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Immunology Letters 50 (1996) 1–15

Immunology letters

Mini-review

The development of Cop 1 (Copaxone®), an innovative drug for the treatment of multiple sclerosis: personal reflections

Ruth Arnon

Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

Received 15 December 1995; accepted 25 January 1996

Keywords: Copaxone®; Multiple sclerosis; Immunospecific drug

1. Introduction

The 14th of June 1995 was a milestone for me – as on this day the file on Copolymer 1 (Cop 1) was submitted, under the name COPAXONE®, by the TEVA Pharmaceutical Company to the FDA for approval as a New Drug Application for the treatment of Multiple Sclerosis. For Prof. Michael Sela and myself, together with our colleague Dr. Dvora Teitelbaum, this was a high point after over 27 years of persistent research effort, perseverance and tenacity of purpose. The purpose of this paper is to describe the history of the development of this drug, interspersed with a review of the scientific findings along the way, all from a personal perspective, when I am "a quarter of a century wiser". Since multiple sclerosis is an immunological disease and Cop 1 is an immunospecific drug, my perspective is that of an immunologist, and it delineates the course of events in a more or less chronological order. Beyond the story as such, the paper shows that studies with Cop 1 advanced side by side with the progress of immunology as a discipline and hence, that the increasing sophistication of the research tools and methods that emerged from time to time permitted a more in-depth study of the immune processes involved in activity of this innovative drug. However, the therapeutic potential of this material was evident to us already 25 years ago.

It all started in the early 1960s. I was then a budding immunologist, or, to put it more precisely, a young chemist 'corrupted' into immunology. Immunology in those days was a completely different science from what it is today, much simpler, with more 'yes' or 'no' answers to issues that even now are not yet completely understood. Antibody structure and function were just being revealed and elaborate studies on the structural aspects of antigens were conducted, under the coined term 'immunochemistry', to arrive at better understanding of their interaction with the antibodies. In parallel, information on the cellular compartment of the immune system was just starting to accumulate, with the fundamental discoveries of Burnet and Jerne.

In our laboratory we were deeply involved in studies on the structural basis of the antigenicity of proteins, utilizing synthetic antigens comprising polymers and copolymers of amino acids [1]. Research with these polymers had been pioneered by Prof. Ephraim Katchalski, in whose laboratory both Michael Sela and myself received our training. Employing these synthetic protein-like molecules we (Michael Sela and I) could induce immune responses of almost any desired specificity, including that of non-protein moieties. Of specific interest was the immune response to lipid components which, due to solubility problems, was not easy to either elicit or investigate. However, conjugates in which synthetic lipid compounds were attached onto synthetic copolymers of amino acids elicited specific response to lipids such as cytolipin H, which is a tumor-associated glycolipid [2], or sphingomyelin [3]. Furthermore, we demonstrated that both the sugar and lipid components of such molecules contributed to their immunological specificity. The resultant anti-lipid antibodies were capable of detecting the corresponding lipids both in water-soluble systems and in their physiological milieu. This was fascinating, since it gave us a glimpse into some disorders involving lipid-containing tissue and consequently led to our interest in demyelinating diseases, namely disorders in which the myelin sheath, which constitutes the lipid-rich coating of all axons, is damaged, resulting in various neurological dysfunctions.

PII S0165-2478(96)02506-0

Multiple sclerosis (MS) is the most frequent demyelinating disease. Not much was known at the time, or even to date, about its aetiology or mechanism of triggering. It is a chronic inflammatory disease of the central nervous system (CNS), in which infiltrating lymphocytes lead to damage of the myelin sheath by means of immune processes. Hence, it is considered an autoimmune disease. In studies performed at a later stage, while I was spending my sabbatical leave with Dr. John Fahey at UCLA, in collaboration with Drs. George Ellison, Lawrence Myers and W. Tourtellotte, we showed that anti-ganglioside serological activity might also be involved in this disease, since antibodies against several brain gangliosides were detected in sera of MS patients, but not in normal individuals. (This activity was demonstrated by the capacity of the sera to cause complement-dependent lysis of liposomes containing the respective ganglioside in their lipid bilayer.) Moreover, an apparent correlation was indicated between the severity of disease and the extent of liposome lysis [4].

Since MS occurs only in the human species, it was necessary to develop animal models of the disease for the purpose of research. Already in 1937 Rivers [5] had observed that a single inoculation of laboratory animals with brain or spinal cord tissue in Complete Freund's adjuvant (CFA) led to an acute neurological autoimmune disease resembling MS, which was designated Experimental Allergic Encephalomyelitis (EAE). Cell-mediated immune responses were shown to be involved in the pathogenicity of EAE, since the disease could be transferred by a single inoculation of sensitized lymphoid cells [6,7]. In the early 1960s one of the myelin components, myelin basic protein (MBP), was identified as an encephalitogenic agent, since when injected in its purified form it induced EAE in guinea pigs [8]. Moreover, the disease proved to be the result of cell-mediated response to the MBP, and its specificity was emphasized by the ability to prevent or suppress EAE by MBP or its modified derivatives [9–11].

Our previous successes in the development of synthetic antigens and their valuable contribution to the understanding of several immunological phenomena, prompted us to take a similar approach with regard to EAE. We actually intended to synthesize an encephalitogen, and anticipated that if the encephalitogenic activity of MBP could indeed be mimicked by a synthetic molecule – it might provide us with a useful tool for investigating the mechanism of EAE. In parentheses I would like to acknowledge the support that we received from two people already at this stage of the research. The first was Prof. Otto Westphal who was excited by our original approach and even helped us obtain the first grant for these studies, from a small private foundation – the Freudenberg Foundation. The second person was the late Prof. Elisabeth Roboz-Einstein. She was deeply involved in research on EAE from the viewpoint of a neurochemist. She was so taken with our research approach that years later she bequeathed to us her entire scientific and reprint collection.

In the late 1960s methods for the synthesis of sequenced polypeptides were not yet available. However, as graduates of the laboratory of Prof. Ephraim Katchalski, we were experts in the synthesis of random copolymers of amino acids, and hence we prepared a series of such copolymers, with compositions approaching that of MBP, all with a highly basic nature due to their high lysine contents. However, efforts over the course of more than a year led to negative results – none of these synthetic copolymers possessed any encephalitogenic activity [12]. Furthermore, even the conjugation of sphingolipid moiety – which could potentially enhance the anti-sphingomyelin response and consequently the demyelination process – did not endow these polymers with any encephalitogenic activity whatsoever. Disappointment. Was our hypothesis wrong? Did the synthetic approach fail us in this case? Should we give up?

Concomitantly, we were expanding our studies on EAE and its characterization: in collaboration with the late Dr. H. Hirshfeld, we developed a simplified procedure for the purification of MBP from myelin [13], based on the use of a new ion-exchange resin, Sulphoethyl-Sephadex, and it enabled us to prepare large quantities of the purified protein for more extensive investigation of EAE. The purified MBP was highly potent in the induction of EAE, as contrasted with the complete lack of such activity in any of the synthetic copolymers. Did these polymers possess any other form of biological activity?

It is a common practice in immunology, once an interaction between antigen and antibody is established, to elucidate its specificity by competition, or inhibition studies. Only substances of a similar specificity will evince inhibitory properties. This had been the procedure by which we had identified the specificity of the synthetic antigens, and the contribution of various parts of the macromolecules as 'antigenic determinants', or epitopes, to their overall antigenic activity. This technique had been employed to characterize the specificity of blood group antigens and many other systems. In most cases such inhibition studies are performed with relatively short molecules – sugars, or peptides. Would it apply to the macromolecular copolymers? Furthermore, the issue we were addressing was not a relatively simple antigen-antibody interaction, but rather a more complex biological process, namely the in vivo induction of encephalitogenic activity. Nevertheless, the earlier findings of Elisabeth Roboz-Einstein and Marian Kees that MBP, as well as some other brain basic proteins, can inhibit EAE [9,10] indicated that this could be a plausible approach.

2. Suppression of EAE, the animal model for multiple sclerosis

The results of the inhibition experiments were overwhelming – not one, but several of the synthetic copolymers showed high efficacy in suppressing EAE! The most active among the series was Copolymer 1 (Cop 1), composed of L-alanine, L-lysine, L-glutamic acid and L-tyrosine in a residue molar ratio of 6.0:4.7:1.9:1.0, and hence most of our subsequent research was conducted only with this substance. It had a marked suppressive effect on EAE when injected to guinea pigs in incomplete Freund's adjuvant or even in aqueous saline solution, after an initial challenge with a disease-inducing dose of MBP [12]. It reduced the incidence of EAE from about 75% in the control group to only 20% in the treated group. Was the effect real? Could it be an artefact? A second batch of Cop 1 was immediately synthesized, very similar in its composition and molecular size to the original one, and was found to be identical in its suppressive effect on EAE, indicating that the observed suppressive effect is a real one. This seemed interesting indeed. Not only did we have in hand a tool for studying the mechanism of EAE and the immune processes involved in it, but already at that early stage we realized that this might lead eventually to a therapeutic agent. We submitted patent applications in Israel and abroad and were granted them in various countries during 1972 to 1974.

It was now necessary to learn more about the effect of Cop 1 and how it exerts it. Cynthia Webb, a Ph.D. student who joined our team, showed that the suppressive activity of Cop 1 could be explained by its immunological cross-reactivity with the MBP. Cross-reaction was clearly manifested on the cellular level, in both in vitro (lymphocyte transformation) and in vivo (delayed hypersensitivity) assays [14]. In studies involving the series of copolymers, there was a good correlation between the level of such cross-reactivity and the capacity to suppress EAE. As for the humoral antibody response, we could not detect cross-reactivity between MBP and Cop 1 using the methods available at the time, namely the precipitin test or the Farr test (which is the 'ancestor' of the radioimmunoassay). However, the more sensitive passive cutaneous anaphylaxis test showed that guinea pig anti-Cop 1 sera did cross-react to a certain extent with MBP, but not vice versa [14]. All these tests were performed, of course, with polyclonal antibodies, the only methodology available at the time. Later studies, using monoclonal antibodies, showed a highly significant cross-reactivity between MBP and Cop 1 – about a third of the hybridomas raised against rat MBP cross-reacted with Cop 1 to the same level of reactivity as with the homologous antigen, and a proportion of the anti-Cop 1 antibodies reacted with MBP [15]. Moreover, some of the monoclonal antibodies raised against either MBP or Cop 1 reacted in a heteroclitic manner and favoured the cross-reacting antigen over the immunogen. It is of interest that the cross-reactivity was observed only with the monoclonal antibodies – antisera of the immunized mice from which these antibodies originated showed no cross-reactivity. Thus, the use of monoclonal antibodies uncovered specificities that were not evident in the polyclonal response and revealed the pronounced cross-reactivity between Cop 1 and MBP, on the B-cell level as well as the previously observed T-cell level. This provided a plausible basis for the suppressive effect of Cop 1 on MBP-induced EAE.

3. Specificity of EAE suppression by Cop 1

The results described above, for the suppressive effect of Cop 1 on EAE, were demonstrated for the disease induced in guinea pigs by the inoculation of bovine MBP. It was known, however, that the induction of EAE is species dependent, in respect to both the species from which the MBP is derived and the species in which the disease is induced. The specificity is reflected not so much in the actual susceptibility to the disease, which is relevant to most species, but in the particular region in the MBP molecule which is responsible for the encephalitogenic activity. The encephalitogenic determinants for guinea pigs, mice, rats and primates are all different [16].

It was therefore interesting to observe that Cop 1 was effective in suppression of EAE in guinea pigs also when it had been induced by MBP of human origin [17]. These results are of particular interest, since when EAE was induced in guinea pigs by the human encephalitogen, the histological changes observed included demyelinations and fibrosis in the guinea pig brain, thus resembling the symptoms of MS more than when the disease is induced by either bovine or rodent MBP. In later experiments we have induced EAE in guinea pigs with MBP of other species and demonstrated that the suppressing effect of Cop 1 was firm and abiding.

Is the effect of Cop 1 then a specific one, or is it due to some non-specific immunosuppressive properties? This is an important question, since it reflects on the mechanism of its activity in the suppression of EAE. Evidence for its specificity were provided by two experiments: The first demonstrated that Cop 1 lacked any suppressive effect on the immune response in several systems – a particulate antigen such as bacteriophage T4, soluble proteins such as BSA and RNase, and carrier-hapten systems such as DNP-BGG or polyalanyl HSA. In all cases the level of the immune response was not affected by the presence of Cop 1. Neither was skin graft rejection in rats affected by the

injection of Cop 1 [17,18]. The second evidence is derived from an experiment using a copolymer identical to Cop 1, but composed of D-amino acids instead of the natural L-form, and denoted D-Cop 1. Both composition and size of Cop 1 and D-Cop 1 were identical. D-Cop 1 was devoid of any suppressive effect on EAE, nor was it cross-reactive with MBP at either the humoral or the cellular level (unpublished results).

Equally, or even more important is the question whether Cop 1 would be effective in suppressing EAE in species other than guinea pigs. Indeed, in a detailed study we showed that Cop 1 demonstrated effective suppression of EAE in rabbits [17], in mice [19] and in two species of primates – rhesus monkeys [20] and baboons [21] (Fig. 1). It is thus apparent that Cop 1 does not manifest species specificity, either for the source of encephalitogen or for the animal tested.

The results in primates were very significant, since, as shown in Fig. 1, these animals are highly susceptible to EAE and *all* those sensitized with MBP succumbed to the disease. The experiment with Rhesus monkeys included 10 animals, of which five served as controls and the other five were treated with Cop 1. The treatment was given daily starting immediately after the onset of the first stages of paralysis. All five monkeys in the control group deteriorated very rapidly and died within 4–11 days after the onset of symptoms. In contrast, four out of the five Cop 1-treated monkeys showed improvement after 4–5 days of treatment and finally recovered completely from the paralysis. The fifth Cop 1-treated monkey continued to deteriorate after an initial improvement, suffered a relapse 35 days later, and finally died of EAE.

A similar level of efficacy of Cop 1 was observed in the experiments with the baboons which included a total of 15 animals. The six baboons in the control group developed EAE with progressive paralysis and died within 4–11 days after initial symptoms were noted. In nine baboons daily treatments were started immediately after the first paralysis symptoms were observed. Although initially they continued to deteriorate and most of them reached the state of full paralysis, they eventually began to recuperate, and seven out of the nine showed full recovery. I remember these experiments very vividly since for us they constituted a considerable effort: baboons are large animals, weighting about 30 kg each and require special cages equipped with appropriate fixtures for experimental manipulation of the animals. Since we had only five such cages, the above experiment was actually performed in three stages – each one including five baboons, of which two served as controls and three were treated with Cop 1. After the second stage we knew what to expect and hence during the third one we filmed one of the Cop 1-treated baboons through all the phases of the trial – before the EAE-inducing challenge with the MBP, during the paralysis period (2–3 days) and up to its complete recovery when he jumped vivaciously in his cage. The film was quite effective and helped me later in demonstrating the effect of Cop 1 and its therapeutic potential. Furthermore, it helped me in raising interest among neurologists and motivating them to conduct a clinical trial with Cop 1.

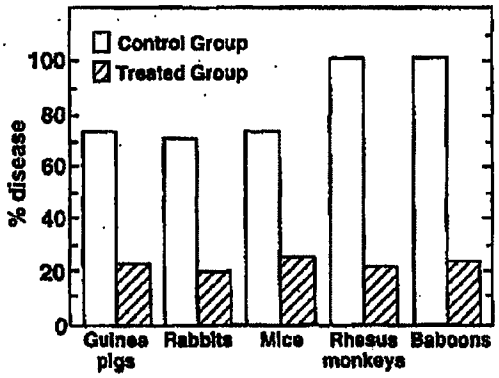

Fig. 1. Suppression of EAE by Cop 1 in various species. Incidence of disease in Cop 1-treated animals as compared to untreated controls.

The results achieved in the sub-human primates are highly significant for two reasons: (1) since they are the species closest to humans, the effect of Cop 1 on primates is more relevant to multiple sclerosis; (2) in all primates the treatment with Cop 1 was begun only *after* symptoms of disease were evident and hence its effectivity was a positive indication, since any treatment suggested for MS would be feasible only after disease had been diagnosed. Furthermore, one of the Cop-1 treated rhesus monkeys which had fully recuperated after being paralyzed was examined and showed no histological damage in its brain. A baboon similarly tested showed very minimal histological lesions. In contrast, drastic damage and multiple lesions were noticed in the brains of all rhesus monkeys and baboons of the control groups, as well as those treated with Cop 1 who died of EAE. This indicates that brain lesions which are not long-lasting could be amenable to remyelination.

Another aspect of the experimental evidence which lends support to the potential of Cop 1 in relation to MS, is its beneficial effect in the chronic-relapsing form of EAE (CR-EAE). This type of EAE [22] is induced in guinea pigs by sensitizing juvenile animals with the encephalitogenic challenge, and is characterized by initial onset followed by a reversal stage and subsequent relapses. Due to its relapsing nature CR-EAE is considered a more faithful experimental model for MS. In collaboration with Dr. Wisniewski and his colleagues we studied the effect of Cop 1 on this type of disease. We showed that pretreatment had a marked effect both in delaying the initial onset and in preventing the

appearance of relapses. Therapeutic treatment, which was given after the onset of initial symptoms, reduced both the occurrence and the severity of relapses [23].

The next logical step was to investigate whether Cop 1 was of any benefit to MS patients. We therefore conducted some basic toxicological studies which are prerequisite for any clinical trial. A full 'toxicological package' is an extremely costly affair (millions of dollars), which we couldn't afford. We did, however, carry out LD_{50} determinations and experiments of acute and subacute toxicity in mice and rats as well as in seven beagle dogs, which were performed at the Weizmann Institute, with the expertise of Dr. Asher Meshorer (unpublished results).

The LD_{50} tests actually failed, since no death occurred at doses up to 2000 mg/kg (the highest dose that could be administered). The acute and subacute toxicity tests showed that Cop 1 can be administered by either single or several successive administrations in doses up to 8000-fold higher than the expected recommended dose for treatment, producing neither pathological effects nor any other macroscopic or microscopic changes. Furthermore, in the Ames test, Cop 1 showed no mutagenic effect. The conclusion was therefore that Cop 1 is a non-toxic material, and the results fulfilled the requirements for a Phase I clinical trial.

4. Initial clinical trials

Our first clinical trial was conducted in Israel, at the Hadassah Medical School, in collaboration with Dr. Oded Abramsky [24]. Dr. Abramsky, an enthusiastic neurologist at Hadassah who is now the Chairman of the Department of Neurology there and currently serves as the Dean of the Medical School, was at the time at the Weizmann Institute, working towards his Ph.D. thesis under my supervision. In this capacity he took part in some of the experiments with Cop 1. Impressed by the experimental data he was interested in testing the effect of Cop 1 in patients. This Preliminary Trial, according to the approval conditions of the Israeli Helsinki Committee, included only four MS patients in the terminal stages of the disease. They were treated with 2–3 mg of Cop 1, 2–3 times a week, for 4–6 months (the initial 3 weeks were under hospitalization). Under these conditions no beneficial effect was expected. Indeed, the patients did not show any significant change in their motor function. Two of them exhibited some improvement in vision and speech capacity, but in the absence of a control group, it was impossible to relate this improvement to the treatment. However, the most important finding was that no side effect was observed in any of the patients. There were no changes in blood pressure, heart rate and ECG, or in liver and kidney functions. Nor were any toxic or allergic reactions observed. This information paved the way for further clinical trials in less severe patients.

The difficulty was to find a clinician to perform such a trial. I recall this time as the 'peddling period'. I participated in almost any conference, large or small, which dealt with MS. I presented our experimental data, wherever possible I screened the film on the baboon and talked to everyone who was prepared to listen. I had success with two neurologists: Dr. Helmut J. Bauer from Göttingen in Germany and Dr. Murray B. Bornstein of the Albert Einstein College of Medicine in New York.

I met Dr. Bauer at one of the meetings which I attended in Europe. He had a large clinic for the treatment of MS and was excited by the opportunity to test the effect of Cop 1 and the glimmer of hope for the otherwise desperate patients. The trial he conducted was an open-label one, involving in all 21 patients, 10 of whom (with DSS range 2–6) received a daily dose of 2 mg Cop 1 and the other 11 (with DSS range 5–7) received a daily dose of 20 mg Cop 1, for the duration of one month. The results were indicative of some improvement, particularly in the group of relapsing-remitting patients and those with lower DSS. Due to the short duration of the trial and to the lack of a control group, the significance of the beneficial effect is not clear. However, the trial was important for demonstrating the safety of Cop 1 – there were only a few minor local reactions and two cases with transient fever without any other adverse effects.

Dr. Bornstein, who passed away recently, was a very dynamic personality, whom I also met at a conference in Europe. He was a renowned neurologist, who was also in charge of a large MS clinic. He was interested in the pathogenic mechanisms leading to MS and their association with EAE. His previous studies in tissue culture had indeed served to relate MS to EAE [25], demonstrating that mammalian CNS tissue cultures respond with identical patterns of demyelination when exposed to serum from EAE-affected animals or from MS patients. Hence, the rationale for his willingness to launch a trial with an agent which suppresses EAE and CR-EAE, looking for its effect on MS patients. Altogether, Dr. Bornstein and his colleagues conducted three clinical trials, a preliminary one and two pilot double-blind controlled trials, one involving exacerbating remitting (E-R) patients and the second involving chronic/progressive (C-P) patients, as summarized in a recent review article [26].

The preliminary trial [27] involved 16 patients (four E-R and 12 C-P) and was conducted as an open study. The first patients were hospitalized for the first 3 weeks to look for any significant local or systemic effects, but since no undesirable side effect was observed, subsequent patients were hospitalized for only 24–48 h, and continued the Cop 1 treatment as outpatients for the

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