High	0.14	No or Minimal	**49.72**
Anthracene (50 mM)	50.27	High	5.80	No or Minimal	**44.47**
Photoporhyrin IX, disodium	55.97	High	29.04	Moderate	**26.94**
Norfloxacin (33.33 mM)*	49.61	High	7.07	Low Sensitiser	**42.54**
Non Phototoxic					
L-Histidine	0.00	No or Minimal	0.09	No or Minimal	**0.00**
Hexachlorophene	62.12	High	50.85	High	**11.28**
Sodium lauryl sulphate	47.47	High	47.54	High	**0.00**

*** Acetate buffer pH 10.2**

Photo-DPRA analysis

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Test Item (100 mM)	After UV treatment increase in	
	% Cys depletion	% Lys depletion
Phototoxic		
Chloropromazine	96.66	9.65
Amiodarone HCl	99.71	0.00
Anthracene (50 mM)	89.72	0.00
Photoporhyrin IX, disodium	59.70	0.00
Norfloxacin (33.33 mM)*	85.08	0.00
Non Phototoxic		
L-Histidine	0.00	0.00
Hexachlorophene	10.12	12.43
Sodium lauryl sulphate	0.00	0.00

Work in progress

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Test Item (100 mM)	% Depletion				
	5 J UV treated		Untreated		
	Mean (Cys+Lys)	DPRA Reactivity	Mean (Cys+Lys)	DPRA Reactivity	Δ
Non Sensitizers					
1-Butanol	0.33	No or Minimal	4.73	No or Minimal	-4.40
Lactic acid	0.97	No or Minimal	4.89	No or Minimal	-3.92
6-Methylcoumarin	56.98	High	2.20	No or Minimal	54.78
4-Methoxyacetophenone	20.08	Low reactive	2.71	No or Minimal	17.38
Sensitizer					
Cinnamaldehyde	66.36	High	64.35	High	2.02
Bindi - Phenols					
4-Benzyloxy Phenol	59.90	High	6.16	High	53.74
4-Tert Butyl Phenol	2.94	No or Minimal	3.38	No or Minimal	-0.44

6-Methylcoumarin ? 4-Methoxyacetophenone ?

6-Methylcoumarin as a photosensitiser

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Journal of Applied Toxicology

Research article

Development of novel *in vitro* photosafety assays focused on the Keap1–Nrf2–ARE pathway

Kyoko Tsujita-Inoue, Morihiko Hirota, Tomomi Atobe, Takao Ashikaga, Yoshiki Tokura, Hirokazu Kouzuki

Photo-KeratinoSens

ELSEVIER

Toxicology and Applied Pharmacology

Volume 81, Issue 2, November 1985, Pages 295-301

Mechanism for 6-methylcoumarin photoallergenicity ☆

Shinobu Kato, Toshihiko Seki, Yoshio Katsumura, Toshiaki Kobayashi, Kazuo Komatsu, Shoji Fukushima

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GPMT and human

4-Methoxyacetophenone as a photoinitiator

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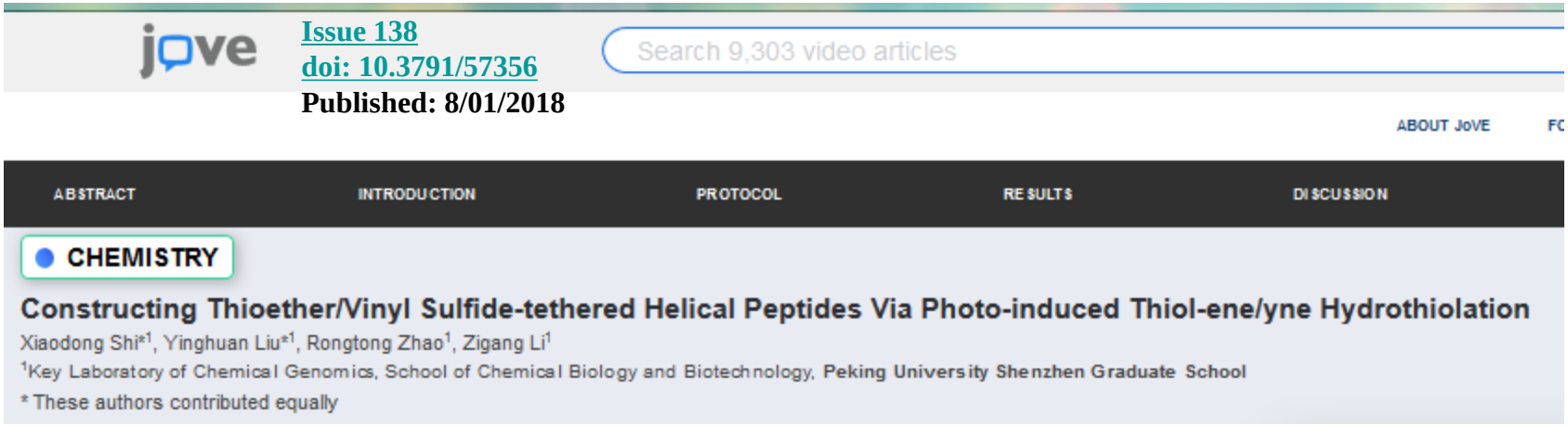

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ABSTRACT INTRODUCTION PROTOCOL RESULTS DISCUSSION

CHEMISTRY

Constructing Thioether/Vinyl Sulfide-tethered Helical Peptides Via Photo-induced Thiol-ene/yne Hydrothiolation

Xiaodong Shi*[1], Yinghuan Liu*[1], Rongtong Zhao[1], Zigang Li[1]

[1]Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School

* These authors contributed equally

UV irradiation using photoinitiator 4-methoxyacetophenone (MAP) and 2-hydroxy-1-[4-(2-hydroxyethoxy)-phenyl]-2-methyl-1-propanone (MMP)

Summary

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- Photo DPRA assay can be used

  - to evaluate the skin sensitisation potential of photosensitive compounds

  - to study photo sensitisation kinetics

- *In chemico* assay to differentiate photo reactive compounds into sensitizers and non sensitizers based only upon the protein reactivity property

- Large dataset is needed to validate …….

Acknowledgement

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Shukriya!