**ABSTRACT**

Ingenol mebutate represents a topical treatment for fields with actinic keratosis (AK). The biological effects of ingenol mebutate in AK, subclinical (SC)-AK, and reference-skin were assessed and graded by in vivo reflectance confocal microscopy (RCM) and histology. Patients with AK and SC-AK lesions in one 25 cm² field on hands or forearms, and with an area of reference skin on the inner upper arm, were included. The two fields were each treated with ingenol mebutate 0.05% gel (n=16), or vehicle (n=8), on 2 consecutive days; clinical and RCM assessments were performed on days 1, 2, 3, 8, and 57, and biopsies on day 3. Local skin responses were more pronounced in AK fields (6.1 (mean) ± 2.6 (SD)) compared with reference skin (3.5 ± 1.5). The clinical AK lesion reduction was 43.8% and 6.3% with ingenol mebutate and vehicle, respectively. RCM and histology evaluations showed that ingenol mebutate induced a significant pronounced cell death and immune response in AK and SC-AK lesions, compared with reference skin. Ingenol mebutate induced RCM-measured reduction in (investigator-1/investigator-2): AK lesions (34/28%), SC-AK lesions (72/56%), and solar elastosis in AK fields (mean, -0.22/-0.25). In conclusion, ingenol mebutate showed selective pronounced biological responses in AK and SC-AK as compared with reference skin. 