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Miracle molecules of our age: ethylenediaminetetraacetic acid

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In his outstanding book, Power Aging[1], Gary Null spends much of the early part of the volume discussing the numerous man-made chemicals and components that constitute a major part of the problems with pollution and damage to human health. It is almost as though anything chemical which we manufacture and use in our world can damage us. Even the pharmaceuticals which have been designed and synthesised, especially to cure us, always have side-effects some of which appear to be at least as bad as the conditions that they were designed to abolish.

In view of what Dr Null says, can we find any synthetic chemicals which are harmless to man and yet have useful and important health benefits for which they were not designed?

Here I discuss one agent, namely ethylenediaminetetraacetic acid (EDTA) that seems, at present, to fit the above criteria.

1. EDTA as a bacterial permeabiliser

It is as a permeabiliser, particularly of Gram-negative bacteria, that EDTA is best known. Numerous inhibitory agents are hydrophobic, and because these fail to enter Gram-negative organisms, they cannot kill them nor even inhibit their growth *i.e.* they cannot have a bactericidal nor a bacteriostatic effect. Generally, failure of hydrophobic agents to penetrate into the Gram-negative cell results from the outer membranes (OMs) being impenetrable to them, hence preventing their passage across the envelope[2,3]. Outer membrane impenetrability as the basis of the above effects can be proven, for hydrophobic agents, by comparing their effects on normal strains and those (lps mutants) altered in the lipopolysaccharide (LPS) component of the OM; whereas unmutated strains are insensitive to hydrophobic agents, they readily cross the envelopes of the mutants with a lethal effect[2,3].

Strikingly, the OMs of some Gram-negative bacteria can also be permeabilised by treatment with EDTA. This molecule is a chelator and its removal, by chelation, of magnesium, manganese and calcium ions from the OMs leads to permeabilisation. These ions are intimately involved in the LPS–LPS and LPS–protein interactions which stabilise the OMs, and so removal of them leads to loss of LPS from the envelopes and permeabilisation to the normally non-penetrating hydrophobic agents[2–4].

The removal of these ions and the loss of LPS from the OMs, in the presence of EDTA, is particularly marked in pseudomonads, so that these organisms show the most marked sensitisation by EDTA[3,4]. Hydrophilic antibacterials normally cross the OMs via the porins, provided that these antibacterials are less than *ca* 600 molecular weight[2]. If this is so, then Gram-negatives show sensitivity to them in the absence of EDTA. Many such agents will, however, also show an increased effect on EDTA-treated organisms, as the hydrophilic molecules will use both entry routes *i.e.* entering via both the porins and the permeabilised OMs. Hydrophilic antibacterials above 600 molecular weight normally fail to affect Gram-negatives; after permeabilisation by EDTA, however, some of them will use the OM route and cause lethality[2].

As a result of its permeabilising effect, EDTA generally sensitises Gram-negatives to an array of potentially lethal agents to which they are inherently resistant. Such agents can be divided into four classes namely disinfectants, antiseptics, preservatives and antibiotics[4]. Many of these agents are hydrophobic, and therefore, cannot enter Gram-negatives, especially pseudomonads, without permeabilisation. The permeabilising effect of chelators such as EDTA is especially important for antiseptics and antibiotics, because organisms are more and more commonly becoming resistant to such agents that originally killed them. The ability of chelators such as EDTA to reverse this effect is of great importance.

EDTA and enhanced lethal effects of disinfectants, antiseptics and antibiotics on organisms on inert surfaces, on wounds and on burns

Because phenols and cresols are hydrophobic, they generally fail to kill Gram-negatives and therefore cannot be used to disinfect inert surfaces. The ability of EDTA to permeabilise OMs, however, means that disinfection by phenols or cresols plus EDTA can be highly effective.

Similarly where Gram-negatives are infecting wounds or burns, and an antiseptic such as a QAC (quaternary ammonium compound) or chlorhexidine is ineffective, EDTA will allow the agent to cross the OMs and make the antiseptic effective. Thus EDTA has been shown to allow QACs to enter inherently resistant pseudomonads, and preparations containing EDTA and various QACs are available for therapeutic use[4].

Similar studies have been undertaken using EDTA to enhance killing by antibiotics of Gram-negative pathogens on wounds, burns or surface infections in animals. Several studies have shown that the chelator can greatly enhance the effect of neomycin on pseudomonad-infected wounds and burns, in one case, the m.i.c. for the antibiotic being 100-fold less plus the chelator. In another study, EDTA enhanced the lethal effects of *e.g.* tetracycline, chloramphenicol and erythromycin on pseudomonads infecting ulcerated skin in rabbits, whilst in a third study, cephaloridine being used to treat otitis (caused by pseudomonads) in dogs was more effective plus EDTA, surprisingly, since this antibiotic generally enters via the porins[5]. Similar results showing EDTA-enhanced killing by antibacterial lavages has been shown for cats or dogs with cystitis[5].

Clearly, therefore, in a wide range of situations, pseudomonads colonising inert surfaces or infecting wounds, burns or surface organs such as eyes, ears or genitalia in animals, are poorly affected by disinfectants, antiseptics or antibiotics alone. In contrast, when the potentially lethal agent is supplemented with EDTA, the organisms are rapidly killed, because the chelator allows the agents to cross the OMs[5].

EDTA and its effects on biofilms in catheters

Catheters are highly susceptible to the formation of biofilms, with a range of Gram-positive and Gram-negative bacteria making-up such films.

Several studies have established that EDTA aids killing of biofilm bacteria by antibiotics; for example, minocycline fails to abolish biofilm bacteria from catheters when used alone, but plus EDTA it effectively kills Gram-positives, including MRSA, as well as Gram-negative bacilli. Strikingly, in one study, EDTA alone (without an antibacterial) killed MRSA, *Enterobacter* spp and *Enterococcus* spp on catheters, and had the same effect on catheters infected with pseudomonads and streptococci. Also, minocycline plus EDTA has been shown to prevent a range of catheter-related diseases *e.g.* bacteremias, phlebitis and endocarditis[6].

EDTA in aerosols

One situation where pseudomonads cause major disease problems is in the respiratory tract of patients with cystic fibrosis; mucoid strains in the airways lead to substantial breathing problems and, as expected, the mucoid derivatives (because antibiotics are impeded in entry by the polysaccharides in the envelope which cause the mucoid phenotype) are intransigent to treatment by most antibacterials. Strikingly, EDTA used in aerosol sprays has been shown to greatly reduce the levels of such organisms in the respiratory tract and aid the breathing of the patient[7].

Permeabilisation by EDTA of Gram-negative bacteria which are causing systemic infections

Several of the studies mentioned above could be considered *in vivo* studies, but it would be far more important if orally administered EDTA permeabilised organisms that were causing systemic infections. EDTA is safe to administer orally, provided that: (i) it is given as a low dose on the first day, with the dose gradually being increased; and (ii) that it is administered with low amounts of ions such as magnesium, manganese and calcium. So can EDTA act to permeabilise pseudomonads and other Gram-negative pathogens in the body to hydrophobic agents. The answer is "Yes" at least in some cases.

I have emphasised that EDTA is particularly adept at permeabilising pseudomonads, almost certainly because it removes a much higher proportion of LPS from their OMs than from the OMs of other Gram-negatives. When considering a role for orally administered EDTA acting *in vivo*, its effects on pseudomonads are particularly important, since few antibacterials act on pseudomonads in the body. Also, most of those which do act cannot be administered orally (but have to be injected) because when administered orally they are destroyed by stomach acid.

The major effective group of oral agents against most pseudomonads are the fluorquinolones such as ciprofloxacin. Pseudomonads can cause a number of significant systemic human and animal infections, as indicated above, and one group of interest here is urinary tract infections (UTIs). Following urinary tract surgery, especially if the patient is catheterised even for a short time after surgery, UTIs are very common, and often involve pseudomonads.

A recent operation[8], led to a long-standing pseudomonad UTI. Although the causative organism was ciprofloxacin-sensitive *in vitro*, the agent was ineffective *in vivo* at 500 mg/day, with intravenous pipericillin being offered as the only solution. Knowing that EDTA permeabilised pseudomonads *in vitro* and was effective with several antibacterials for pseudomonad-infected wounds, burns, *etc*., the patient tested (on himself) 500 mg/day ciprofloxacin and 50 mg/day EDTA. The infection was abolished in 24 h and did not return[8]. One possibility was that permeabilisation by the chelator allowed more ciprofloxacin to cross the pseuomonad OMs, *i.e*. using both the porins and the permeabilised OMs for passage, after removal of LPS by the chelator[4,8].

There are other possible explanations for the EDTA effect, however. First, EDTA could itself have killed the infecting pseudomonad, as a result of the substantial damage that it causes to the OMs. More likely, it might have enhanced the potentially lethal effects of any of the patient's own defensive components, some of which are likely to be on the mucous membrane surfaces of the UT.

In view of the fact that EDTA sensitises pseudomonads and other Gram-negatives to hydrophobic agents *in vitro*, and that the chelator has been proven to enhance killing of pseudomonads associated with wounds, burns and other surface infections, it is highly likely that EDTA will act with antibacterials other than ciprofloxacin, to kill Gram-negative pathogens causing systemic infections as it did on the UTI discussed above[8]. It could, for example, act to cause lethality by such agents as fusidic acid, novobiocin, erythromycin and any of a range of hydrophobic beta-lactams, which generally alone do not kill Gram-negatives.

Lethality of oral EDTA (administered alone) on pathogenic Gram-negatives in vivo

Such killing could occur, as mentioned above, as a result of the chelator enhancing entry, across the OMs, of potentially lethal host defence components. Several host components are known to enter Gram-negatives better in the presence of EDTA *in vitro*. This is particularly true for lysozyme. This enzyme, which occurs widely in the body, degrades the murein of the bacterial envelope, causing lysis. It fails to act on Gram-negatives, such as pseudomonads, salmonellas and *E. coli*, because it cannot cross the OMs to reach the murein. EDTA by permeabilising the OMs allows the enzyme to cross the OMs and cause lysis. Thus, *S. typhimurium* on incubation with (i) no additions, (ii) lysozyme 25 μg mL^{-1} alone, (iii) EDTA 270 μg mL^{-1} alone showed no lysis in 20 min. With the same amounts of EDTA and lysozyme added together, however, optical density fell by 75% in 20 min showing marked lysis[9].

Other potentially lethal host enzymes are produced in the intestine *e.g.* phospholipase A2 is secreted there as a host defence mechanism. This enzyme would not cross Gram-negative OMs but EDTA would permeabilise them *in vivo* and allow the enzyme to destroy the cytoplasmic membranes and kill the organisms.

Orally administered EDTA may also aid killing *in vivo* by co-operating with host transferrins. This seems likely, since it is known that the chelator enhances the lethal effects of ovotransferrin on *E. coli* O157:H7 in broth[10], and the killing effects of transferrins on organisms on meat surfaces.

In the intestine, amongst the potentially antimicrobial agents are the bile components[11]. Because of their hydrophobicity, these agents would generally not kill Gram-negative bacteria, but EDTA should allow them to cross the OMs, which are known to impede their passage, and kill by causing damage to the CMs and possibly to the DNA[11].

Another group of toxins found in the intestine are the colicins[12]. Some Eastern European groups have proposed that colicin preparations could be orally administered for therapy *in vivo* against Gram-negative pathogens[13]. However, some potentially sensitive pathogens are unaffected by these agents

in the upper intestine, because of the acidity of this location[14], which may prevent the lethal agent from reaching its site of action. If this were because the OMs impeded its passage, then EDTA orally administered with the colicin would probably lead to effective therapy.

2. EDTA in chelation therapy and in chelox therapy

Blocking of blood vessels by plaque leads to a substantial risk of heart attacks, strokes and other serious disorders such as intermittent claudication. The problems are clearly considerable. Plaque contains appreciable amounts of calcium salts, but pollutant metals such as copper, iron, mercury, cadmium and aluminium are also present. Whilst the build-up of the plaque itself, and the presence of the above metals, which can give rise to free-radical production, leads to the risk of serious disease, the ability of certain agents such as EDTA to remove the plaque constituents by chelation gives a simple opportunity to alleviate the problem.

For over 50 years now, chelation therapy has been available as a treatment for conditions where the blood vessels are seriously blocked; used correctly, it prevents the build-up of plaque, but also removes appreciable amounts of plaque already attached and causing partial blocking. It can, therefore, act both to prevent further damage and to abolish damage already incurred. Thus such therapy has a major advantage over using statins, which only stop new plaque from building-up.

The efficacy of EDTA against blood vessel blockage was originally discovered when a patient being treated with EDTA for lead poisoning (the EDTA binds the lead and the complex is excretedby the kidneys) began to show amelioration of angina symptoms[15]. It was a Eureka moment for the physician involved, Dr Norman Clarke, and further studies indicated that severe heart disease could be treated without surgery, as well as offering hope that other severe symptoms of blood vessel blockage could be reversed[15,16]. Within 5 years, Clarke had treated nearly 300 patients with EDTA, and almost 90% of them quickly showed amelioration of their symptoms[15,16]. Originally, EDTA was administered by intravenous infusion, but now is often given orally.

It is now believed that removal by EDTA of metals such as iron, copper and cadmium prevents and reverses the damage which free-radicals arising from the polluting metals cause, and therefore allows the already damaged blood vessels to recover, and prevents further damage. In any case, at the right level of EDTA, appreciable plaque is removed, circulation is improved and blood pressure falls.

It is not only angina and other heart conditions that benefit from EDTA therapy, but blood flow throughout the body improves so that the legs and feet improve, whilst better circulation in the head and neck

enhances the conditions of patients with cerebral vascular disease, so that strokes are less likely.

Chelation therapy with EDTA is also beneficial to the eyes. With age, the capillaries in the eyes suffer from decreased circulation, and several of the degenerative diseases of the eye (*e.g.* cataracts, glaucoma and macular degeneration) result partly from such decreased circulation.

Chelox therapy

Chelation therapy, at the present time, often involves oral administration of EDTA. In contrast, chelox therapy still involves intravenous infusions, and both EDTA and hydrogen peroxide are used. Very low intravenous doses of hydrogen peroxide, when administered with EDTA, lead to more efficient plaque removal than the use of EDTA alone. It appears that EDTA works best on smaller vessels, whereas hydrogen peroxide is particularly effective on larger blood vessels.

3. EDTA and cancer

So can EDTA reduce the incidence of other diseases? A small statistical study undertaken by two of the most distinguished chelation therapy workers[17], suggests that chelation therapy with EDTA can greatly reduce subsequent cancer deaths.

The study involved 231 persons who lived in the same Swiss city and were exposed to roughly the same levels of pollution, especially from traffic. 59 of these persons who had earlier been treated with chelation therapy were followed-up over an 18 year period, as were 172 patients who had not been treated with EDTA. In the 18 year period, only one of the 59 chelation therapy-treated patients died from cancer (1.7%), whereas 30 of the 172 untreated persons (17.4%) died from cancer. Statistics showed the difference to be highly significant at almost the 99.9% confidence level ($P = 0.002$). Attempts had been made to ensure that the two groups were of similar ages and backgrounds.

It was speculated that the EDTA effects were due to the fact that the treated group would have had less exposure to free radicals because the EDTA would have removed most of the pollutant metals from the bodies of this group.

4. EDTA and the leaching of metals from food and drink containers

The FDA has approved the addition of EDTA to cans of carbonated soft drinks, but not to non-carbonated drinks. It is believed that the lower pH of the carbonated drinks means that the leaching of metals from the cans will

be greater and pose a health hazard. Other countries do not allow EDTA addition and presumably the leached metal will be ingested and deposited in the body.

Metals could, of course, also be leached from cans of food, especially if the pH were low *e.g.* in the case of foods in tomato sauces.

5. The future for the use of EDTA in man

It is extraordinary that the use of EDTA in man is still not appreciable, despite its being 55 years since Clarke's pioneering studies. In the States, chelation therapy is appreciable, although it is often looked at with mistrust, In contrast, this therapy is almost unknown in the UK, mainly being used on a self-heal basis, with the knowledgeable patient obtaining supplies through herbal companies. With our being so far ahead in our studies of molecular biology and molecular genetics in medicine, it is a surprise to say the least, that a cheap, safe, simple, effective chemical remedy for disease is not widely used.

Does lightning strike twice in the same place? Apparently it does, when we are considering EDTA. As with its role in chelation therapy, its use in permeabilising Gram-negative bacteria has been known for a generation. I worked on EDTA whilst studying permeabilisation by EDTA *in vitro*, this being in the early part of my career[9], and yet nearly 10 years after my retirement the likely benefits of the use of EDTA *in vivo* are almost totally overlooked. EDTA will almost certainly enhance the effects of antibacterials *in vivo*, and it is highly likely that it will also aid the lethal effects of some of the body's own defences. This is likely to be particularly the case for pseudomonads, because EDTA has such a substantial effect on pseudomonad OMs.

Other likely beneficial effects of EDTA in man

There is some evidence, firstly that chelation therapy with EDTA reduces the incidence of Alzheimer's disease. Secondly, some studies suggest that cognitive function is improved by chelation therapy. Thirdly, and most interestingly, some researchers believe that longevity is increased by chelation therapy. In each case, the likelihood is that removal of polluting metalsfrom the body is responsible for the effect.

Conclusions

As stated above, many of the possible benefits of EDTA are widely overlooked. More research is needed and a much more open-minded approach needed. In particular, a much different view might have been taken if Norman Clarke's outstanding work[15,16], which was published in major

journals and has the ring of truth and validity about it, were read again and studied in detail.

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