Enzymes as Drugs

Edited by

JOHN S. HOLCENBERG
Medical College of Wisconsin

JOSEPH ROBERTS
Sloan Kettering Institute for Cancer Research

A WILEY-INTERSCIENCE PUBLICATION

JOHN WILEY & SONS New York • Chichester • Brisbane • Toronto

Library of Congress Cataloging in Publication Data:

Main entry under title:

Enzymes as drugs.

"A Wiley-Interscience publication."
Includes indexes.
1. Enzymes—Therapeutic use. I. Holcenberg, John S., 1935- II. Roberts, Joseph, 1936- [DNLM: 1. Enzymes—Therapeutic use. QU135 E615]

RM666.E55E59 615'.35 80-20641
ISBN 0-471-05061-X

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

chapter thirteen

Soluble Polymer-Enzyme Adducts

ABRAHAM ABUCHOWSKI AND FRANK F. DAVIS
Rutgers University
New Brunswick, New Jersey

1 INTRODUCTION

It is not often realized that todays advances in the modification of enzymes for therapeutic use are an outgrowth of the pioneering work of Landsteiner (1) and van der Scheer (2–5), who studied the effect on antigenic specificity of short peptides coupled to proteins. This led to a great body of work by Sela and others [for review see (6)] on the elucidation of the immune response through the use of synthetic polypeptides, both free and coupled to proteins. During the course of this work, it was found that the coupling of certain polyamino acids to proteins could dramatically decrease immunogenicity and antigenicity.

This information was very important to investigators in the field of enzyme therapy as immune complications, invariably associated with the

injection of heterologous enzyme, have proved the greatest obstacles to effective enzyme therapy (7). To overcome the problem of immunogenicity and other shortcomings of enzymes such as limited circulating life in the blood, unsatisfactory pH optima, instability, and nonaccumulation at target sites, investigators have turned to chemical modification of enzymes. Today, many different polymer systems are used in an attempt to overcome these problems and improve therapeutic performance.

2 IMPROVED BIOLOGICAL PROPERTIES

The main thrust of this type of research is the production of soluble adducts with biological properties suitable for *in vivo* use. This is usually taken to mean that the conjugates have long circulating half-lives, do not produce or react with antibodies and are themselves nontoxic. Each polymer and its corresponding enzyme conjugate will be discussed individually. The effect of the various polymers on the circulating life of the enzymes is summarized in Table 1.

2.1 Poly(*N*-Vinylpyrrolidone)

A single enzyme, β-D-*N*-acetyl-hexosaminidase A, has been modified by the covalent attachment of poly (*N*-vinylpyrrolidone) (PVP), and tested *in vivo* (8). The native enzyme and the conjugated enzyme exhibited strikingly different clearance rates following intravenous injection into rabbits. Approximately 90% of the native human enzyme was cleared from the blood within 10 minutes after injection and the remainder was cleared within 1 hour. During the same time period (1 hour), 40% of the conjugated enzyme was still circulating in the blood. After 5 hours approximately 21% remained in the circulation. The clearance rate then steadily decreased so that 14% was detectable after 48 hours. Unfortunately, the polymer-bound enzyme reacted weakly with anti-hexosaminidase A indicating that this preparation may be immunogenic as well as antigenic. It is not known, however, if the clearance rates would be altered by the presence of antibodies that would invariably be produced upon repeated injections of the conjugate.

2.2 Glycosylation

Glycosylated enzymes also show enhanced plasma persistance as compared to the native enzymes. Sherwood et al. (9) prepared dextran conjugates of carboxypeptidase G from *Pseudomonas* sp. and arginase from *Fusarium solani,* which exhibited enhanced circulating lives. The half-life ($t_{1/2}$) of carboxypeptidase G in DBA/2 mice was 3.5 hours, while the $t_{1/2}$ for the corresponding dextran conjugate was 17 hours. Similarly, the $t_{1/2}$ for native arginase was 1.4 hours, as compared with 12 hours for its dextran conjugate.

Table 1 Effect of Various Polymers on the Circulating Life of Enzymes

Enzyme	*Polymer*	*Clearance from Blood (% remaining/time or half-life, t½)*	*Ref.*
β-D-*N*-Acetyl hexosaminidase A	Poly (*N*-vinylipyrrolidone)	40%/1 hr, 21%/5 hr, 14%/48hr	8
β-D-*N*-Acetyl hexosaminidase A	None	0%/1 hr	8
Carboxypeptidase G	Dextran	$t½$ = 17 hr	9
Carboxypeptidase G	None	$t½$ = 3.5 hr	9
Arginase	Dextran	$t½$ = 12 hr	9
Arginase	None	$t½$ = 1.4 hr	9
Arginase	Polyethylene glycol	15%/72 hr	24
Acinetobacter glutaminase-asparaginase	Glycopeptide	t½ = 16 hr[a], 8.2 hr[b], 19 hr[c]	10
Acinetobacter glutaminase asparaginase	None	t½ = 1.1 hr[a], 1 hr[b], 4.3 hr[c]	10
Lysozyme	Desialized fetuin	Targeted to liver within 10 min	12
Pseudomonas 7A glutaminase-asparaginase	Desialized orosomucoid	$t½$ = 7 min (targeted to liver)	13
Pseudomonas 7A glutaminase-asparaginase	None	$t½$ = 7 hr	13
E. coli asparaginase	Lactose (17 residues/mol)	Targeted to liver within 10 min	14
E. coli asparaginase	None	$t½$ = 4 h	14
Indolyl-3-alkane ∝-hydroxylase	Polymaleic acid	$t½$ = 2 hr[a]	37
Indolyl-3-alkane ∝-hydroxylase	None	$t½$ = 6 hr[a]	37
E. coli asparaginase	Poly-DL-alanine	$t½$ = 21 hr[a]	43
	None	$t½$ = 3 hr[a]	43
Erwinia carotovora asparaginase	Poly-DL-alanine	$t½$ = 36 hr	43
	None	$t½$ = 5 hr	43
Uricase	Albumin (homologous)	$t½$ = 26 hr	15
Uricase	None	$t½$ = 6 hr	15
Uricase	Polyethylene glycol	20%/48 hr	19
Catalase	Polyethylene glycol	10%/48 hr	17
Catalase	None	6%/6 hr	17
Adenosine deaminase	Polyethylene glycol	$t½$ = 30 hr	25
Adenosine deaminase	None	$t½$ < 0.5 hr	25
Superoxide dismutase	Polyethylene glycol	40%/72 hr	26
Superoxide dismutase	None	6%/4 hr	26

[a]Mice.
[b]Rats.
[c]Rabbits.

Glycosylation of *Acinetobacter glutaminasificans* glutaminase-asparaginase with glycopeptides from human fibrin and γ-globulin produced adducts with prolonged half-lives when tested in either mice, rats, or rabbits (10). Increasing the extent of modification produced conjugates with increased plasma half-lives and correspondingly decreased isoelectric points. A maximal increase in the half-life was obtained at a pI of 5, which was 15-fold that of the native enzyme (1.1 hours; pI = 8.2). Similarly in rats, the $t½$ of the native enzyme was 1 hour as compared with 8.2 hours for the conjugate (pI = 4.7). In rabbits the $t½$ of glycosylated enzyme was 19 hours compared with 4.3 hours for the native enzyme. Unfortunately, the glycosylated enzyme cross-reacted with rabbit antiserum raised against the native en-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.