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Superoxide dismutase for therapeutic use: Clinical experience, dead ends and hopes

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Summary

The clinical trials performed with bovine superoxide dismutase (SOD) are reviewed. SOD, applied intraarticularly at a dosage of 2–16 mg, proved to be effective in osteoarthritis of the knee joint in three placebo-controlled and one steroid-controlled double-blind trials. Its efficacy in other inflammatory joint disorders is documented by uncontrolled trials. Similarly, some controlled and many open studies support the efficacy of locally injected SOD in periarticular inflammation. Systemic treatment of rheumatoid arthritis by SOD at the dosages indicated yielded disappointing results. Well documented, though open uncontrolled studies demonstrated beneficial effects of locally administered SOD in radiation cystitis, interstitial cystitis and Peyronie's disease. Tolerance is good, but allergic reactions at low incidence have to be anticipated. Human SOD derived from recombinant microorganisms is being developed to explore its therapeutic potential particularly in ischemia-reperfusion damage, adult respiratory distress or similar conditions.

Introduction

The therapeutic principle of preventing free radical injury by superoxide dismutase (SOD) is at risk from unfounded speculations, poorly designed and correspondingly disappointing clinical trials, exaggerated promises, and theoretical controversies before its actual potential has been fully explored. It, therefore, appears timely to review the achievements and scrutinize the therapeutic options.

To narrow down the scope of this article, it will not deal with SOD pills sold in US health food stores for longevity and having the approximate composition and pharmacodynamic potential of a 'medium-rare' piece of calf's liver. Neither will we elaborate on the attempts to improve the intracellular oxidant defense system by liposome-encapsulated or microinjected SOD since these approaches, despite their theoretical interest, are not considered to be widely applicable in medical practice. Therapeutic use of SOD shall here mean either local or systemic injection of the plain enzyme to interfere with pathological events presumed to be caused by unbalanced superoxide production.

Admittedly, the brief introductory statements already reveal the bias of a former enzymologist now working in drug development. Specifically, the following assumptions are being made and suggested for general acceptance: 1) Superoxide dismutase, if it acts therapeutically, does so by means of its only documented catalytic function, i.e. by dismutating superoxide radical to yield O_2 and H_2O_2. 2) As a protein of 32000 Daltons it has little, if any chance at all to survive the gastrointestinal tract when taken orally. 3) For the same reason it will not enter cells with the exception of those specialized to absorb protein, e.g. the tubule cells of the kidney or phago-

cytes. This implies that the injected enzyme can only interact with superoxide radicals released into the extracellular space, and 5. needless to say, this interaction can only be a therapeutic one, if the products of the enzymatic dismutation are less toxic than O_2^- itself or products formed therefrom non-enzymatically.

The apparent triviality of these assumptions, however, should neither mislead us to restrictive dogmatism not to undue extrapolations from in vitro experiments. Although our theoretical knowledge of the molecular and functional characteristics of superoxide dismutases is highly advanced (for review see [1]), our understanding of the occurrence and biological role of the superoxide radical in a living organism is almost exclusively based on circumstantial evidence, since the radical in a complex system is not accessible to direct chemical detection. In fact, testing the inhibition by SOD of a biological effect is considered the most adequate way to support its mediation by superoxide radicals. Due to these analytical restrictions the development of SOD as a drug has not been, and will not likely be a straightforward strategy based on established theoretical knowledge. Rather, the concept is slowly emerging from a patchwork of serendipities, discoveries, trials and errors [2].

SOD in inflammatory joint diseases

Rationale

The antiinflammatory properties of SOD were discovered by Huber and Schulte [3] long before the enzymatic nature of the metallo-protein was recognized by McCord and Fridovich [4], and clinical pilot trials with SOD isolated from bovine liver (Orgotein) in rheumatoid arthritis and osteoarthritis had been initiated [5, 6] before the release of O_2^- by inflammatory cells such as polymorphonuclear leukocytes [7] and macrophages [8] was detected. A posteriori, emerging knowlege on the role of O_2^- in inflammatory processes [9] has provided a reasonable rationale for the therapeutic use of SOD which may be summarized as follows:

- All phagocytosing cells so far investigated, i.e. polymorphonuclear leukocytes [7], monocytes [10], macrophages [8] and endothelial cells [11] are capable of producing O_2^- by NADPH oxidase [12] and to release it into the phagosome or the extracellular space.
- O_2^- release by such cells is triggered not only by phagocytosis but by a large variety of compounds known to stimulate or mediate inflammation, like aggregated IgG [13], antigen-antibody complexes [14], the complement component C5a [13], N-formylated chemotactic peptides [15], leukotriene B_4 [16] and the platelet activating factor (PAF-Acether) [17].
- O_2^- by generating more aggressive oxygen-centered radicals, may cause oxidative destruction of biomembranes [18, 19] and fragmentation of extracellular macromolecules like hyaluronic acid [20, 21], collagen and proteoglycan [22, 23]. Similarly, it causes inactivation of serine protease inhibitors thereby facilitating protein degradation [24].
- O_2^- released from activated phagocytes appears to amplify the initial inflammatory response by inducing cell recruitment [9, 25] or denaturing proteins, i.e. by generating new inflammatory stimuli [26].
- Injected SOD will distribute in the extracellular space, which is poorly protected by endogenous SOD [27], and may there prevent both the O_2^--mediated tissue injury and the amplification of the inflammatory reaction [9, 28].

This brief outline of the rationale of SOD therapy is not to ignore the host of mediators contributing to inflammation. Not surprisingly, SOD does not consistently exhibit therapeutic effects in some animal models of inflammatory edema which reliably responds to inhibitors of prostaglandin synthesis, whereas the antiinflammatory effect of SOD, e.g. in the reversed Arthus reaction, proved to be so consistent that it was initially used to monitor purification of SOD (Huber, personal communication) and to standardize samples [3]. This simply indicates that the relative contribution of O_2^- and other mediators of inflammation largely varies with the model and phase of inflammation, as well as with the species and even the strain of animals used. Conflicting animal data (for review see [3, 9, 28]) thus demonstrate

that our rationale may, but does not necessarily apply to all kinds of clinical inflammation, and that the potential benefit of SOD therapy has to be worked out separately for different clinical situations.

Clinical verification

Osteoarthritis

Efficacy of bovine SOD injected intraarticularly into the synovial cleft of patients suffering from active osteoarthritis of the knee joint was first reported by Lund-Olesen and Menander [5]. In this pilot trial the patients were treated with 2–3 mg SOD 3 times. Out of the 19 patients, 16 improved and the remission appeared to last for about 3 months after cessation of treatment.

The promising results of this open uncontrolled study was later substantiated by three placebo-controlled, randomized, double-blind trials performed independently in central Europe [29,30], the United Kingdom [31] and Scandinavia [32]. In these trials, either 2 mg [32], 4 mg [29–31] SOD or placebo was injected intraarticularly at biweekly [32] or weekly intervals [29–31], respectively, and the clinical improvement was monitored at each visit. This therapeutic regimen was essentially dictated by its feasibility in medical practice and the reluctance of the physicians to puncture the knee joint more frequently. In each trial comparison of initial and consecutive assessments revealed a pronounced placebo effect but also a clearcut and significant superiority of the SOD treatment in clinically relevant parameters like pain [29–32], functional improvements in terms of maximum walking ability and climbing stairs [29, 30, 32], joint circumference [29, 30], and physician's and patient's global ratings [29–32]. Superiority of drug versus placebo usually became obvious after the third injection. As expected, no difference in the radiological appearance of placebo- and drug-treated joints was seen, indicating that SOD ameliorates the superimposed inflammation rather than the underlying degenerative process of osteoarthritis.

In a fourth randomized double-blind study, intraarticular SOD at two dosage levels (8 and 16 mg) was compared to intraarticular corticoid injection (40 mg methylprednisolone acetate [33]). The patients were treated every second week up to the sixth week and observed for a total period of 6 months. During treatment, all patients continuously improved, as judged by stiffness after rest or in the morning, pain, and patients' assessment of overall results, while warmth, effusion and tenderness of the joints did not change markedly. 16 mg SOD tended to be most effective during the treatment period. After cessation of treatment the corticoid group deteriorated rapidly and reached pretreatment scores in patients' assessments at 24 weeks, whereas the therapeutic effect of SOD at least at the 16 mg dose was sustained for the whole follow up period.

Based on a biased selection of papers reviewed, Greenwald [34] recently concluded that 'therapeutic benefits of oxygen radical scavenger treatments remain unproven'. This statement cannot remain unchallenged: 1) Compiling some conflicting data from experimental models of inflammation and clinical pilot trials for different indications does not allow such a generalized conclusion. 2) out of the four controlled trials in osteoarthritis only one [33] has been quoted and heavily misinterpreted. Gammer and Broback [33] definitely did not describe a deterioration of their patients when treated with 8 mg SOD or 40 mg methylprednisolone acetate, but, as outlined above, an improvement during therapy in all of the three treatment groups and a deterioration in two groups after cessation of treatment. 3) Greenwald [34] insists on inactivated SOD as the only legitimate placebo control. This is an unacceptable postulate: Inactivated SOD, irrespective of its mode of preparation, proves notoriously unstable. It would never survive unchanged the obligatory stability testing and subchronic animal safety studies and can thus not be used as a control in clinical trials for legal and ethical reasons.

In our view, the controlled trials unequivocally prove that SOD, if applied locally to the site of inflammation, ameliorates the symptoms of osteoarthritis. It cannot, of course, eliminate the underlying degenerative disorder leading to inflammatory episodes. But, with regard to its sustained therapeutic effects it may be suggested that SOD may also slow down the progression of the disease, as far as it is caused by inflammation-dependent tissue destruction.

Based on the results obtained with SOD in os-

teoarthritis of the knee joint, the therapeutic potential of SOD has been evaluated in analogous conditions of other joints like the hip, vertebrae and fingers (for review see [35, 36]). In view of the complicated mode of application in those instances, blinded placebo-controlled trials could not be performed. The overall experience from controlled and uncontrolled trials, however, appears to justify the generalization that therapeutic benefits of SOD in osteoarthritis cannot only be expected for the knee joint, but whenever an intraarticular injection of an inflamed joint is anatomically feasible.

Rheumatoid arthritis

With regard to the generalized character of rheumatoid arthritis systemic, i.e. intramuscular, treatment by SOD was investigated first. SOD (8 mg i.m. 3 or 4 times weekly) or placebo injections were superimposed on an aspirin or corticoid treatment which was kept constant over the study period (12 weeks). The additional SOD treatment was reported to yield a small therapeutic increment which reached statistical significance in some of the parameters investigated [6, 37]. Similar results were described when systemic SOD treatment was compared to gold or penicillamine [6]. Other investigators, however, could not verify any therapeutic effect of SOD administered systemically to rheumatoid patients [38].

Thus, the evidence of any therapeutic benefit of SOD given i.m. in the milligram dosage range to rheumatoid arthritis patients is at best controversial. This seemingly surprising outcome of the clinical studies may be attributed to inefficient tissue levels of active drug which can be estimated to be lower by orders of magnitude when compared to those obtained after intraarticular application [36]. But it cannot be concluded that O_2^- and the radicals it generates play a less important pathogenic role within the realm of mediators in this particular type of inflammatory disorder [28]. A certain contribution of oxygen-centered radicals to the pathogenic events in rheumatoid arthritis is suggested by pilot trials in which SOD was administered intraarticularly to the primarily affected joints of rheumatoid patients [39, 40]. These trials, however, have been duly criticized in respect to their unconventional therapeutic controls, i.e. intraarticular injection of acetyl salicylic acid or low dose corticoids [34].

In conclusion, then, SOD is not – and will probably never become – the drug of choice for the treatment of rheumatoid arthritis, and its efficacy in this disease may remain an academic problem. Local treatment may be helpful, but will be restricted to a small minority of patients in view of the generalized nature of the disease. Systemic treatment, if it is to work, might require dosages in the 100 mg range, and it can reasonably be doubted whether the benefit of this approach will ever outweigh the costs and the potential hazards of a chronic therapy with high doses of a protein.

Periarticular inflammations

Once a drug has been approved for therapeutic use in a defined indication and made available to the public, its use tends to get out of control and its profile will become less well defined. Since 1981, therefore, SOD has been reported to cause beneficial effects in a variety of periarticular diseases such as sports injuries, tennis elbow (epicondylitis), frozen shoulder (periarthritis humeroscapularis), tendosynovitis, and the like. Commonly the drug was injected locally into the site that was presumed to be the focus of an ongoing inflammation. The vast majority of pertinent reports is not easily evaluated since they are based on uncontrolled or unblinded studies or just on case reports (for review see [35, 36]). Theoretically, scattered results must be anticipated due to unpredictible local kinetics of the injected drug, the variability of the disorders and diagnostic uncertainties. Surprisingly, however, this therapeutic approach became widely accepted in European countries and was even supported by some controlled trials. A randomized double blind trial in epicondylitis, comparing the effects of SOD (4 mg in 4 ml) and procain (4 ml of a 2% solution), established a substantial superiority of the weekly SOD infiltrations, particularly in respect to pain relief and restoration or working ability [41]. As expected, a retrospective analysis of the data also revealed that the more acute cases with obvious signs of inflammation responded markedly better to SOD therapy. In another randomized controlled trial on periarticular inflammations, SOD infiltrations (2 and 4 mg) were reported to be as effective as with methylpred-

nisolone (20 and 40 mg, respectively), but superior in terms of duration of action and tolerance [42].

Radiation cystitis and interstitial cystitis

The documentation of efficacy of SOD in the treatment of chronic cystitis (radiation cystitis and interstitial cystitis) suffers from its history, i.e. from convincing results obtained in open pilot trials. In the early seventies Marberger *et al.* [43] reported that pain, micturition frequency and bladder capacity in such patients could be markedly improved by infiltration of SOD into the affected sites of the urinary bladder. The outstanding results of SOD, particularly in radiation cystitis, were soon reproduced by the same group of investigators [44, 45], and others [46–50]. Surprisingly, even radiation ulcers were reported to heal [49, 50]. Usually 8–40 mg SOD are administered under cystoscopic control and general or local anesthesia at weekly intervals. As radiation cystitis was known to be notoriously resistant to any kind of therapy, these reports on open trials and case reports were considered sufficient proof of efficacy and accepted with enthusiasm among European urologists and health authorities. The other side of the coin is that physicians for ethical reaons now have to refuse to perform blinded controlled trials in order to formally prove the efficacy of an apparently effective therapy, much to the regret of more bureaucratic health authorities and those depending on them.

Induratio penis plastica

The first encouraging results from repeated SOD instillations in patients suffering from induratio penis plastica have also been reported by Marberger *et al.* [43]. Induratio penis plastica or Peyronie's disease is characterized by fibromatous plaques and deformations of the penis causing considerable discomfort during erections and sexual intercourse. Although its etiology is unknown, histological evidence suggests a chronic inflammatory process [51–53]. Further studies, therefore, appeared justified and by and large confirmed the initial observations (for review see [54]). The delicate nature of the disease, the mode of application which often requires anesthesia, the difficulties in evaluating the results objectively and, again, the early success so far precluded blinded and controlled clinical trials, but the overall experience leaves little doubt about the efficacy of SOD therapy in Peyronie's disease [36, 54, 55].

Prevention of radiation damage

The knowledge that superoxide anions are generated by radiation of oxygenated watery solutions has raised considerable interest in the possibility of preventing radiation damage by superoxide dismutase. Pertinent clinical trials, however, cannot be interpreted to interfere with this radiation-dependent radical formation, since SOD has usually been administered following the irradiation, i.e. long after the superoxide anions generated during the irradiation process itself have been decomposed. These trials should be considered as attempts to prevent inflammatory reactions resulting from the irradiation.

Two double-blind placebo-controlled trials were performed by Edsmyr [56–58] in patients subjected to radiation therapy for bladder or prostate tumors. 4 or 8 mg SOD injected intramuscularly 15–30 minutes after the irradiations significantly reduced the incidence of acute proctitis, diarrhea and cystitis. The results were supported by a similar study aiming at the prevention of mucositis in patients irradiated for head and neck tumors [59]. In this context also, the reports on beneficial effects of liposome-encapsulated SOD in cases of severe accidential overexposure to radiation merit consideration [60].

Despite its attractiveness, however, the concept of prophylaxis of irradiation damage has not been widely explored. The underlying reasons for this shortage of documentation are numerous. Just to exemplify the logistic difficulties in performing such trials it should be mentioned that Edsmyr (unpublished) undertook a further attempt to broaden his documentation but failed, since improved radiation techniques resulted in lack of acute radiation side effects in both SOD-treated and untreated patients. Further the acute side effects of radiotherapy do not necessarily correlate with the more relevant chronic radiation damage diagnosed later, usually by another physician. This implies that conclusive clinical trials would have to enrole huge numbers of patients and to observe them over long periods of time in ord-

er to establish relevant differences of small incidences of radiation injuries. The alternative approach, i.e. raising the irradiation dose to a more dangerous level and trying to counteract the corresponding risk would not be acceptable for ethical reasons.

Safety of therapeutic use of bovine SOD in humans

SOD, irrespective of its origin, appears to be nontoxic even when administered intravenously in excessive amounts [3]. Parenteral application of an heterologous protein, however, should always raise concern, and correspondingly an intravascular administration of bovine SOD should be strictly avoided.

Based on more than ten years of clinical experience the risk of severe immunological complications from highly purified bovine SOD*, injected locally, as recommended, can be rated as acceptable. The last documentation of side effects was presented by Wilsmann at the 1985 Superoxide Dismutase Meeting in Rome [61] and can be updated as follows [62]: In 7715 patients monitored during clinical trials of any kind up to December 1986 a total of 491 (6.4%) incidences of side effects were reported. The majority consisted of local irritations at the injection site. Most others were mild and uncharacteristic and did not allow any solid conclusion as to whether or not they were related to treatment. More importantly, 64 reports on side effects (0.8%) were rated allergic or questionably allergic and three cases of anaphylactic shock (0.04%) were observed. In an extensive drug monitoring study on 11024 patients, the corresponding figures were: total side effects 264 (2.4%), allergic or questionably allergic reactions 62 (0.6%), and no cases of anaphylactic shock. Spontaneous reports on side effects since marketing of the drug in 1981 were 318 in total, 118 were on allergic reactions and 46 on anaphylactic shocks. These numbers have to be compared to the delivery of more than four million ampoules of drug and an approximate average of four ampoules per treated patient. In agreement with the observations from clinical trials and the drug monitoring study, the incidence of the only serious side effect, i.e. anaphylactic shock, in general practice is thus far below 0.1% and so far the anaphylactic reactions could always be managed by conventional therapy. Surprisingly, in about half of the cases the anaphylactic reaction occurred after the first injection suggesting a preexisting sensitization.

The immunogenic risk of the heterologous SOD, although surprisingly low, has to be balanced against the expected therapeutic benefit and precludes the use of bovine SOD in bagatelle indications. Further, intravascular applications of high doses of the heterologous protein may be presumed to cause more severe reactions and theoretical indications requiring such treatments (see below) have therefore not yet been explored.

Perspectives

Considerable experimental evidence has accumulated that SOD treatment might be beneficial in the treatment of postischemic reperfusion injury [63, 64], septicemia [65, 66] or adult respiratory distress syndrome [67]. According to available animal data, in these conditions SOD has to be administered intravenously at a dosage somewhere between 1–10 mg/kg.

With the advance of gene technology, human SOD has become available from recombinant microorganisms [68–70]. Particularly, the human SOD isolated from recombinant yeast* proved to be exactly identical with authentic human SOD in being N-terminally acetylated [70], whereas human SOD prepared from recombinant E. coli exhibited different electrophoretic mobility due to the inability of E. coli to process the enzyme correctly [70]. In the meantime 'therapeutic' equivalence of the yeast-derived human SOD to bovine SOD has been demonstrated in a rat model of renal ischemia/reperfusion injury [71] and in salvage of the reperfused canine myocardium [72, 73]. The preparation has passed animal and human safety studies without any complications (Grünenthal GmbH, un-

* Peroxinorm® , Grünenthal GmbH, Aachen, FRG

* Sudismase (INN)

published) and may at present be considered the optimum tool to further explore the therapeutic potential of SOD.

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