Inhibition of TNF-α production in THP-1 macrophages by glatiramer acetate does not alter their susceptibility to infection by Listeria monocytogenes and does not impair the efficacy of ampicillin or moxifloxacin against intracellular bacteria

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Sir, Listeriosis is one of the potential adverse effects of TNF-α-neutralizing treatments. Glatiramer (copolymer 1; COPAXONE), a mixed, random polymer of Ala, Glu, Lys and Tyr used in the treatment of relapsing–remitting multiple sclerosis blocks the secretion of TNF-α from IFN-γ- and endotoxin-stimulated THP-1 macrophages. We have, therefore, examined the influence of glatiramer on the ability of IFN-γ to contain Listeria infection and on the activity of ampicillin and moxifloxacin to kill intraphagocytic bacteria in THP-1 macrophages. Glatiramer [CAS Registry no. 147 245-92-9; batch no. 242908102: average molecular weight, 7500Da (limits, 4200–16 350); amino acid content (molecular fraction) L-Glu, 0.139; L-Ala, 0.432; L-Tyr, 0.091; L-Lys, 0.338; total amino acid residue content, 87.9%; bacterial endotoxin content, <0.25 endotoxin units/mg] was kindly received from Teva Pharmaceuticals Industries (Petah Tiqua, Israel). All experimental procedures and assay methods have been described in our previous publications. We used a concentration of glatiramer of 20 mg/L, which was both non-toxic (based on lactate dehydrogenase release) and effective in blocking the production of TNF-α in THP-1 cells.

Glatiramer (20 mg/L) did not influence the intrinsic antimicrobial activity of ampicillin or moxifloxacin towards L. monocytogenes, based on MIC determinations in broth [0.3±0.1 and 0.5±0.1 mg/L (arithmetic dilutions) for ampicillin and moxifloxacin, respectively]. Unstimulated cells produced only negligible amounts of TNF-α and glatiramer did not alter this behaviour. In contrast, the medium of cells exposed to IFN-γ (100 units/mL; 24 h) contained 38.3±6.0 ng/L of TNF-α, and this concentration was decreased by ~2/3 in the presence of glatiramer. With infected cells, TNF-α production remained low in unstimulated cells and unaffected by the presence of glatiramer, whereas it amounted to 31.6±1.2 ng/L (5 h post-phagocytosis) in IFN-γ-stimulated cells (24 h prior to infection). This production was again decreased by 2/3 if glatiramer was present. Glatiramer did not significantly modify the capacity of THP-1 macrophages to phagocytose L. monocytogenes. Figure 1 shows that glatiramer did not modify the growth of intracellular L. monocytogenes compared with untreated cells in the 24h model (this model uses gentamicin at a concentration of 2× its MIC to prevent the extracellular growth of L. monocytogenes). No change was seen either in the 5h model (data not shown). As previously described, IFN-γ impaired the intracellular growth of L. monocytogenes after 24 h (phagocytosis) and then returned to fresh medium for 24 h before cell collection and enumeration of cell-associated bacteria. The growth of extracellular bacteria, which would otherwise occur after 5–6 h through the release of dying cells, was prevented by addition of gentamicin [2 mg/L (2×MIC); this concentration does not prevent the intracellular growth of L. monocytogenes, data not shown]. The four left blocks refer to cells unexposed to ampicillin and treated as follows: ctrl (control), no treatment; gtr (glatiramer), cells incubated with glatiramer (20 mg/L) for 24 h, and then maintained in the presence of the same concentration of glatiramer during the phagocytosis of L. monocytogenes as well as during the post-phagocytosis period (fresh solutions of glatiramer were used each time); IFN (IFN-γ), cells exposed to IFN-γ (100 units/mL) for 24 h before phagocytosis (IFN-γ was not present during phagocytosis or during the post-phagocytosis period); gtr+IFN, cells treated as for the IFN group but with glatiramer present throughout the experiment as in the gtr group. The four right blocks refer to experiments with the same design but for which ampicillin (50 mg/L) was added during the 24 h post-phagocytosis period. The ordinate shows the change in bacterial counts from original inoculum (post-phagocytosis). All data points are the mean of three determinations ± S.D. Blocks with the same letters denote groups that are not significantly different from one another by one-way ANOVA (P>0.05). This experiment was performed twice with essentially similar results.
L. monocytogenes by ~50%, and glatiramer did not modify this effect. When infected cells were exposed to ampicillin for 24 h after phagocytosis, the bacterial load was reduced by ~1.7 log compared with the original, post-phagocytosis inoculum. Glatiramer, IFN-γ, or the combination of glatiramer and IFN-γ did not significantly modify this effect of ampicillin. In the next series of experiments, we examined the activity of moxifloxacin (4 mg/L) using the 5 h model. We observed a decrease in the post-phagocytosis inoculum of 1.34 ± 0.03, 1.26 ± 0.16, 1.31 ± 0.07 and 1.32 ± 0.07 log10 units for cells treated with moxifloxacin alone, glatiramer and moxifloxacin, IFN-γ and moxifloxacin, and the combination of glatiramer, IFN-γ and moxifloxacin, respectively. In parallel experiments, we examined the influence of glatiramer on the accumulation of moxifloxacin and no effect was seen [apparent cellular to extracellular drug concentration ratios at 2 h of 9.6 ± 2.0 in controls versus 9.4 ± 1.1 and no effect was seen [apparent cellular to extracellular drug concentration ratios at 2 h of 9.6 ± 2.0 in controls versus 9.4 ± 1.1 in cells exposed to glatiramer (20 mg/L) during the uptake period; similar values were found for cells pre-exposed to glatiramer (20 mg/L) for 24 h]. Glatiramer did not influence the accumulation of three other quinolones (ciprofloxacin, levofloxacin and garenoxacin).

Our data, therefore, show that the production of TNF-α is not critical in IFN-γ-stimulated THP-1 cells for anti-Listeria activity. The model used has been validated to analyse the behaviour of intracellular L. monocytogenes with respect to the action of cytokines5,6 and to the influence of antibiotics. THP-1 cells display functional receptors for TNF-α and their presence in the cell line used here has been confirmed (J. Zanon, unpublished data). TNF-α may be more a potentializer of IFN-γ than a true effector for the control of L. monocytogenes growth in THP-1 cells. Because intracellular multiplication of L. monocytogenes is an important determinant in the persistence and the spread of the infection, our results suggest that glatiramer (i) may actually not increase this risk, and (ii) may not adversely affect ampicillin or quinolone-based antibiotic treatments should the necessity arise. This will need to be confirmed by in vivo studies.

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Serotonin syndrome due to co-administration of linezolid and venlafaxine

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Sir,

Linezolid is an oxazolidinone antibiotic with non-selective, reversible monoamine oxidase inhibitor (MAOI) action. It has been reported to interact with selective serotonin reuptake inhibitors (SSRIs) and other sympathomimetic drugs resulting in serotonin syndrome. We report the first published case of serotonin syndrome due to co-administration of linezolid and venlafaxine.

An 85-year-old man was referred for management of a chronically infected total hip joint prosthesis. He had a past history of Parkinson’s disease, ischaemic heart disease, atrial fibrillation, diabetes, previous stroke and a permanent pacemaker. The hip prosthesis was removed and surgical specimens were sent for pathological examination. The infection was caused by Listeria monocytogenes. The patient had not received any antibiotic treatment prior to surgery. Consequently, the isolated organism was highly susceptible to ampicillin, clindamycin, cephalosporins, penicillins, and second generation cephalosporins. Linezolid was chosen for treatment of the infection due to its broad spectrum activity against many Gram-positive and Gram-negative bacteria, including L. monocytogenes. Oral linezolid (600 mg) was administered twice daily for 6 weeks. Intravenous antibiotics were given for 6 weeks. Following closure of the wound, oral therapy was commenced with ciprofloxacin 750 mg twice daily, rifampicin 300 mg twice daily for a further 6 weeks. After the completion of oral therapy, serum levels of linezolid were checked and were found to be within the therapeutic range.

Serum levels of venlafaxine were also measured and were found to be within the therapeutic range. During the course of treatment, the patient did not complain of any gastrointestinal symptoms, and there were no other adverse effects reported. The patient tolerated oral linezolid well and a follow-up MRI scan showed resolution of the hip joint infection.

Serotonin syndrome due to linezolid and venlafaxine is a relatively rare complication, and may present with symptoms such as tremors, muscle rigidity, increased blood pressure, tachycardia, diaphoresis, hyperthermia, and altered mental status. Early recognition and treatment with serotonin antagonists such as cyproheptadine or triptans is important to prevent serious adverse outcomes. It is recommended that clinicians be aware of this potential interaction and consider alternatives to linezolid.

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289