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Correspondence

Reply to J. Gordon et al.

Dear Sirs,

We should like to address the points raised by Gordon and colleagues as follows:

There is some common ground:

(i) Based on our data [1], we agree that SSRIs (selective serotonin transport inhibitors) can kill many different cell types, when present in concentrations in the low μM range.
(ii) This action is independent of their eponymous actions, i.e. inhibition of the serotonin transporter.
(iii) While Bcl-2 overexpression may render a given cell line more resistant (quite unsurprising), it is also rather unsurprising that Bcl-2 levels, *per se*, do not predict the susceptibility of a given cell type to the toxic effects of SSRIs. These data were supplied during the review process to satisfy the curiosity of one of our referees.

We find the comments of Gordon on our apoptosis data and on the use of fluoxetine versus paroxetine somewhat disingenuous: (i) annexin V-staining was not the sole measure of apoptosis reported, the FACS data that we show also include propidium iodide uptake. In addition, from the patient's perspective, i.e. for clinical outcome, differences between assays for *in vitro* growth inhibition, induction of apoptosis or of necrosis become an academic pre-occupation. (ii) In their original data set [2], paroxetine was certainly not less potent ($IC_{50} = 6.9 \pm 1.2$ μM) than fluoxetine ($IC_{50} = 9.3 \pm 2.3$ μM) in inhibiting growth of L3055 (biopsy-like Burkitt's lymphoma) cells. There is no specific reason why we should favour fluoxetine over any other SSRI, in particular given that fluoxetine also has appreciable affinity, *e.g.*, for $5HT_{2C}$-receptors [3] and HERG-channels [4] and is therefore considered the most "dirty" SSRI.

We are aware of the difference of between established cell lines and primary cells: as pointed out by Gordon and colleagues, the cells derived *ex vivo* from lymphomas arising in Eμ-myc mice were as susceptible to the action of paroxetine as their L3055 (biopsy-like Burkitt's lymphoma) cells. It is however also worth pointing out that the shape of the concentration-response curve was shallow in the Eμ-myc driven tumour cells, while all other concentration-response curves were steep. This suggests that the susceptibility of the heterogeneous primary tumour cells varies widely. Thus, resistant cells are likely to emerge rapidly unless concentrations of SSRIs can be achieved *in vivo*, which will kill <90% of the cells (see below). Dr. Gordon and his co-workers – we assume – will agree that a high fractional killing rate is essential for curative cancer chemotherapy.

The crucial point of this whole discussion, however, is whether concentrations of SSRIs (specifically fluoxetine according to Gordon et al.) can be dosed safely to reach levels, which allow for effective killing of Burkitt's lymphoma cells (fractional killing >99%). When taken at 1 mg/(kg day), fluoxetine levels (R- and S-isomer combined) can reach maximum total blood levels of 1.2 μM at steady state (i.e., after repeated dosing). For the benefit of the doubt, the metabolites (R- and S-norfluoxetine) may be added, although their action on Burkitt's lymphoma cells remains unknown: in this instance, total plasma concentrations (fluoxetine + norfluoxetine) amount to ~2 μM [5]. *In vitro*, this concentration *per se* does not suffice to achieve fractional killing rates close to or >90%. Factoring in only total concentrations, however, ignores that 95% of fluoxetine is bound to plasma proteins: thus, at 2 μM total concentration, the free concentration is ~100 nM. At the usual therapeutic dose (20 mg/day) fluoxetine reaches peak plasma levels at steady state of ~100 nM, resulting in a free concentration of 5 nM [5]. With a K_D of 1 nM, the law of mass action predicts that ~80% of SERT in the brain should be occupied at this free concentration: quite unsurprisingly, *in vivo*, PET (positron emission tomography) studies with patients taking 20 mg fluoxetine have visualized an occupancy of SERT of ~80% [6]. In other words, total plasma levels of fluoxetine must be corrected for the protein bound fraction. We therefore consider it unlikely that fluoxetine (or any other SSRI) can be dosed to reach concentrations, which kill Burkitt's lymphoma *in vivo*.

We may err, because our assumptions are conservative, but we do not consider our conclusions inaccurate and misleading

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and we assume that our colleagues who read Biochemical Pharmacology can follow our argument and judge for themselves. We also feel that cautionary notes are warranted given that, in many instances, resources and hopes are wasted on overrated novel therapeutic approaches. We obviously wish Dr. Gordon and his colleagues – and in particular their patients – good luck in their phase II trial and we should be happy, if proven wrong. In the meantime, we will put their hypothesis on the usefulness of SSRIs to another acid test, namely treat mice harbouring the Eμ-myc transgene with SSRIs.

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