The Impact of Inflammation on the Absolute Concentration of Albumin and α1-Acid Glycoprotein in Porcine Skin

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Introduction

The efficacy of dermal drugs strongly depends on the free drug fraction in the skin which is accessible by sampling dermal interstitial fluid (dISF) using dermal open flow microperfusion (dOFM). The free drug fraction in dISF is influenced by the drug's protein binding properties and the amount of protein present.

As inflammation can alter the protein content in dISF, it will also influence protein binding and subsequently the free drug fraction and drug efficacy.

This study aimed to determine the absolute concentration of the main binding proteins in dISF namely albumin and α1-acid glycoprotein (AGP) in normal and inflamed skin of pigs.

Results

Mean IL-6 concentration in inflamed skin (10.8 ± 4.6 ng/ml) was significantly higher than in normal skin (0.4 ± 0.2 ng/ml) (P<0.05) (Figure 1b).

Mean absolute protein concentrations were significantly higher in inflamed skin (albumin: 19.0 ± 6.9 mg/ml, AGP: 0.56 ± 0.13 mg/ml) than in normal skin (albumin: 11.0 ± 1.3 mg/ml, AGP: 0.31 ± 0.06 mg/ml) (P<0.05) (Figure 1c).

Mean ISF-to-plasma ratio was significantly higher in inflamed skin (albumin: 0.67 ± 0.21, AGP: 0.86 ± 0.15) than in normal skin (albumin: 0.39 ± 0.06, AGP: 0.49 ± 0.13) (P<0.05) (Figure 1d). In inflamed skin, mean ISF-to-plasma ratio for AGP was significantly higher than ISF-to-plasma ratio for albumin (P<0.05).

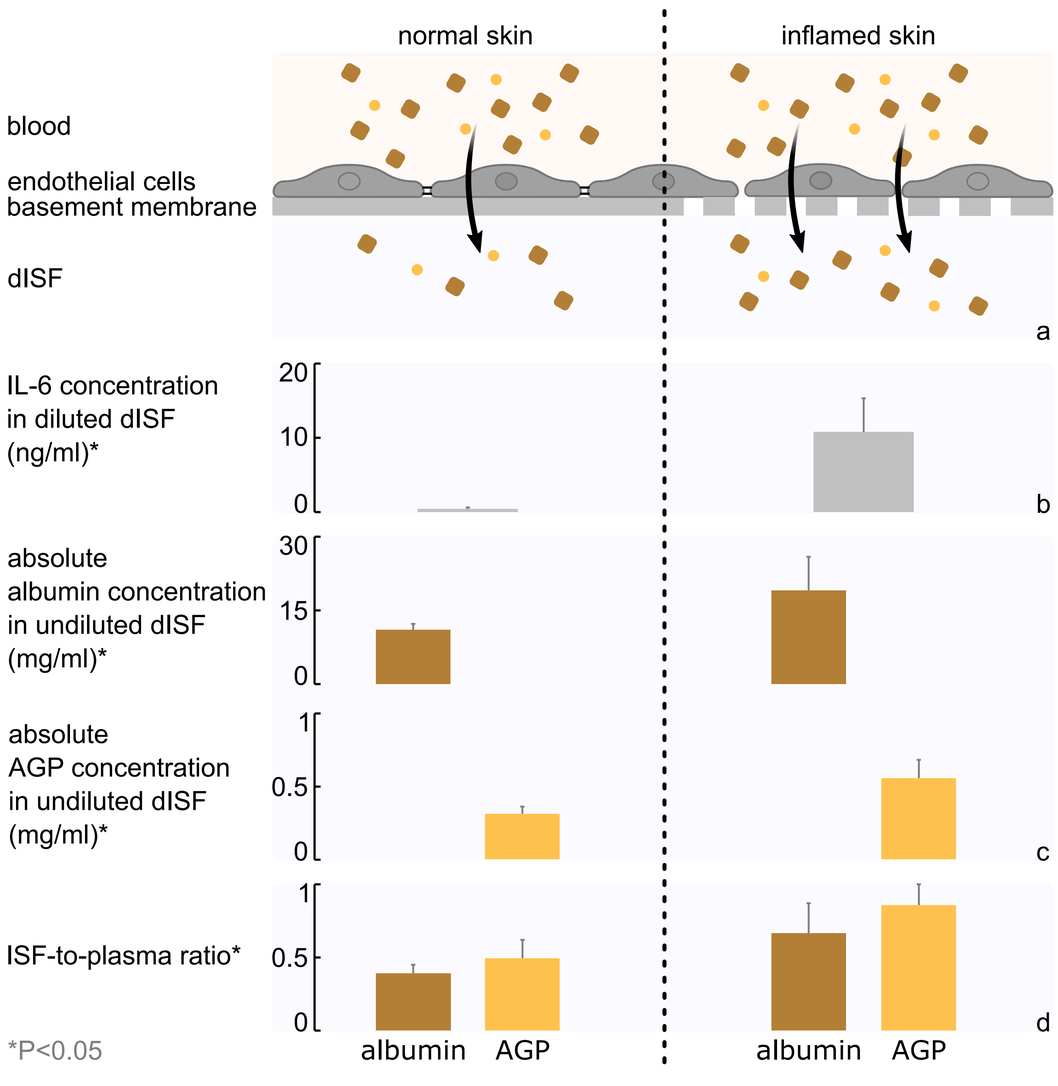

Figure 1: (a) Schematic of capillary permeability of proteins in normal and inflamed skin, (b) IL-6 concentration of dISF samples in normal (n=12) and inflamed skin (n=12) collected by standard dOFM (P<0.05), (c) absolute albumin and AGP concentration in dISF in normal (n=6) and inflamed skin (n=11) collected by dOFM recirculation (P<0.05), (d) ISF-to-plasma ratio of albumin and AGP in normal (n=6) and inflamed skin (n=11) (P<0.05), inflamed skin ISF-to-plasma ratio of AGP higher than albumin (P<0.05)

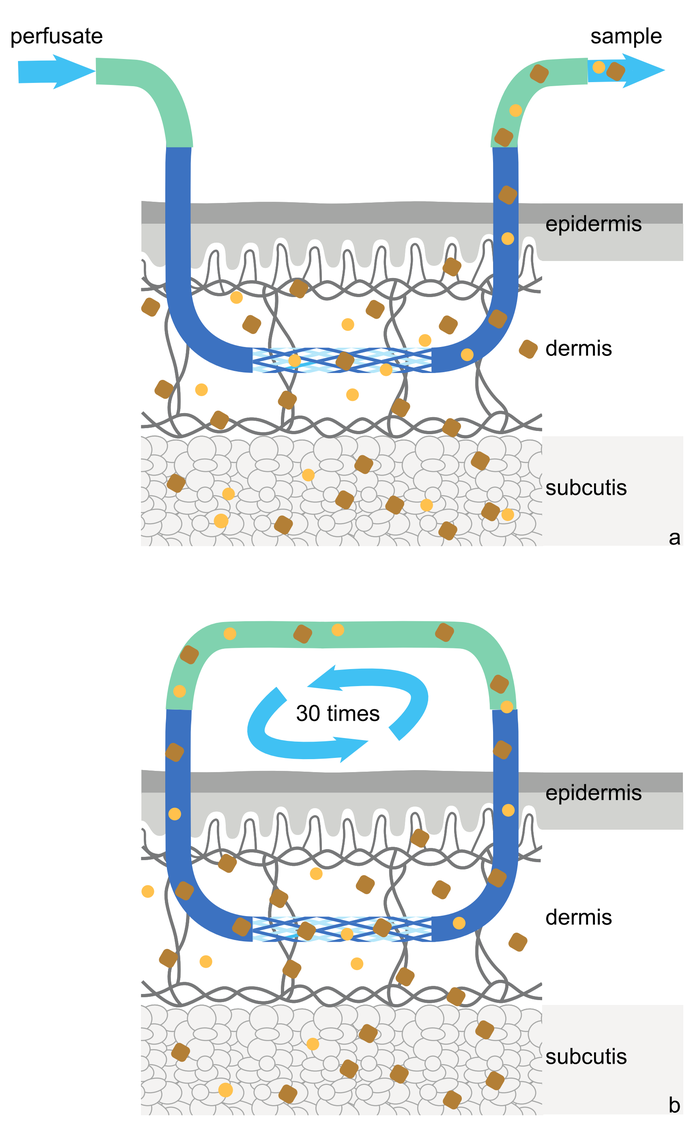

Figure 2: (a) standard dOFM setup to collect diluted dISF samples (b) dOFM recirculation setup to collect undiluted dISF samples

Methods

- **preclinical study**
  - landrace pigs (n=3, female, 10 weeks)
  - normal skin vs. inflamed skin
  - skin inflammation: split thickness wounds were treated with resiquimod for 2 days
  - IL-6 as biomarker for inflammation
- **sampling**
  - diluted dISF by standard dermal open flow microperfusion over 12 h (Figure 2a)
  - undiluted dISF by dOFM recirculation (30 recirculations over 12 h, Figure 2b)
  - blood plasma samples
- **Analytical assays**
  - IL-6 from diluted dISF via V-PLEX Plus Proinflammatory Panel 1 Human Kit (MSD)
  - Albumin from undiluted dISF via colorimetric bromcresol green assay (Roche)
  - AGP from undiluted dISF via MAb 1.62

Conclusion

These findings indicate that higher protein concentrations in inflamed skin may result in higher protein binding and can thus be of crucial importance for drug development for inflammatory skin diseases, especially for highly protein bound drugs.

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