THE CHEMISTRY OF THE COPAXONE DRUG

Ruth Arnon and Michael Sela

1. Introduction

Copaxone is the commercial name of an FDA approved drug for the treatment of multiple sclerosis (1). It consists of a synthetic polymer of amino acids, denoted Copolymer 1 (Cop 1), composed of L-alanine, L-lysine, L-glutamic acid and L-tyrosine. This is a novel and unique drug not only since it is the first drug based on an antigen-specific suppression of an autoimmune disease, but also because this is the first case in which a synthetic polymeric substance comprises the main ingredient of a drug (2).

We are familiar with the use of biopolymers, for packaging a drug, for slow and controlled release, and for many other uses, but never as the active ingredient against a disease. In the following, we intend to discuss the chemistry of this substance, its polymeric nature, and its development into a drug against the exacerbating-remitting type of multiple sclerosis [3].

Cop 1 is effective because it is related immunologically to the myelin basic protein (MBP), a substance in the myelin sheath of the brain that seems to be the main cause of the autoimmune phenomena in multiple sclerosis. In this particular case the agent is a synthetic product, a copolymer of amino acids. Hence, the drug is specific for this disease. But, it can serve as a prototype of specific drugs-vaccines against autoimmune diseases. Vaccines against infectious diseases are known to be highly specific. We have extended this concept to autoimmune diseases: whenever it is possible to identify the putative cause of the diseases, it should be possible to find a close molecular analog which will combat the disease. In this particular case the agent is a synthetic product, a copolymer of amino acids.

2. Chemistry of polyamino acids - Polymeric aspects

In a typical polycondensation or polyaddition, the polymerization occurs through the reaction of growing chains with growing chains, resulting in a very broad distribution of molecular weights. The situation is totally different in polymerization of ethylene oxide, where a growing chain can react only with a monomer, leading to a much narrower, Poissonian, distribution of molecular weights. Poly-a-amino acids are prepared usually from N-carboxy-a-amino acid anhydrides [4], and this polymerization occurs through the growth of chains by reaction only with monomers and not with each other [5]. The polymerization is essentially devoid of a termination reaction, but two types of termination reactions have been shown to occur: a general intermolecular termination reaction in which a growing peptide chain reacts with an N-carboxyamino acid anhydride to yield a ureido compound with a terminal carboxyl group [6]; and a specific intramolecular termination reaction, in the case of glutamic acid, leading to an unreactive terminal pyrrolidone ring [5]. As these occur very rarely, the experience is that polymers and copolymers of amino acids possess a narrow molecular weight distribution.

The length of the polymer will depend on the ratio between the monomer and the initiator which is usually a primary or secondary amine. Keeping this ratio constant leads to high reproduciblity of molecular size in different batches of the polymers. Furthermore, the rate of polymerization is an intrinsic property of the different N-carboxyanhydride derivatives, and hence, different samples of a polymer with the same composition of amino acids, although of random sequence in their nature, will be very similar in their physical and chemical properties.

3. Synthetic antigens - Use of amino acid copolymers in immunology

As early as 1960 we showed that a multichain amino acid copolymer, composed of a backbone of poly-L-lysine, with chains of poly-DL-alanine attached to the amino groups of polylysine, and elongated with short chains of L-tyrosine and L-glutamic acid - all prepared by polymeric techniques - is immunogenic, leading to the production of specific antibodies in a variety of

experimental animals [7]. This led to a series of studies towards the elucidation of the molecular basis of immune phenomena [8,9], as well as to the discovery of the determinant-specific genetic control of the immune response [10,11]. The role of size, composition, optical configuration of the component amino acids, electrical charge, etc. has been defined, and the special importance of the steric conformation has been stressed, leading to the definition of sequential and conformational antigenic determinants (epitopes) [12]. This led in turn to the development of synthetic vaccines against infectious diseases [13,14], and to efforts to deal with autoimmune diseases [15].

It all began as basic research into the mechanisms involved in the induction and suppression of experimental allergic encephalomyelitis (EAE), which is the primary animal model for MS. EAE is an acute neurological autoimmune disease, induced by the injection in complete Freund's adjuvant (CFA) of brain - or spinal cord-derived substances which constitute the encephlitogenic antigens. These include several proteins such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendritic glycoprotein (MOG) and others. The disease is mediated by CD4+ autoreactive T cells, which recognize the encephalitogenic antigen(s) in association with major histocompatibility complex (MHC) class II molecules. These autoreactive cells migrate into the central nervous system (CNS) and mediate the pathogenic process. When we started our research in 1967, the only encephalitogenic material identified in the CNS was the MBP, and the only information available about it was its overall amino acid composition. It is of interest that MBP, under different conditions (e.g. in the absence of CFA) was capable of suppressing EAE rather than inducing the disease [16].

Our approach to the study of EAE and its suppression was the synthetic one, using copolymers of amino acids whose composition resembled to a certain extent that of natural MBP, in order to simulate its ability to induce or suppress EAE. None of the copolymers proved to be encephalitogenic even after conjugation with brain lipids, but some, particularly Cop-1, showed high efficacy in suppressing EAE [17].

Cop-1 is a synthetic amino acid copolymer composed of L-alanine, L-lysine, L-glutamic acid and L-tyrosine in a residue molar ratio of 4.2:3.4:1.4:1.0. It was shown to suppress EAE induced by MBP in a variety of animals, including guinea pigs, rabbits, mice and two species of monkeys - rhesus monkeys and baboons [18]. The results clearly indicated that there was a remarkable degree of suppression of EAE by Cop-1 in all species studied, although different encephalitogenic determinants of MBP were involved in disease induction in the different species. Indeed, our studies have shown that the suppressive effect of Cop-1 in EAE is a general phenomenon and is not restricted to a particular species, disease type or the encephalitogen used for EAE induction [3,15]. Furthermore, Cop 1 was effective in suppressing also the chronic-relapsing EAE, a disease which shows a closer resemblance to MS, that can be induced either in guinea pigs by MBP, or in mice by PLP. In both species Cop1 reduced both the incidence and the severity of the relapses[1].

4. Immunological cross-reactivity between MBP and Cop 1

Since EAE is autoimmune in nature, and its pathogenicity involves T cells sensitized to MBP, the specific inhibition by Cop 1 may be explicable in terms of an immunological cross-reaction between Cop 1 and MBP. Studies have been performed to test this hypothesis at both the cellular and humoral levels of the immune response.

Using monoclonal antibodies raised against MBP, we could demonstrate clearly that several monoclonal anti-MBP antibodies reacted with Cop 1 and vice versa [19]. At the cellular level, a marked cross-reaction was observed both in vivo in the delayed hypersensitivity skin test and in vitro by measuring lymphocyte transformation [20]. Of particular interest is the very good correlation between the extent of immunological

cross-reactivity and suppressive effect on EAE of various materials. Thus, D-Cop 1, a polymer resembling Cop 1 in all parameters except that it is composed of D-amino acids rather than L-amino acids, does not cross-react with MBP and has no suppressing activity whatsoever [21]. It is therefore plausible that the immune response/intervention by Cop 1 is the basis for its suppressive effect on EAE.

5. Clinical studies with Cop 1 in MS

In view of the putative resemblance between EAE and MS and the assumption that MBP may be involved in the pathogenesis of MS, preliminary clinical trials using Cop-1 were conducted on MS patients. These were begun after toxicity studies in experimental animals showed that Cop 1 was nontoxic after both acute and subchronic administration to mice, rats, rabbits and beagle dogs [1], and that there was no significant uptake by any of the animal organs.

Our clinical trials have included two preliminary open trials and two double blind phase II trials, one involving exacerbating- remitting (ER) patients [22] and another one on chronic progressive (CP) patients [23]. The results of the phase II trial on ER patients demonstrated a remarkable decrease in the number of relapses during the two years of the trial, as well as in the rate of progression of the disease in Cop 1-treated patients compared with the placebo control. After a successful pivotal multicenter phase III clinical trial [24,25], which was conducted in 11 medical centers in the United States and involved 251 patients, the US Food and Drug Administration decided to approve Cop 1 (Copaxone) as a drug for MS. Copaxone has since been approved in Israel, Canada, Argentine, and several countries in Europe. It is being used by thousands of patients with highly successful results.

6. Mechanism of activity of Cop 1

a. Induction of antigen-specific suppressor cells

We have demonstrated that mice pretreated with Cop 1 in incomplete adjuvant became resistant to further EAE induction. This state of unresponsiveness could be adoptively transferred to normal recipients by spleen cells from Cop 1 treated donors, and the cells responsible for the suppressive activity were identified as T lymphocytes [26]. Furthermore, we have demonstrated the generation of suppressor T-cell hybridomas and lines from spleen cells of mice rendered unresponsive to EAE by Cop 1. Both cell types produce in vitro inhibition of MBP specific effector lines and in vivo inhibition of clinical EAE [27]. Recent results revealed that these T suppressor cells secrete Th2 cytokines after exposure to either Cop 1 or MBP [28]. These cytokines may mediate the therapeutic effect of Cop 1 in disease induced not only with MBP but also with other encephalitogens by the mechanism of bystander suppression . The induction of such specific suppressor cells by Cop 1 is therefore one mechanism by which its therapeutic effect is delivered.

b. Inhibition of antigen-specific T-cell responses

It has been demonstrated that Cop 1 can competitively inhibit the response to MBP of diverse MBP-specific murine and human T cell lines and clones, which have different MHC restrictions and respond to different epitopes of MBP, while having no effect on PPD-specific T cell clones [29, 30]. These studies suggest that the site of competition between MBP and Cop 1 is most likely to be the MHC-binding site. In order to demonstrate the direct binding of Cop 1 to MHC molecules on antigen-presenting cells (APC) and to study the specificity, affinity and time course of these interactions, we used a biotinylated derivative of Cop 1 and a fluorimetric method to follow the binding [31]. Cop 1 exhibited a very high and indiscriminate binding to different types of APC of various H-2 and HLA haplotypes. The specificity of the binding was confirmed by its inhibition with either the relevant anti-MHC class II antibodies or unlabelled analogs. The binding of Cop 1 to MHC class II molecules was more rapid and efficient than that of MBP. Moreover, Cop 1 inhibited the binding of MHC and other

encephalitogens to the MHC molecules and could even efficiently displace MBP from the MHC class II binding groove. This inhibition of binding to the APC is, however, of rather broad specificity, and not limited to the MBP.

Specificity for the MBP was recently displayed, however, by the competition that occurs at the level of the T-cell receptor between the complex of MBP-derived peptides with class II MHC antigen, and the complex of Cop 1 with class II antigens. This was corroborated by the antagonistic effect of Cop1, where it inhibited the presentation of MBP or its encephalitogenic epitope to the T-cell receptor in a strictly antigen-specific manner (32).

7. Proposed mode of action of Cop 1 in EAE and MS

Cop 1 affects EAE, and therefore by extrapolation MS, at various levels of the immune response involved, which differ in their degree of specificity. Binding of Cop 1 to the MHC class II molecules, which is the least specific step, is a prerequisite for its effect by any mechanism. Following this interaction, two mechanisms were clearly shown to be effective: 1) Cop 1 binding to the relevant MHC leads to the activation of T suppressor cells, which are activated by suppressive determinants shared between MBP and Cop 1. This mechanism is a specific one and results from the cross-reactivity between Cop 1 and MBP. 2) Cop 1 can compete for binding to MHC class II molecules with several myelin-associated antigens, resulting in inhibition of antigen-specific T cell effector functions (i.e. proliferation, interleukin secretion and cytotoxicity).

This mechanism may be less specific, as MHC blockade may lead to interference with other immune responses. However, this does not seem to be the case, as Cop-1 did not inhibit responses to ovalbumin or lysozyme. Furthermore, D-Cop 1, which bound to MHC class II molecules as efficiently as Cop 1 and competed with MBP for binding, did not inhibit MBP-specific T cell lines, and did not inhibit EAE when coinjected with the encephalitogenic emulsion. These findings may suggest that the nonspecific MHC blocking is a necessary but not sufficient step, which requires an additional step involving antigen-specific mechanisms such as induction of crossreactive T cell tolerance, or T cell receptor antagonism. Regardless of the mechanism involved, the ability of Cop 1 to suppress disease which is induced not only by MBP but by other myelin-associated proteins as well, is very important, since these antigens might be potential autoantigens in MS.

8. Conclusion

Copaxone is the only non-interferon novel drug for the treatment of multiple sclerosis. It is a synthetic polymer of amino acids, and has a specific effect on the autoimmune process involved in EAE and in MS. The results of clinical trials with Cop 1 indicate that it is a promising low-risk MS-specific drug for the treatment of relapsing MS, capable of slowing progression of disability and reducing the relapse rate. As an antigen-specific intervention, Cop 1 has the advantage of reduced probability of long-term damage to the immune system.

As for the chemistry angle of this drug, it is of interest that Copaxone is the first drug of a polymeric nature approved for treatment of a disease. This is a macromolecular preparation obtained by polymeric techniques, in which probably no two molecules are completely identical. The microheterogeneity of Cop 1 can actually be part of its success, as it may contain sufficient different amino acid sequences that could successfully compete with the encephalitogenic antigens for class II MHC antigens of many different genetic backgrounds. This, as well as its high safety profile, make Copaxone a first choice drug that will hopefully alleviate the suffering of many MS patients.

References

[1] R. Arnon, Immun. Lett. 1996, 50, 1

[2] M. Sela, Acta Polym .1998, 49, 523

[3] D. Teitelbaum, R. Arnon, M. Sela, CMLS, Cell Mol. Life Sci. 1997, 53, 24.

[4] E. Katchalski, M. Sela, Adv. Protein Chem. 1958, 13, 243.

[5] E. Katchalski, M. Sela, H.I. Silman, A. Berger, Polyamino Acids as Protein Models , in The Proteins (Ed. H. Neurath), Academic Press, New York 1964, pp. 405-602.

[6] M. Sela, A. Berger, J. Am. Chem. Soc. 1955, 77, 1893.

[7] M. Sela, R. Arnon, Biochim. Biophys. Acta 1960, 40, 382.

[8] M. Sela, Acta Polym. 1998, 49, 523-525.

[9] M. Sela, Science 1969, 166, 1365.

[10] H.O. McDevitt, M. Sela, J. Exp. Med. 1965, 122, 517.

[11] H.O. McDevitt, M. Sela, J. Exp. Med. 1967, 126, 969.

[12] M. Sela, B. Schechter, I. Schechter, F. Borek, Cold Spring Harbor Symp. Quant. Biol. 1967, 32, 537.

[13] M. Sela, Bull. Inst. Pasteur 1974, 72, 73.

[14] M. Sela, R. Arnon, Vaccine 1992, 10, 991.

[15] D. Teitelbaum, M. Sela, R. Arnon, Israel J. Med. Sci. 1997, 33, 280.

[16] C.M. Shaw, E.C. Alvord, W.J. Fahlberg and M.W. Kies, J. Immunol. 1962, 89, 54.

[17] D. Teitelbaum, A. Meshorer, T. Hirshfeld, R. Arnon, M. Sela, Eur. J. Immunol. 1971, 1, 242.

[18] M. Sela, R. Arnon, D. Teitelbaum, Bull. Inst. Pasteur 1990, 88 303.

[19] D. Teitelbaum, R. Aharoni, M. Sela, R. Arnon, Proc. Natl. Acad. Sci. USA 1991, 88, 9528.

[20] C. Webb, D. Teitelbaum, R. Arnon, M. Sela, Eur. J. Immunol. 1973, 3, 273.

[21] C. Webb, D. Teitelbaum, A. Herz, R. Arnon, M. Sela, Immunochemistry 1976, 13, 333.

[22] M.B. Bornstein, A. Miller, S. Slagle, M. Weitzman, H. Crystal, E. Drexler, M. Keilson, A. Merriam, S. Wassertheil-Smoller, V. Spada, W. Weiss R. Arnon, I. Jacobsohn, D. Teitelbaum, M. Sela, New England J. Med. 1987, 317, 408.

[23] M.B. Bornstein, A Miller, S. Slagle, M. Weitzman, E. Drexler, M. Keilson, V. Spada, W. Weiss, S. Appel, L. Roolak, Y. Harati, S. Brown, R. Arnon, I. Jacohsohn, D. Teitelbaum, M. Sela, Neurology 1991, 41, 533.

[24] K.P. Johnson, B.R. Brooks, J.A. Cohen, C.C. Ford, J. Goldstein, R.P. Lisak, L.W. Myers, H.S. Panitsch, J.W. Rose, R.B. Schiffer, T. Vollmer, L.P. Weiner, J.S. Wolinsky and The Copolymer 1 Multiple Sclerosis Study Group, Neurology 1995, 45, 1268.

[25] K.P. Johnson, J. Neurol. 1996, 243 S32.

[26] Z. Lando, D. Teitelbaum, R. Arnon, J. Immunol. 1979, 132, 2156.

[27] R. Aharoni, D. Teitelbaum, R. Arnon, Eur. J Immunol. 1993, 23, 17.

[28] R. Aharoni, D. Teitelbaum, M. Sela, R. Arnon, Proc. Natl. Acad. Sci. USA 1997, 94, 10821.

[29] D. Teitelbaum, R. Aharoni, R. Arnon, M.˚Sela, Proc. Natl. Acad. Sci. USA 1988, 85, 9724.

[30] D. Teitelbaum, R. Milo, R. Arnon, M. Sela, Proc. Natl. Acad. Sci. USA 1992, 89, 137.

[31] M. Fridkis-Hareli, D. Teitelbaum, E. Gurevich, I. Pecht, C. Brautbar, O.J. Kwon, T. Brenner, R. Arnon, M. Sela, Proc. Natl. Acad. Sci. USA 1994, 91, 4872.

[32] R. Aharoni, D. Teitelbaum, R. Arnon and M. Sela. Proc. Natl. Acad. Sci (in press)

Ruth Arnon

1955 M.Sc. Chemistry, from the Hebrew University, Jerusalem; 1960 - Ph.D. Hebrew University, (work carried out at the Weizmann Institute). Since 1960 Department of Immunology, The Weizmann Institute of Science, 1985-1993: Dean of the Faculty of Biology; 1988-1993: Vice-President; 1995-1997: Vice-President for International Scientific Relations.

Positions in Scientific Bodies: WHO Task Force on Immunological Methods for Fertility Control (Steering Committee)1972-1977; President of European Federation of Immunological Societies (IUIS) 1989-1993, Elected Member of EMBO. Chairman of the Science Division of the Israel Academy of Science and Humanities (1995-present). Prizes include Robert Koch prize in Medical Sciences (Germany); Jimenez Diaz Award (Spain); Chevalier of l Ordre de la Legion d Honneur , (France); Hadassa Women of Distinction Award; Wolf Prize (Israel); Rothschild Prize (Israel).

Published more than 400 articles, chapters and books in the field of Immunology and Biochemistry.

Michael Sela

1946 - M.Sc., Chemistry, Hebrew University, Jerusalem; 1954 - Ph.D., Hebrew University, (work carried out at the Weizmann Institute). Since 1960 at the Weizmann Institute of Science in Rehovot. 1970-1973: Dean, Faculty of Biology; 1970-1971: Vice-President; 1975-1985: President; 1985-2001: Deputy Chairman of the Board of Governors. From 1967 - Foreign Member of the Max-Planck Institute for Immunobiology; 1975-1979 - Chairman, EMBO Council; 1977-1980 - President, International Union of Immunological Societies; 1979-1982 - Member, WHO Global Advisory Committee on Medical Research; from 1989 - President, Scientific Council Institut Pasteur-Weizmann Institute.

Prizes include Israel Prize, Rothschild Prize, Otto Warburg Medal, Emil von Behring Prize, Gairdner Award (Canada), Prize of the Institut de la Vie (France),
Jaubert Prize (Switzerland), Interbrew-Baillet Latour Health Prize (Belgium), The Wolf Prize.

Academy memberships include Israel Academy of Sciences and Humanities, U.S. National Academy of Sciences, Pontifical Academy of Sciences, Deutsche Akademie der Naturforscher Leopoldina, American Academy of Arts and Sciences, Russian Academy of Sciences, Romanian Academy, French Academy of Science, American Philosophical Society, Academia Nazionale dei Lincei (Italian). Honorary Doctorates from the Universities of Bordeaux, Strasbourg, Mexico, Tufts, Colby College (Maine), and the Hebrew University, Jerusalem. Cross of the order of Merit of the Federal Republic of Germany; Officer of l Ordre de la Legion d Honneur , France; UNESCO Albert Einstein Golden Medal; Harnack Medal of the Max Planck Society (Germany).

Published more than 700 articles, chapters, books, in the fields of immunology, biochemistry and molecular biology.