Supplementary Data

Comparing naturally occurring glycoforms of proline rich antibacterial peptide, Drosocin

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Mass spectra:
Di-drosocin
NMR spectra:

$^1$H-NMR

$^{13}$C-NMR
(c) Effect of PBS and DMEM on the bactericidal activity of glycosylated and non-glycosylated forms of drosocin:

It has been reported that the antibacterial activity of AMPs is affected by buffer/media used in the assay [1-3]. In order to analyze whether different forms of drosocin retained their antibacterial activity in PBS buffer and Dulbecco’s Modified Eagle’s Medium (DMEM), used for haemolytic and cytotoxic/immunomodulatory assays, respectively, the killing kinetics was carried out in this buffer or media. In PBS, M-drosocin and Di-drosocin were able to completely eradicate the bacterial cells in 120 and 360 min, respectively, at their MIC values, whereas n-drosocin could display the bactericidal effect at its 4 fold MIC and completely killed the bacteria in 360 min (Fig S1). The results showed that M-drosocin had the faster bactericidal activity than that of Di-drosocin and n-drosocin in PBS.

We observed that the bactericidal activity of different forms of drosocin against E. coli ATCC 25922 reduced significantly in DMEM. Glycosylated and non-glycosylated forms of drosocin failed to totally eliminate the bacteria at their 4 fold MIC in DMEM (data not shown). M-drosocin took 360min to completely kill the bacteria at its 50µM, whereas Di-drosocin and n-drosocin required 360min and 720min, respectively to totally eliminate the bacteria at 200µM (Fig S2). Thus, M-drosocin displayed the faster bactericidal activity in comparison with the Di-drosocin and n-drosocin in DMEM.
Fig S1: Effect of PBS buffer on the bactericidal activity of different forms of drosocin.

Time-kill study of *E. coli* ATCC 25922 in PBS challenged with n-drosocin, M-drosocin and Di-drosocin. M-drosocin shows the faster bactericidal activity than that of Di-drosocin and n-drosocin in PBS.
Fig S2: Effect of DMEM on the bactericidal activity of different forms of drosocin.

Time-kill study of *E. coli* ATCC 25922 in DMEM challenged with (a) 50µM (b) 200µM of n-drosocin, M- drosocin and Di-drosocin. M-drosocin displays faster bactericidal activity against *E. coli* in DMEM in comparison with that of Di-drosocin and n-drosocin.
Fig S3: Effect of drosocin forms on secretion of TNF-α and IL-6 in macrophages. RAW 264.7 cells were treated with different concentrations of drosocin forms and LPS (20ng/ml) for 24h. Concentrations of (a) TNF-α and (b) IL-6 in the cell supernatant were measured by ELISA. LPS served as a positive control which induced the secretion of cytokines. Levels of cytokines present in the supernatant of macrophages treated with medium alone as depicted by only cells or with different concentrations of peptides were found to be similar. Data
shown are representative values of three independent experiments performed in triplicate. n.d. (not detectable) indicates that cytokine levels were below detection level. **: p<0.01 compared to only-cells control; ‘ns’ means there was no statistical significance from the only-cells control.
(e) Effect of 200µM of drosocin peptides on secretion of TNF-α and IL-6 in macrophages stimulated with or without LPS:

It was observed that both glycosylated and non-glycosylated drosocins could not stimulate the secretion of TNF-α and IL-6 (Figs S4a and S4b) and even did not modulate LPS-induced levels of TNF-α and IL-6 in macrophages at their concentration of 200µM (Figs S4c and S4d), the concentration at which all the drosocin forms exhibited the bactericidal activity against *E. coli* ATCC 25922 in cell culture medium, DMEM.
Fig S4: Effect of 200μM of drosocin peptides on secretion of TNF-α and IL-6 in macrophages stimulated with or without LPS. RAW 264.7 cells were treated with medium alone or LPS (20ng/ml) alone or with 200μM of different forms of drosocin in the absence or presence of LPS (20ng/ml) and combination of polymyxin B (7.2μM) and LPS for 24h. Concentrations of (a and c) TNF-α; (b and d) IL-6 in the cell supernatant were measured by
ELISA. Polymyxin B served as a positive control which inhibited the LPS induced secretion of cytokines. Data shown are representative values of three independent experiments performed in triplicate. n.d. (not detectable) indicates that cytokine levels were below detection level. ‘ns’ means there was no statistical significance from the LPS. (p<0.01)
References:

