7

T-cell costimulation by derivatives of benzoic acid through an antioxidant-sensitive mechanism. D. Kinchington, T. Ng, N. Mathews and W. O. Ayuko. Medical College of St. Bartholomew's Hospital, London, UK.

Two benzoic acid derivatives were found to costimulate the anti-CD3-induced proliferation of PBMC. Structure-activity relationships of a number of compounds are compared. T-cell costimulation correlated with an increase in tyrosyl phosphorylation of a number of cellular protein substrates. Both costimulation and tyrosyl phosphorylation were inhibited by the antioxidant butylated hydroxytoluene. Studies with blocking monoclonal antibodies against B7-1 or B7-2 on antigen presenting cells indicate that the mode of action requires the macromolecular interactions of T-cell costimulatory molecules such as CD28/CTLA-4. We speculate that these compounds generate reactive oxygen intermediates which in turn activate intracellular signalling events. This finding is discussed in relation to HIV infection.

8

Linking AZT to Ceramide Increases Drug Uptake, Retention and Antiviral Action While Decreasing Bone Marrow Toxicity
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A ceramide-conjugated, ester-linked prodrug (AE_6C) of zidovudine (AZT), has therapeutic attributes not found in free AZT. It is more readily taken up and retained by murine 3T3 cells, indicating that it could serve as an intracellular reservoir. Consistent with that possibility, AE_6C pre-treatment reduced infection in 3T3 cells exposed to murine virus (M-MuLV) 24 and 48 hrs after drug removal, but not in cells pre-treated with AZT. AE_6C blocked infection as well as AZT when human CD4+ Hela or 3T3 cells were continuously exposed to the drugs during infection with either HIV or M-MuLV. Marrow and peripheral blood progenitor cell [erythroid (BFU-E) and myeloid (CFU-GM)] toxicities were evaluated after 7 (murine) and 14 (human) days incubation in either drug. The ID_{50} results showed that AE_6C was much less toxic to progenitors than AZT. After oral administration AE_6C, relative to AZT, attained and retained much higher drug concentrations in mouse brain and thymus. Thus a reservoir is established that should provide prolonged maintenance of effective levels of esterase released active drug. The greater AE_6C uptake, retention and prolonged release in cells and target organs should allow lower and/or less frequent doses of the AE_6C prodrug to be administered, thereby decreasing nausea and vomiting. Furthermore, because it is less toxic to progenitor cells, AE_6C is less likely to induce hematopoietic suppression. Because AE_6C addresses the major limitations AZT it could prove to be a clinically useful drug.
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9

Randomized Phase II Studies of VIRACEPT™, an HIV Protease Inhibitor, Given as Monotherapy and in Combination with Stavudine (d4T)

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A total of 63 patients were randomized in two clinical trials evaluating the safety and efficacy of VIRACEPT given either as monotherapy (30 patients) or in combination with Stavudine versus Stavudine alone (33 patients). Patients in both trials had CD_4 cell counts $\geq$ 200 cells/mm^3, quantitative HIV RNA titers $\geq$ 15,000 copies/ml, and two weeks of antiretroviral therapy wash-out prior to enrollment. Patients on the monotherapy study were randomized to receive either 500 mg tid, 750 mg tid, or 1000 mg tid of VIRACEPT. Patients on the combination study were randomized to receive either d4T alone or d4T plus 500 mg, 750 mg, or 1000 mg tid of VIRACEPT. In the first 28 days, the median log decrease in viral RNA from baseline using the bDNA method with a 100 copies/ml minimum, ranged from 1.5 to 2.4 for patients on monotherapy while patients on combination therapy at all three doses had log decreases in excess of 2.0 logs. The median log decrease for patients on d4T alone was 0.9 logs. A large proportion of patients on combination therapy had viral load values that fell below the standard assay limit. Mean increases in absolute CD_4 cell counts from baseline ranged from 37 to 195 cells/mm^3 during the first 28 days. The most common adverse events observed in both studies were loose stool and mild to moderate diarrhea but the diarrhea was well-controlled by anti-diarrheal medication. Results suggest that VIRACEPT is both safe and effective when administered as monotherapy or in combination with d4T. Controlled Phase II/III trials are ongoing.