

Use of IL-8 release and p38 MAPK activation in THP-1 cells to identify allergens and to assess their potency *in vitro*

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ABSTRACT

The local lymph node assay (LLNA) has been developed to assess skin sensitization, and based on the EC₃ value, it can also be used to evaluate allergen potency. Therefore, in the development of *in vitro* alternatives to the LLNA assay, one should not only consider the hazard identification but also the possibility to classify allergens relatively to their potency.

We have recently described a selective release of interleukin-8 (IL-8) by chemical allergens in THP-1 cell line, and identified the activation of p38 mitogen-activated protein kinase (p38 MAPK) as a common pathway. Therefore, the purpose of this study was to expand the number of chemicals tested and to investigate whether IL-8 production and p38 MAPK activation can be used to classify allergens according to their potency.

THP-1 cells were exposed to the contact allergens (*p*-benzoquinone, 2-aminophenol, isoeugenol, diethyl maleate, citral and imidazolidinyl urea), selected according to their potency in the LLNA, and to lactic acid and propylene glycol as non-sensitizers. p38 MAPK activation was evaluated 5–15 min after treatment by FACS analysis, while IL-8 release was assayed by ELISA following 24 h of incubation. p38 MAPK was activated by all contact allergens, including the pro-apten isoeugenol, whereas IL-8 release was significantly increased after stimulation with all allergens tested, except for isoeugenol. The failure of isoeugenol may be due to decrease in the stability of IL-8 mRNA. Irritants exposure, as expected, failed to induce both p38 MAPK activation and IL-8 release.

A significant correlation between IL-8 release and the LLNA EC₃ was found (Pearson correlation $r = 0.743$, $p = 0.0036$, $n = 12$). On the contrary, the activation of p38 MAPK showed no significant correlation between LLNA data and vigor of p38 MAPK activation.

Overall, data presented confirm our previous observations and reveal IL-8 as potential tool not only to identify sensitizers, with the exception of pro-haptens, but also to classify them according to their potency, while p38 MAPK activation allows the identification of all sensitizers, including pro-haptens, but was not useful for potency classification.

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1. Introduction

The possibility that chemicals used in the workplace and in consumer products might cause skin sensitization is major concern. Chemical allergens are small molecules able to form a sensitizing complex once they bound to proteins (Corsini and Kimber, 2007). One of the most frequent manifestations of chemical allergy is contact hypersensitivity, which can have serious impact on the quality of life and represents a common occupational health. For this reason, evaluation of the potential of a compound to cause allergic

contact dermatitis has to be integral part of the development of consumer products and their ingredients.

To date the identification and evaluation of unknown sensitizers relies on animal testing, mainly by the local lymph node assay (LLNA) (Kimber et al., 1986), as no validated alternative exists. The additional testing of chemicals for their allergenic potential required by the new EU-legislation on chemicals (REACH, EU, 2006) is expected to significantly increase the use of animals. It has been estimated that skin sensitization testing is among those effects that would require large numbers of animals. Conversely, the 7th amendment to the cosmetics directive (Directive 76/768/EEC) completely bans all animal testing for cosmetic ingredients for all human-health related effects by 2009 for all endpoints except the repeated-dose toxicity endpoints for which the marketing ban deadline is 2013. Therefore, in the screening of drugs, cosmetics and other chemicals for human use, it is very important,

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both from safety and economic point of view, to have biological markers to discriminate allergy and irritation, events that have different impact on human health. Development of methods for prediction of skin sensitization potential without animal testing has been an active research area for some time, but has received further impetus with the existing and pending EU regulations. The ultimate goal is the establishment of *in vitro* assays ready for validation and approval by ECVAM.

Allergic contact dermatitis (ACD) is an adaptive inflammatory response of the skin after exposure to allergens. It is a T cell-mediated disease and recognized as a delayed-type hypersensitivity immune response (type IV), which develops in two phases defined operationally as induction and elicitation (Toebak et al., 2009). Dendritic cells (DCs) are the main antigen-presenting cells in adaptive immunity being their capacity to stimulate naïve lymphocytes the most definitive functional characteristic of these cells (Banchereau et al., 2000). Langerhans cells (LCs) are resident immature DCs in the skin capable to take up and process contact allergens. During this process they differentiate into mature immunostimulatory cells by up-regulating the expression of several co-stimulatory molecules such as CD80, CD86 and CD40 and various adhesion molecules including CD2, CD11a, CD54, CD58 (Quah and O'Neill, 2005), maturing LCs secrete various cytokines, such as IL-1 β and IL-8, and down-regulate proteins involved in antigen uptake, such as aquaporins. Activated LCs move out of the epidermis into the dermis, and into the regional lymphatic system. In the lymph node, LCs differentiate into mature dendritic cells and present antigen to specific T lymphocyte, using MHC class II molecules. Adhesion molecules on both the antigen-presenting cell (i.e. CD86) and the T-cell (i.e. CD28) ensure appropriate contact and co-stimulation. Following appropriate stimulus, a clone of T cells is produced with the ability to react to the antigen, which caused their expansion.

Based on the understanding of the processes occurring *in vivo* it is intended to develop assays systems that model sensitization, rather than irritation and toxicity of chemicals and proteins. Conceptually, the *in vitro* test method must address the key steps involved in the initiation of ACD. That is, the alternative non-animal test methods should be mechanistically based. Recently many *in vitro* protocols have been developed to measure the activation of DC-like cells upon exposure to chemicals (reviewed in Galvão dos Santos et al., 2009). Among the different assays proposed, we have recently described a selective release of IL-8 and the activation p38 MAPK by allergens in THP-1 cells as a common pathway (Mitjans et al., 2008). However, the understanding of relative sensitization potency of chemicals is of great importance for risk assessment (Kimber et al., 2002), and it has been shown that LLNA EC3 values correlate closely with the ability of the materials to induce ACD. The purpose of this study was, therefore, to expand the number of chemicals tested and to investigate whether IL-8 production and p38 MAPK activation can be used to classify allergens according to their potency.

2. Materials and methods

2.1. Chemicals

p-Benzoquinone (BZQ), 2-aminophenol (AM), isoeugenol (Iso), diethyl maleate (DEM), citral (Cit) and imidazolidinyl urea (IMZ) were selected as skin sensitizers according to their LLNA potency. Lactic acid (LA) and propylene glycol (PG) were used as non-sensitizers compounds. All reagents were purchased from Sigma (St. Louis, MO, USA) at the highest purity available. BZQ, AM, Iso, DEM, Cit and IMZ were dissolved in DMSO (final concentration of DMSO in culture medium <0.2%), while Lac and PG, were dissolved in PBS. Cell culture media and all supplements were from Sigma.

The selected contact sensitizers were classified according to their potency on the basis of the EC₃ LLNA value data, covering different potencies from extreme to weak (Table 1).

2.2. Cells

Cells were incubated with increasing concentrations of the selected chemicals, or DMSO as vehicle control for different times as previously described (Mitjans et al., 2008).

2.3. Cell viability

Cytotoxicity was assessed by flow cytometric evaluation of propidium iodide (PI)-stained cells following 24 h of treatment. After centrifugation, cells were resuspended in 500 μ l PBS containing 1 μ g/ml PI. The percentage of positive cells was analyzed using a FACSCalibur flow cytometer, and data were quantified using CellQuest software (Becton Dickinson). Based on cell viability, cells were treated with concentrations of chemicals resulting in 75, 85 > 90% of cell viability.

2.4. Cytokine production

IL-8 release was assessed in cell free supernatants by a specific sandwich ELISA, commercially available (Immunotools, Friesoythe, Germany), being 15.6 pg/ml the limit of detection. Results are expressed in pg/ml.

2.5. Flow cytometric analysis of p38 MAPK activation

For p38 MAPK flow cytometric analysis, 1 ml of cells suspension in FCS free medium were treated, after 1 h of equilibration 37 °C, with increasing concentrations of the selected chemicals or DMSO as vehicle control. After 15 min, cells were centrifuged, and fixed in 2% *p*-formaldehyde in PBS for 10 min at 37 °C, washed with 2 ml of cold PBS and permeated at –20 °C overnight with 1 ml 90% methanol. Cells were then washed with 2 ml of cold PBS and resuspended in 100 μ l of PBS with 1% FCS and 0.1% NaN₃. 2×10^5 cells were stained for 1 h with specific anti-phospho-p38 MAPK antibody (Becton Dickinson) following supplier's instructions. Cells were then washed and suspended in 0.4 ml of PBS supplemented with 1% FCS and 0.1% NaN₃. The intensity of fluorescence and the percentage of positive cells were analyzed using a FACSCalibur flow cytometer and data were quantified using CellQuest software (Becton Dickinson).

2.6. Real time RT-PCR

Total RNA was isolated after 3 h of treatment using a commercial available kit (TriReagent from Sigma) as described by the man-

Table 1
Classification of chemicals according to the LLNA EC3 values.

Chemical	Abbreviation	EC3 value (%) ^a	LLNA classification
Benzoquinone	BZQ	0.01	Extreme
2-aminophenol	AM	0.4	Strong
Isoeugenol	Iso	1.2	Moderate
Diethyl maleate	DEM	5.8	Moderate
Citral	Cit	9.8	Weak
Imidazolidinyl	IMZ	24	Weak
Urea	Lac	–	Non sensitizer
Lactic acid propylene glycol	PG	–	Non sensitizer

^a Gerberick, G.F., Ryan, C.A., Kern, P.S., Schlatter, H., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A., 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 4, 157–202.

ufacturer. For the synthesis of cDNA, 2.0 µg of total RNA was retro-transcribed using a high-capacity cDNA archive kit from Applied Biosystems (Foster City, CA, USA) following the supplier's instructions. IL-8 gene expression was evaluated by Real time reverse transcription-polymerase chain reaction (Real Time PCR). For PCR-analysis, Taq-Man™-PCR technology was used. PCRs were performed in duplicate and according to the standard protocol suggested by the manufacturer. For each PCR reaction, 10 ng of total RNA were used. The 18S ribosomal RNA transcription was used as endogenous reference and the quantification of the transcripts was performed by the $\Delta\Delta C_T$ method.

2.7. mRNA stability

To assess IL-8 mRNA stability, cells were treated with isoeugenol 75 µg/ml or DMSO as vehicle control for 3 h ($t = 0$), then 5,6-dichlorobenzamidoazole riboside (DRB, Sigma), an inhibitor of transcription, (50 µM) was added and total RNA extracted at different time thereafter. Total RNA was isolated using the TriReagent. IL-8 gene expression was evaluated by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). For the synthesis of cDNA, 2.0 µg of total RNA was suspended in a 20 µl final volume of the reaction buffer 25 mM Tris–HCl, pH 8.3, 37.5 mM KCl, 10 mM dithiothreitol, 1.5 mM MgCl₂, 1 mM concentration of each dNTP (Sigma), and 0.5 µg oligo d(T) 12–18 primer (Invitrogen, Carlsbad, CA, USA). After the reaction mixture reached 42 °C, 200 U of SuperScript II reverse transcriptase (Invitrogen) was added into each tube and incubated for 50 min at 42 °C and stopped by denaturing the enzyme at 70 °C for 10 min. PCR primers for IL-8 were synthesised (PRIMM, Milan, Italy) and contained the following sequences:

IL-8 forward 5'-CCAAGCTGGCCGTGGCTCTC-3'

IL-8 reverse 5'-TCTCTTGGCCCTTGGCCTCA-3'

cDNA concentrations were titrated to establish standard curves to document linearity and to permit semi-quantitative analysis of signal strength. In particular, for IL-8 were used 100 ng of corresponding RNA. The PCR mixture (25 µl) contained 2.5 µl 10X PCR buffer, 2 µl deoxynucleotides (1 mM each), 0.25 µl of specific primers (each 0.15 µM), 1.88 µl MgCl₂ (1.25 mM final concentration) and 0.25 µl of TaqGold DNA polymerase (5U, Applied Biosystem, Branchburg, NY, USA). Amplifications were initiated with 5 min of denaturation at 94 °C for 1 cycle followed by 35 cycles at 94 °C for 30 s, 30 s at 60 °C and 30 s at 72 °C using Gene Amp PCR System 2700 (Applied Biosystem). After the last cycle of amplification, the samples were incubated for 7 min at 72 °C. The PCR products (10 µl) were visualised by UV illumination following electrophoresis through 2.0% agarose at 80 V for 1 h and staining in Tris Borate EDTA buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.2) containing 0.5 µg/ml ethidium bromide. The amplified PCR product was 614 base pairs (bp) long. The image of the PCR products was acquired with the Molecular Imager Gel Doc XR (BioRad). The optical density of the bands was calculated and analyzed by means of the Image 1.47 program for digital image processing (Wayne Rasband, Research Service Branch, NIMH, NIH, Bethesda, MD, USA). The $t_{1/2}$ was evaluated by linear regression analysis of data.

2.8. Data analysis

All experiments were performed at least three times, with representative results shown. The data presented are expressed as mean $\pm$ SD. Statistical significance was determined by Dunnett's

multiple comparison test or student's t test as reported in the figure legends. Differences were considering significant for $p \leq 0.05$.

3. Results

3.1. Effect of the selected chemicals on cell viability

THP-1 cells were treated with increasing concentrations of the selected chemicals for 24 h. Cell viability was then assessed by flow cytometry using the PI staining. The concentration of chemical resulting in 75% of viability or CV₇₅ respect to vehicle treated cells was calculated by linear regressions analysis of data (Table 2). According to the results, non-sensitizers were the least cytotoxic compounds while BZQ, which is regarded as an extreme sensitizer, was the most cytotoxic one. Based on cell viability, cells were then treated with concentrations of chemicals resulting in 75, 85 > 90% of viability.

3.2. Effects of the selected chemicals on IL-8 release and p38 MAPK activation

In Figs. 1 and 2, the effects of the selected chemicals on IL-8 release and p38 MAPK activation are reported. The release of IL-8 was measured in supernatants 24 h after treatment by ELISA, while p38 MAPK activation was assessed by flow cytometry following 5 or 15 min of treatment.

As shown in Fig. 1, a dose-related increase in IL-8 release was observed following exposure to all contact allergens tested, with the exception of the pro-hapten isoeugenol. In contrast, Lac and PG failed to induce significant release of IL-8 (Fig. 2), confirming our previous results (Mitjans et al., 2008).

Alternatively to IL-8 release, the effect of the selected chemicals on p38 MAPK activation was investigated by FACS analysis. In this case, all contact sensitizers including pro-haptens, resulted in a statistical significant activation of p38 MAPK (Fig. 1) whereas no activation was found for the non-sensitizers (Fig. 2), confirming our previous observations obtained by Western blot analysis (Mitjans et al., 2008). With AM a significant p38 MAPK activation was observed at 5 min of treatment (Fig. 1 inset), while no activation was found at 15 min.

3.3. Use of IL-8 release and p38 MAPK activation for potency classification

The possibility to use IL-8 release as a possible mean to classify allergens in terms of potency was investigated. For this reason, the

Table 2
Concentration of chemical resulting in 75% of viability as assessed by PI staining.

Chemical	CV ₇₅ (µg/ml)
BZQ	0.38 $\pm$ 0.17
AM	5.7 $\pm$ 2.7
Iso	64.0 $\pm$ 6.9
DEM	63.6 $\pm$ 7.3
Cit	9.5 $\pm$ 0.9
IMZ	30.2 $\pm$ 3.8
LAc	>500*
PG	>1000*

THP-1 cells were treated for 24 h with increasing concentrations of the selected chemicals. Cell viability was assessed by PI staining. CV₇₅ was calculated by linear regression analysis of data. Each value represents the mean $\pm$ SD of three independent experiments. *Maximal concentration assayed with no effects on cell viability.

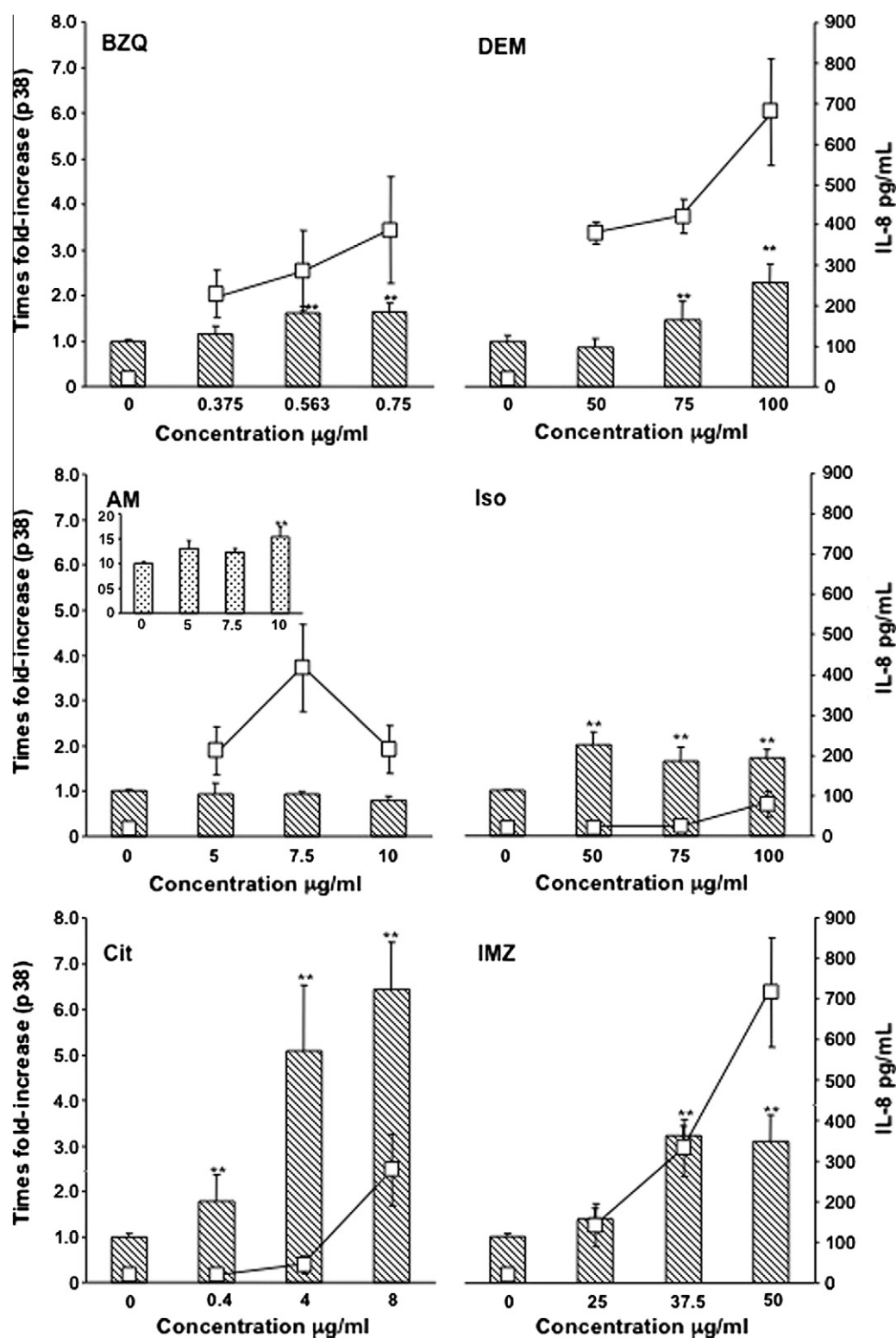


Fig. 1. IL-8 release and activation of p38 MAPK induced by sensitizers. THP-1 cells were treated with increasing concentrations of the selected chemicals or DMSO as vehicle control for different times. Interleukin-8 secretion (open squares) was measured by ELISA in culture supernatants after 24 h of incubation, while p38 MAPK activation (grey boxes) was evaluated by flow cytometry 15 min after treatment (in the inset of AM, p38 MAPK activation at 5 min is reported). Results are presented as mean $\pm$ SD of at least 3 independent experiments. Statistical analysis was performed with Dunnett's multiple comparison test, with $^{**}p < 0.01$.

concentration of allergen that induced a release of IL-8 of 100 pg/ml was calculated by linear regression analysis of data, the following order was obtained: BZQ 0.2 µg/mL, AM 5 µg/mL, Cit 3.6 µg/mL, IMZ 12 µg/mL and DEM 13.8 µg/mL. Then, these values and

previous ones calculated for other skin allergens (Mitjans et al., 2008) were compared with the available in vivo LLNA EC₃ data (Table 1) and, as shown in Fig. 3, a significant correlation was obtained: Pearson correlation $r = 0.743$, $p = 0.0036$, $n = 12$. The other

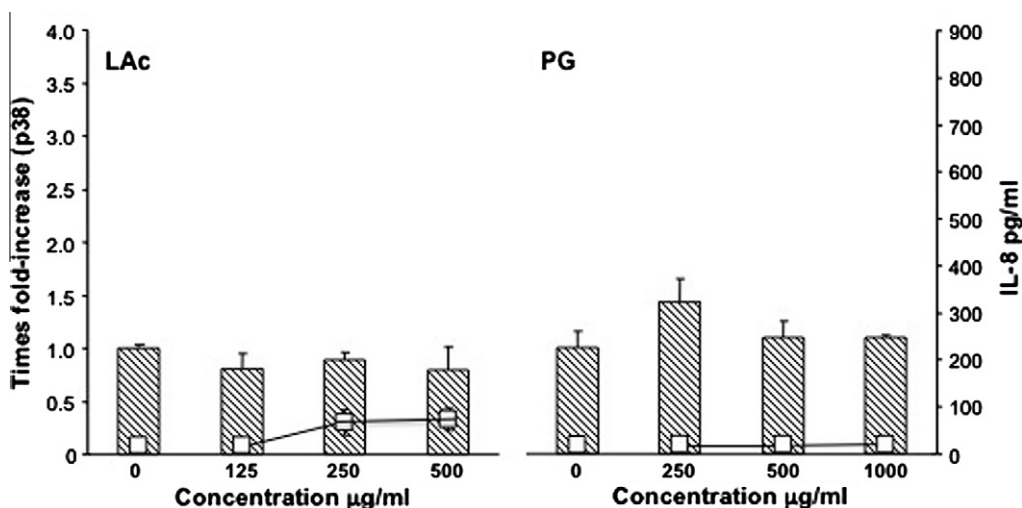


Fig. 2. IL-8 release and activation of p38 MAPK induced by non-sensitizers. THP-1 cells were treated with increasing concentrations of the selected chemicals for different times. Interleukin-8 secretion (open squares) was measured by ELISA in culture supernatants after 24 h of incubation, while p38 MAPK activation was evaluated by flow cytometry 15 min (grey boxes) after treatment. Results are presented as mean $\pm$ SD of at least 3 independent experiments. Statistical analysis was performed with Dunnett's multiple comparison test.

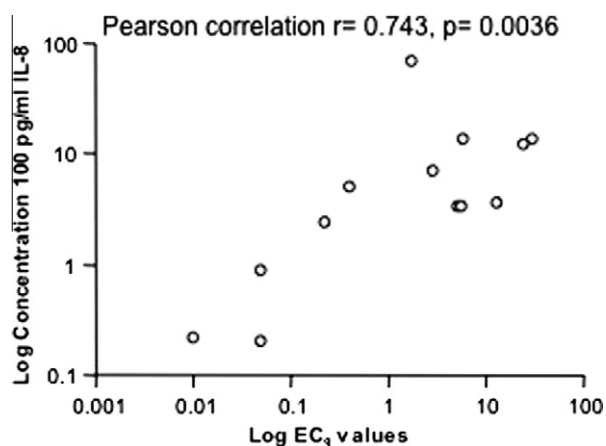


Fig. 3. Correlation between the *in vivo* LLNA EC_{50} values and *in vitro* EC_{100} . The concentration of chemical that induces the release of 100 pg/ml of IL-8 was calculated by linear regression analysis of data and compared with the *in vivo* LLNA EC_{50} values. The chemicals tested in the present manuscript together with the ones previously tested were plotted. The following chemicals were included: BZQ (EC_{100} = 0.2 μ g/mL), AM (EC_{100} = 5 μ g/mL), Cit (EC_{100} = 3.6 μ g/mL), IMZ (EC_{100} = 12 μ g/mL), DEM (EC_{100} = 13.8 μ g/mL), DNCB (EC_{100} = 0.9 μ g/mL), PPD (EC_{100} = 2.4 μ g/mL), TMTD (EC_{100} = 3.4 μ g/mL), HClPt (EC_{100} = 6.8 μ g/mL), Cinn (EC_{100} = 6.9 μ g/mL), Pen G (EC_{100} = 13.4 μ g/mL), Resorcinol (EC_{100} = 170 μ g/mL). Pearson correlation r = 0.743, p = 0.0036, n = 12.

EC_{100} values included were: DNCB 0.9 μ g/mL, PPD 2.4 μ g/mL, TMTD 3.4 μ g/mL, HClPt 6.8 μ g/mL, Cinn 6.9 μ g/mL, Pen G 13.4 μ g/mL, Resorcinol 170 μ g/mL.

As for IL-8 release, the possibility to use p38 MAPK activation to classify allergens according to their potency *in vivo* was investigated. In this case, the concentration resulting in 1.5-fold increase over vehicle treated cells was calculated, but no significant correlation was found (data not shown).

3.4. Isoeugenol and IL-8 mRNA expression and stability

As shown in Fig. 1, isoeugenol induces p38 MAPK activation, but fails to induce IL-8 release. In order to understand the reason of this behaviour, the mRNA expression of IL-8 was evaluated by Real Time-PCR. As reported in the inset to Fig. 4A, allergen-induced IL-8

mRNA expression was indeed dependent on p38 MAPK activation: the selective p38 MAPK inhibitor SB202190 prevented cinnamaldehyde-induced IL-8 mRNA expression. The effect of isoeugenol on IL-8 mRNA expression was therefore investigated. Isoeugenol induced a significant increase in IL-8 mRNA expression, while the Lac and PG failed (Fig. 4A). The failure of isoeugenol to induce IL-8 protein production and release may be explained by a decrease in IL-8 mRNA stability. As shown in Fig. 4 panel B, a significant decrease in IL-8 mRNA stability in isoeugenol treated cells was observed: 2.3 ± 0.4 h vs. 3.8 ± 0.9 h in vehicle treated cells ($p < 0.05$).

4. Discussion

The replacement of animal tests for the detection of the sensitizing potential of chemicals is of great importance due to current legislations and public concern (Directive 76/768/EEC; REACH, EU, 2006). At present, the identification of potential contact allergens completely relies on animal testing. It is therefore of great urgency to develop reliable *in vitro* alternative methods to replace the animal test. Among the recently proposed tests, the release of IL-8 is one of the most promising biomarkers to distinguish sensitizers from non-sensitizers (Galvão dos Santos et al., 2009). The aim of the present study was to further investigate the performance of our previously developed assay based on the selective release of IL-8 and activation of p38 MAPK by sensitizers in the THP-1 cell line (Mitjans et al., 2008), and to investigate the possibility to classify allergens according to their potency. Overall, the new data confirm the previous results and reveal IL-8 as potential tool not only to identify sensitizers but also to classify them according to their potency, while p38 MAPK activation allows the identification of all sensitizers, including pro-haptens, but is not useful for potency classification. The absence of pro-hapten-induced IL-8 release may be partially explained by a 1.6-fold decrease in IL-8 mRNA stability.

Maturation of DCs/LCs in response to sensitizing agents has been identified as one of the *in vitro* strategies to predict the sensitizing capacity of chemicals (Casati et al., 2005). Different approaches based on the use of dendritic-like cells cultures have been developed to mimic the maturation process of LCs. Among them, the THP-1 cell line is one of the most interesting alternatives because of its availability and abundance (Yoshida et al., 2003). Using this cell line, we have previously shown that exposure of

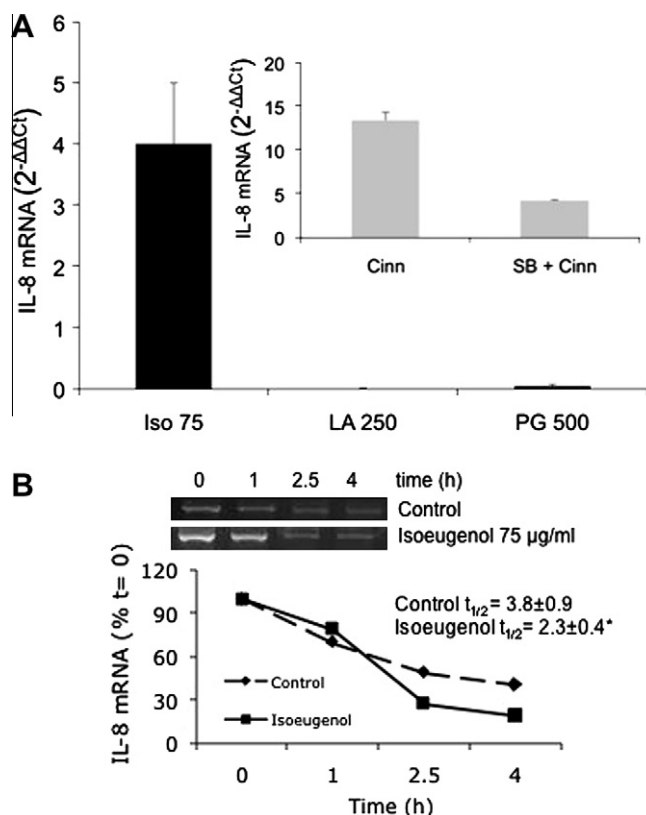


Fig. 4. IL-8 mRNA expression and stability. (A inset) THP-1 cells were treated for 1 h with the selective p38 MAPK inhibitor SB202190 (50 nM) or with DMSO as vehicle control, then cinnamaldehyde (60 µg/ml) was added for 3 h. IL-8 mRNA expression was evaluated by Real Time PCR. Results are presented as mean $\pm$ SD of at least 3 independent experiments. (A) THP-1 cells were treated for 3 h with isoeugenol (Iso, 75 µg/ml), lactic acid (LA, 250 µg/ml) and propylene glycol (PG, 500 µg/ml). IL-8 mRNA expression was evaluated by Real Time PCR. Results are presented as mean $\pm$ SD of at least 3 independent experiments. (B) IL-8 mRNA stability. THP-1 cells were treated for 3 h ($t = 0$) with isoeugenol (75 µg/ml) or DMSO as vehicle control, then DRB, an inhibitor of transcription, was added for different times (1, 2.5 and 4 h). IL-8 mRNA expression was evaluated by RT-PCR as described in the Materials and Methods section. Representative results, expressed as % relative to the expression at $t = 0$, are shown. The $t_{1/2}$ was calculated by linear regression analysis of data. Each value represents the mean $\pm$ SD of at least 3 independent experiments.

THP-1 cells to allergens results in a dose-related release of IL-8, whereas exposure to irritants fails to induce IL-8 secretion. These promising observations are confirmed by the results presented in this work by a new panel of sensitizers and non-sensitizers, with the exception of the pro-hapten isoeugenol, which failed to induce IL-8 release. Isoeugenol was, however, able to activate p38 MAPK and to induce IL-8 transcription. On the contrary, skin irritants failed to induced p38 MAPK activation, IL-8 mRNA expression and IL-8 release.

In our experience, the detection of IL-8 presents better sensitivity than the surface expression of CD54 and CD86, which fail to identify approximately 30% of the sensitizers tested (Mitjans et al., 2008). Therefore, secretion of this cytokine emerges as a tool to accurately identify sensitizers in the toxicological evaluation of hazards.

The relative skin sensitizing potency of chemicals may vary by many orders of magnitude from one to another (Kimber et al., 2001). At present, the only method assay that brings the possibility to classify a chemical on the basis of sensitization potency is the LLNA assay (Basketter et al., 1999). We have, therefore, investigated the possibility to use the release of IL-8 as an instrument not only to identify allergens but also to classify them according

to their potency. The concentration of chemicals resulting in a release of 100 pg/ml of IL-8 was calculated by linear regression of data for each sensitizer and compared with the in vivo LLNA EC3 value, a significant correlation was found, indicating that IL-8 could also be regarded as a way to classify allergen potency.

Kinases are components of signal transduction pathways implicated in changes in surface marker expression and cytokine/chemokine production. For this reason, effects on signal transduction pathways have been proposed as another potential tool to identify allergens. Among the different kinases studied, we have recently shown that p38 MAPK activation plays a crucial role, although not exclusive, in IL-8 production (Mitjans et al., 2008). Moreover, p38 MAPK is considered being a critical player in the initiation of contact hypersensitivity (Toebak et al., 2009). In the present work, the investigated sensitizers, including the pro-hapten isoeugenol, activated this kinase, while no sensitizers failed, thereby confirming p38 MAPK as a promising marker for the identification of contact allergens. This is also consistent with data published by Megherbi et al. (2009). However, with the chemicals we had tested and, the time point investigated for p38 MAPK activation, we failed to show any correlation between LLNA EC3 data and vigour of p38 MAPK activation. Citral, classified as a weak sensitizer, revealed higher increase than the extreme skin allergen BZQ and, in a similar way, imidazolidinyl urea was more potent than the moderate ones isoeugenol and diethyl maleate. We cannot exclude that a different time point or multiple time points may indeed provide indication useful for potency classification. Moreover, the fact that other intracellular pathways are being activated makes more difficult to use p38 MAPK as classification tool.

Overall, data obtained confirmed previous results (Mitjans et al., 2008), indicating not only the possibility to discriminate sensitizers from non-sensitizers but also the possibility to use IL-8 release for potency classification. On the contrary, p38 MAPK activation did not correlate with the in vivo LLNA EC3 values, but it was activated by all allergen tested including pro-haptens, i.e. isoeugenol, and by chemicals which failed to induce a significant release of IL-8, i.e. trimellitic anhydride (data not shown). The added value of assessing p38 MAPK activation, together with IL-8 mRNA expression, is the identification of pro-haptens, for which no significant release of IL-8 was observed in THP-1 cells. For an unknown chemical, therefore, we propose the following approach: after identification of the CV₇₅, the release of IL-8 should be evaluated treating cells for 24 h with concentrations of chemicals resulting in 75, 85 > 90% of cell viability. If a statistically significant release of IL-8 is found, chemical will be classified as potential allergen. If negative, IL-8 mRNA expression (at 3 h) and/or p38 MAPK activation (at 5–15 min) should be evaluated. If negative results were obtained, the chemical lacks sensitization potential.

In conclusion, while reducing the number of laboratory animals used, our test would enable reliable analysis of chemicals using a human system. Overall, our data suggest that THP-1 cells are a good model for screening sensitizers and, that IL-8 release could also be used for potency classification.

Conflict of interest

For the past 4 years since the beginning the work, all Authors declare not having any financial, personal, or association with any of the individuals or organizations, that have could inappropriately influence the submitted work.

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