selective intracellular delivery of dexamethasone into activated endothelial cells using an E-selectin-directed immunoconjugate


Purpose of the Study. To determine if a dexamethasone-anti-E-selectin conjugate can be specifically delivered to activated endothelial cells and exert its biological effects in a cell-specific manner.

Study Population. Experiments were performed using human and mouse endothelial cell cultures.

Methods. Dexamethasone was conjugated to anti-human E-selectin and the resulting immunoconjugate was characterized in terms of its ability to bind to E-selectin and endothelial cells, to be internalized, and to exert biological activity on the endothelial cells. Binding of the dexamethasone-anti-E-selectin conjugate to endothelial cells was analyzed by immunohistochemistry and flow cytometry. Internalization and localization of the dexamethasone conjugate was studied with confocal laser scanning microscopy (CLSM) and immuno-transmission electron microscopy (Immuno-TEM). Activity of the drug was analyzed using a mouse endothelial cell line transfected with a glucocorticoid-responsive reporter gene construct and the drug’s effect on interleukin (IL)-8 expression was assayed using Northern blotting.

Results. The dexamethasone conjugate bound to activated, but not resting, human endothelial cells when the drug-treated cell cultures were stained with antibodies directed against anti-human E-selectin or dexamethasone. Flow cytometry confirmed these results. CLSM and Immuno-TEM demonstrated binding of the dexamethasone conjugate to activated, but not resting, cell surfaces 10 and 20 minutes after incubation and after 1 hour, cell surface staining began to decrease and intracellular staining increased. The dexamethasone conjugate was able to activate a glucocorticoid-responsive reporter gene to a similar degree to that seen with free dexamethasone. Northern blotting demonstrated downregulation of IL-8 expression in activated human endothelial cells treated with the dexamethasone conjugate. Again, this effect was similar to that seen with free dexamethasone.

Conclusions. Conjugated dexamethasone-anti-E-selectin is specifically targeted to active endothelial cells and internalized by these cells. The results also suggest that the conjugate drug retains the ability to regulate transcription.

Reviewers’ Comments. Although targeted drug therapy is not a novel concept, these experiments suggest that a nontoxic drug can be targeted to a specific cell type and retain biological activity. Unlike a drug targeted to kill cancer cells, this approach utilizes an antiinflammatory drug to downregulate proinflammatory cytokines and cell surface receptors with the idea that the toxicity associated with immune suppression would be minimized.

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