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Antiproliferative effect of benzothiphene γ-hidroxibutenolide in skin inflammation via inhibition of STAT3 pathway

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Several studies have implicated Signal Transducer and Activator of Transcription 3 (STAT3) in the pathogenesis of psoriasis. Total STAT3 expression has been proven to be upregulated in psoriatic lesions and a transgenic mouse model constitutively overexpressing activated STAT3 in the basal stem-cell layer of the epidermis was able to reproduce many of the characteristic features of psoriasis. Moreover, several cytokines and growth factors upregulated in psoriasis such as IL-6 and the IL-20 family cytokines (IL-19, IL-20, IL-22, and IL-24) are able to induce STAT3 activation (1).

In the present study we characterized the effect of 4-benzo[*b*]thiophen-2-yl-3-bromo-5-hydroxy-

5H-furan-2-one (BTH), which was previously identified as a potent inhibitor of NF-κB activation in keratinocytes, on STAT3 signalling pathway and keratinocyte proliferation.

The release of IL-6 by normal human keratinocytes was significantly inhibited by BTH (10 μM) (4.72 ± 2.10 pg/ml *vs.* 48.21 ± 5.73 pg/ml in stimulated cells; $p < 0.01$) after 48 hours challenge with TNFα (10 ng/ml). Subsequently, cells were stimulated with IL-6 (50 ng/ml) in order to further characterize the effect of BTH in this signalling pathway. An inhibition of IL-6 induced STAT3 nuclear translocation was observed by immunofluorescence staining after a 30 min pre-treatment with BTH. This effect was mediated by blockage of the phosphorylation on the Tyr705 residue of STAT3 measured by Western Blotting. This modification is mainly carried out by the Janus Kinases (Jak).

STAT3 is a key transcriptional factor involved in the regulation of cell proliferation (2). In accordance with its capability to inhibit STAT3 activation, BTH impaired keratinocyte growth (absorbance 490 nm: 0.73 ± 0.04 *vs.* 1.11 ± 0.03 in control cells; $p < 0.01$) as observed by the MTT assay (3) whereas it maintained cell viability, measured by the LDH assay (1).

To complete its *in vivo* pharmacological profile, we tested the anti-proliferative effect of BTH in the murine TPA-induced epidermal hyperplasia model (4). Topical application (400 μg/site) of BTH significantly inhibited the skin hyperplasia induced by repeated application of TPA (2 nmol/site) in the backs of female Swiss mice (approx. 25 g) as observed in the hematoxylin-eosin and keratin-6 staining.

The inhibition of STAT3 signalling pathway is one of the most promising new therapeutical strategies in the treatment of psoriasis, consisting in the search of molecules capable of blocking Jak phosphorylation of STAT3. The STAT3-mediated antiproliferative effect demonstrated in this study, together with our previous data on its anti-inflammatory capabilities, suggests a potential beneficial pharmacological profile for BTH in psoriasis.

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