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A comparison of the effects of CCR4 antagonists on human platelet and T cell responses to CCL22

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***Introduction**:* We have previously shown that CCR4 antagonists inhibit human T cell responses to the chemokines CCL17 and CCL22 via two distinct allosteric binding sites[1]. CCR4 is also expressed on platelets[2] but the effects of small molecule antagonists on platelet responses to CCR4 agonists have not been studied. We compared the effects of several allosteric CCR4 antagonists (compounds 1, 4, 5, 7, 8 and 9[1]) on CCL22-induced T cell actin polymerisation and platelet aggregation.

Methods: Blood samples were obtained with informed consent from healthy medication-free donors. Actin polymerisation was determined flow cytometrically[1]. Platelet aggregation was determined photometrically[3]. Preincubation with antagonists was performed for 30 min. Data are presented as mean±SEM (n donors).

Results: The maximal aggregation to CCL22 was 23.0%±3.2% (n=7) of that to 100µM ADP. CCL17 was a partial agonist with intrinsic activity 0.70±0.07 (n=5), lower than that previously seen in T cells[4] (0.95±0.03). The potency of CCL22 in actin polymerisation assays, pEC_{50}=10.12±0.06 (n=5), was also higher than that in platelet aggregation assays, pEC_{50}=8.87±0.06 (n=7). The antagonists, whether surmountable or insurmountable, inhibited the two responses very similarly. Deming regression of the apparent pA_2 values gave slope=1.12±0.16 and intercept=-0.66±1.09. Coapplication of compounds 1 and 7 resulted in concentration-ratios that were substantially larger than the sum of those of the individual antagonists in both systems (platelets (n=6): combined log(DR)=1.82±0.17 (mean=65.5) vs 0.89±0.11 (mean=7.8) & 0.63±0.09 (mean=4.2) individually; T cell (n=4): log(DR)=1.21±0.05 (mean=55.6) vs 1.07±0.07 (mean=11.8) & 0.65±0.01 (mean=4.5)). The coapplication of these two compounds also resulted insurmountable antagonism, apparently more so in platelets (55.4%±7.8%, n=6, inhibition vs 28.8%±4.8%, n=4, inhibition in T cells).

Conclusions: The lower potency of CCL22 and partial agonism of CCL17 in platelet aggregation suggest a lower receptor reserve in this assay than in the actin polymerisation assay and possibly therefore a lower receptor density in the platelet. Despite this, there was a very strong similarity between the effects of the antagonists in the two assays indicating that the properties of these allosteric compounds are well conserved between the two systems. The supra-additive effects of compounds 1 and 7 in combination are consistent with their previously described binding to distinct sites on CCR4.

References:

1. Slack *et al.* (2013). Pharmac Res Per **1**: e00019.

2. Clemetson *et al*. (2000). Blood **96**: 4046-4054.

3. Slack *et al.* (2016) Biochem Pharmacol **117**: 88-96

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