TGFβ1 Single Nucleotide Polymorphism C-509T Alters Mucosal Cell Function in Pediatric Eosinophilic Esophagitis

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Wound healing assay

Wound healing assays were done as previously described (1, 2). 4 well Lab-Tek™ II chamber slides (Thermo-Fisher Scientific, MA, USA) were marked with two parallel lines in the external bottom side of the slide to create reference points for imaging. All images were taken at the same points at every time point. Fibroblasts from 4 CC and 4 TT lines were plated at 50,000 cells/well in pre-marked 4 well chamber slides and grown to confluence. Confluent monolayers were switched to basal medium for 24 hours and monolayers were scratched using a 10µl pipette tip. Images were taken at baseline, 8, 24, 32, and 48 hours after scratching with an inverted microscope (Fein Optic, CA) using the previously drawn lines as reference points and at 4 distinct points. The area of the wound at baseline in 4 different points was measured using ImageJ area measuring tool (National Institutes of Health, Bethesda, MD) and the average area in these 4 points calculated. This area was considered as the maximum wound size. The percentage of wound closure was calculated by dividing the area at each time point by the area at time 0, and subtracting the obtained value to 100.

References

1. Manresa MC, Tambuwala MM, Radhakrishnan P, Harnoss JM, Brown E, Cavadas MA, et al. Hydroxylase inhibition regulates inflammation-induced intestinal fibrosis through the suppression of ERK-mediated TGF-beta1 signaling. [corrected]. American journal of physiology Gastrointestinal and liver physiology. 2016;311(6):G1076-G90.
2. Sonnylal S, Shi-Wen X, Leoni P, Naff K, Van Pelt CS, Nakamura H, et al. Selective expression of connective tissue growth factor in fibroblasts in vivo promotes systemic tissue fibrosis. Arthritis and rheumatism. 2010;62(5):1523-32.

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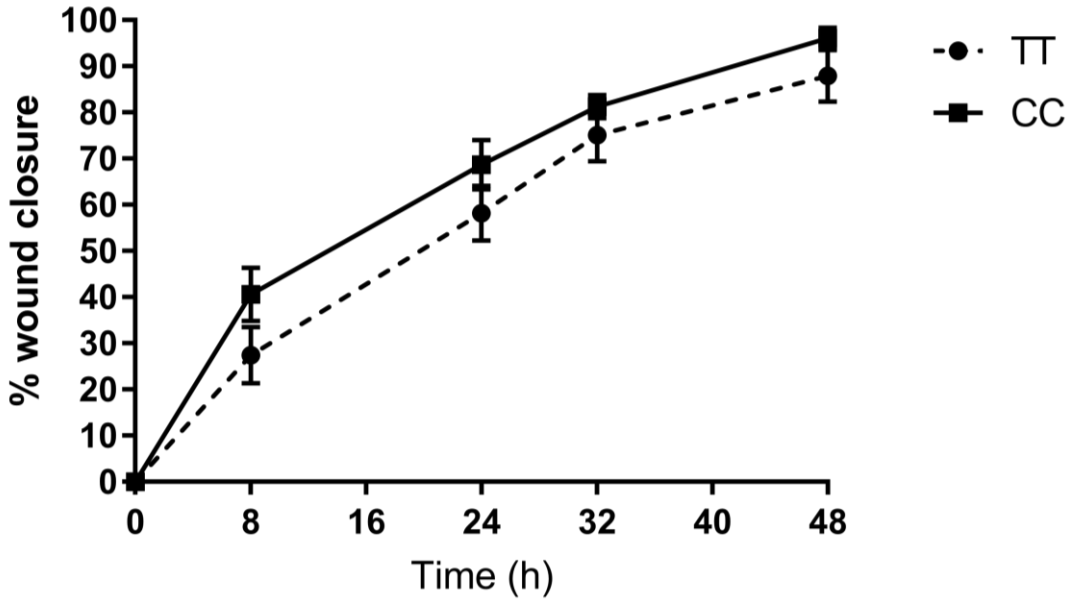
% wound closure
100
90
80
70
60
50
40
30
20
10
0
0
8
16
24
32
40
48
Time (h)
TT
CC