Dimethyl sulfoxide is not necessary to dissolve most sensitizers for their *in vitro* stimulation of dendritic cells

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Abstract

Most of sensitizers are hydrophobic and cannot be dissolved in water. Therefore, whenever the effects of sensitizers are examined *in vitro*, they are dissolved using DMSO. However, when sensitizers stimulate Langerhans cells or dendritic cells *in vivo*, they are exposed to sensitizers dissolved in the interstitial fluid. So, in this study, we examined whether sensitizers dissolved in the culture medium without using DMSO can stimulate dendritic cells or not. To pursue this purpose, we used human monocytic leukemia-derived THP-1 containing a IL-8 reporter gene and a synthetic culture medium, X-VIVO™ 15. The results clearly demonstrated that a synthetic culture medium, X-VIVO™ 15, can replace DMSO for dissolving most water-insoluble chemicals in the IL-8 reporter assay (IL-8 Luc assay) and that the IL-8 Luc assay using X-VIVO™ 15 was significantly better in detecting sensitizers than the IL-8 Luc assay using DMSO. In examining 105 sensitizers and 31 nonsensitizers, the IL-8 Luc assay provided 87.6% of sensitivity, 74.2% of specificity, and 84.6% of accuracy after considering its applicability domain. When we constituted the integrated approach by combining direct peptide reactive assay, the performance was significantly improved, 94.0% of sensitivity, 66.7% of specificity, and 88.7% of accuracy. These studies clearly demonstrated that DMSO is not necessary for dissolving most sensitizers for *in vitro* studies examining their effects on dendritic cells.

1. Purposes

1.1 Can a synthetic culture medium, X-ViVO™-15, replace DMSO as a solvent?

1.2 Can the IL-8 Luc assay using X-ViVO™-15 predict sensitizers with better performance that that using DMSO?

2. Materials and Methods

2.1 Cells and culture

2.2 Procedure of IL-8 Luc assay

Parameters of IL-8 Luc assay

Abbreviation	Definition
GAPLA	SLR luciferase activity reflecting GAPDH promoter activity
IL8LA	SLO luciferase activity reflecting IL-8 promoter activity
nIL8LA	IL8LA / GAPLA
Ind-IL8LA	nIL8LA of THP-G8 cells treated with chemicals / nIL8LA of untreated cells
Inh-GAPLA	GAPLA of THP-G8 treated with chemicals / GAPLA of untreated cells
CV05	The lowest concentration of the chemical at which Inh-GAPLA becomes < 0.05.

2.3. Dilution of chemicals and chemical treatment

2.4 Criteria for positive judgment and to determine sensitizers

3. Results

3.1. Performance of the mL-8 Luc assay

Table 1. Difference of the performance between the IL-8 Luc assay using DMSO (oIL-8 Luc) and that using X-VIVO 15 as a solvent (mIL-8 Luc)

Criteria	oIL-8 Luc	mIL-8 Luc	
		Applicability domain (-)	Applicability domain (+)
Chemical numbers	122	143	136
Accuracy	73%	80%	85%
Sensitivity	72%	86%	88%
Specificity	74%	64%	74%

3.2. The effects of physical properties of chemicals on the sensitivity of the mL-8 Luc assay

Fig. 1. Comparison of chemical properties between false negative and true positive chemicals judged by the mL-8 Luc assay suggests that these properties do not affect the judgment of the mL-8 Luc assay.

3.3. Estimation of MITs using the mL-8 Luc assay

To estimate the concentration of the stock solution insoluble at 20 mg/ml, the solubility of the chemical at 2 mg/ml was examined. If the chemical was dissolved, the concentration of the stock solution was estimated as 2 mg/mL, and if not, the concentration was approximated by 0.2 mg/mL. For liquid chemicals, 1 mg of Sudan black B was added to permit visualization.

Fig. 2. Determination of the solubility of test chemicals at 2 mg/mL

3.4. Correlation between the mL-8 Luc MITs and the oIL-8 Luc MITs

Fig. 3. The significant correlation between the mL-8 Luc MITs and the oIL-8 Luc MITs, which validates the device to estimate the concentration of chemicals

3.5. Correlation between the mL-8 Luc MITs and parameters of other *in vitro* test methods

Fig. 5. No significant correlation between the mL-8 Luc MIT and DPRA

Fig. 6. Only weak correlation between the mL-8 Luc MIT and parameters of h-CLAT

4. Discussion

In all current *in vitro* methods to detect sensitizers, DMSO is used to dissolve water-insoluble chemicals. In this study, we demonstrated the following

- 1) A synthetic culture medium, X-VIVO™ 15, can replace DMSO for dissolving water-insoluble chemicals in the IL-8 Luc assay and that the modified IL-8 Luc assay using X-VIVO™ 15 (mIL-8 Luc) was significantly better in detecting sensitizers than the original assay using DMSO as a solvent (oIL-8 Luc). The mL-8 Luc assay provided 87.6% of sensitivity, 74.2% of specificity, and 84.6% of accuracy after considering its applicability domain.
- 2) There was a significant correlation between the mL-8 Luc minimum induction concentrations (MITs) and the oIL-8 Luc MITs ($r^2 = 0.866$). On the other hand, only weak correlation was observed between mL-8 Luc MITs and CD86 EC150 and CD54 EC200.

5. Conclusion

These results demonstrate that the mL-8 Luc is a unique *in vitro* test for detecting sensitizers in purely aqueous phase and that the obtained data cannot be replaced by the data from any other test methods.

References

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6. The proposed OECD test guideline of the IL-8 Luc assay

6.1 Criteria

6.2 Integrated approach with DPRA

6.3 Performance of the IL-8 Luc assay (Test guideline protocol)

Table 2. The performance of the IL-8 Luc assay, its integrated approach with DPRA, and other *in vitro* test methods when compared using the same chemicals.

	oIL-8 Luc	mIL-8 Luc	IL-8 Luc assay (Test Guideline protocol)	IL-8 Luc + DPRA	h-CLAT (Takenouchi et al. 2015)	KeratinSens (Urbisch et al. 2015)
Accuracy	0.78	0.85	0.86	0.88	0.80	0.75
Sensitivity	0.78	0.88	0.96	0.94	0.82	0.76
Specificity	0.77	0.74	0.41	0.69	0.76	0.71
Total chemical	136	136	118	142	107	122
Total sensitizer	105	105	96	107	82	91
Total negative	31	31	22	35	25	31
Correct Positive	82	92	92	100	67	69
False Negative	23	13	4	7	15	22
Correct negative	24	23	9	24	19	22
False positive	7	8	13	11	6	9

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