Extractable Positive Control for In Vitro Skin Irritation Testing of Medical Devices

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Introduction
Skin irritation is a condition caused by acute damage to keratinocytes following exposure to a chemical. Testing for skin irritation potential of medical devices has typically involved the use of laboratory animals. In an effort to reduce the need for in vivo testing, alternative in vitro skin irritation test methods have been developed and validated [1-3]. While these methods have been shown to provide results consistent with in vivo data, they have involved the use of chemical solutions or spiked extracts as positive controls. In order to provide a method more applicable to medical device testing – in which devices are extracted in polar and non-polar solvents – attempts have been made to find an extractable material that will induce a positive skin irritation response in both polar and non-polar extraction vehicles.

Here we report the findings of polar extraction vehicles.

Methods
Reconstructed human epidermis (RHE) tissues were exposed to material extracts and irritation response was determined by measuring MTT reduction and IL-1α release. In vitro results were compared to in vivo intracutaneous reactivity testing and histopathology.

Sample Extraction
- Y-1, Y-2, Y-3 and Y-4 materials, along with appropriate extraction vehicle controls were extracted for 72 h in both saline and sesame oil at 37°C and 50°C according to ISO 10993-12 [5].
- 6 cm²/ml surface area to extract volume ratio was used.

Skin Irritation Testing

Results

In Vitro Testing

-MTT Viability Assay
- Tissue Exposure
  - RHE tissues were exposed to 100 µL of extracts and incubated at 37°C and 5% CO₂ for 24 h.
  - 100 µL of 1% sodium dodecyl sulfate (SDS) was used as a positive control and 100 µL of the corresponding vehicle control was used as a negative control.

- After exposure, tissues were rinsed with phosphate buffered saline.

-MTT Viability Assay
- Tissues were placed in a 1 mg/mL MTT solution and incubated at 37°C and 5% CO₂ for 3 h.

- Formazan was extracted in isopropanol at room temperature on an orbital shaker for 2 h.

- Formazan extracts were mixed thoroughly and transferred in duplicate to a 96 well plate.

- OD₅₇₀nm was measured and percent viability of each sample was calculated relative to negative control.

- Tissue viability of ≤50% indicates skin irritation.

- Interleukin-1α (IL-1α) Assay
- Thermo Scientific Human IL-1α ELISA kit (Catalog No. EH2IL1A) was used according to manufacturer protocol to measure IL-1α released into culture medium during sample exposure.

- IL-1α values >100 pg/mL indicate skin irritation.

Intracutaneous Reactivity Test
- Samples were extracted in saline and sesame oil at 50°C for 72 h.

- Performed according to ISO 10993-10 [4].

- Reactivity scores >1 indicate skin irritation.

Histopathology
- Skin with dermal layer was collected and fixed in 10% neutral buffered formalin.

- Paraffin-embedded sections were prepared and stained using hematoxylin and eosin staining.

- Three sites were examined for Y-1, Y-2 and Y-3 samples. Six sites were examined for Y-4 samples.

Conclusions
- Y-1, Y-2 and Y-3 do not induce a skin irritation response in vitro as measured by either tissue viability or IL-1α release.
- Y-4 consistently results in a positive skin irritation response in vitro under all extraction conditions tested.
- In vivo results are in line with in vivo results.
- Y-4 should be considered for use as an extractable positive control material for future in vitro skin irritation testing of medical device extracts.

References

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