Comparative Evaluation of Disodium Edetate and Diethylenetriaminepentaacetic Acid as Iron Chelators to Prevent Metal -Catalyzed Destabilization of a Therapeutic Monoclonal Antibody

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ABSTRACT: Understanding the effect of metal chelators with respect to their ability to inhibit metal catalyzed degradation in biologic products is a critical component for solution formulation development. Two metal chelators, disodium edetate (Na_2EDTA) and diethylenetriaminepentaacetic acid (DTPA), were evaluated for their ability to stabilize IgG2 mAb in solution formulations spiked with various levels of iron. Real-time stability attributes such as oxidation, soluble aggregate formation, deamidation and fragmentation demonstrated that DTPA was equivalent to Na_2EDTA with respect to inhibiting iron-induced degradation over the range of iron concentrations studied. When sufficient chelator was present to stoichiometrically complex trace iron contamination, both Na_2EDTA and DTPA exhibited the capacity to reduce protein degradation. However, sub-stoichiometric ratios of both chelators were unable to inhibit the degradation induced by free iron ions, which were found to bind weakly to the mAb. This bound iron did not measurably alter the secondary or the tertiary structure of the mAb, but appeared to decrease its intrinsic thermodynamic stability, probably by causing subtle perturbations in the tertiary structure. These destabilization effects were not observed when the chelators were present at stoichiometric ratios highlighting the feasibility of using DTPA as an alternate trace metal chelator to Na_2EDTA in biologic protein formulations. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:4239–4250, 2010

Keywords: Protein aggregation; oxidation; deamidation; fragmentation; chelator; monoclonal antibody; protein structure; binding; stability; protein formulation

INTRODUCTION

It is well known that transition metal ions such as Fe(II)/(III), Cu(II), Zn(II), Co(II) and Ni(II) etc., can bind to protein molecules to stabilize their structure and play a critical role in modulating physiological functions [1–7]. Iron, an essential constituent of proteins involved in many cellular processes, is crucial for the growth and viability of almost all organisms. However, excessive iron may become toxic or even fatal due to its ability to induce oxidation of lipids and other cellular constituents [8–10]. It has been reported that iron can induce protein degradation via different mechanisms [11–15]. Since iron is only regulated by uptake [6], iron chelators have been introduced into clinical practice to protect patients from toxicity caused by iron overload. Various chelators such as cell-impermeable chelators like ethylenediaminetetraacetic acid (EDTA), dipicolinic acid (DPA), diethylenetriaminepentaacetic acid (DTPA)[16], cell-permeable chelators of hydroxypyridiones such as β-[N-(3-hydroxy-4-pyridone)]-α-aminopropionic acid (L-mimosine) [17], catecholates such as 1,5,10-N, N', N'-tris (5-sulfo-2,3-dihydroxybenzoyl) triazadecane (3,4-LICAMS) [6], and pyrophosphates [6] have been widely studied, although (salts of) EDTA (Fig. 1A) and to a lesser extent DTPA (Fig. 1B) are the most commonly used. EDTA is a hexadentate chelator capable of complexing stoichiometrically

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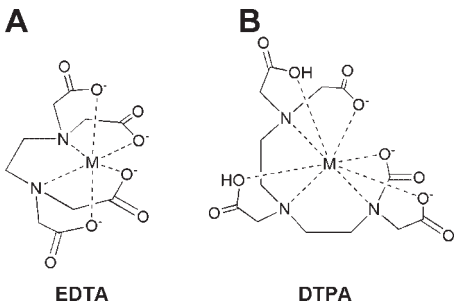

Figure 1. Schematic structures of (A) EDTA and (B) DTPA and the binding mechanism to metal.

with virtually every transition metal in the periodic Table [18]. The effectiveness of EDTA as a chelator for a particular metal ion is governed by the stability constant of the resulting metal complex. The stability constants (log K) of EDTA (acid form) metal complexes for the most commonly observed trace metal ions in parenteral formulations are 25.1 for Fe(III) and 14.6 for Fe(II) [19]. The stability constants for DTPA-Fe (III) is 27.3 and that for DTPA-Fe (II) is 16.0 at 20°C at 0.1 M ionic strength [20].

Many parenteral biologics drug products include metal chelators to improve product stability, with salts of EDTA being the most common. These chelators are generally used to complex trace amount of metals (especially iron ions) which may be introduced into the drug solution from formulation excipients and/or leachables from contact with stainless steel equipment utilized in production and storage. Both disodium edetate (Na_2EDTA) and calcium disodium edetate ($CaNa_2EDTA$) have been approved for human use by the FDA [21,22]. Analysis of drugs approved through the year 2000 reveal that 48 parenteral drugs contain EDTA in various salt forms [23]. Nine contain $CaNa_2EDTA$, 38 contain Na_2EDTA, and 1 product (Folvite®) contains sodium edetate[23].

Na_2EDTA is generally used in pharmaceutical preparations as a chelating agent at concentrations between 0.005 – 0.1% w/v (0.05 – 1 mg/mL). $CaNa_2EDTA$ is used in formulations as a chelating agent in the range 0.01% – 0.1% w/v (0.1 – 1 mg/mL). Na_2EDTA readily chelates calcium and can, in large doses, cause calcium depletion (hypocalcemia) if used over an extended period or if administered too rapidly by IV infusion. $CaNa_2EDTA$ does not chelate calcium. For Na_2EDTA the LD50 (mouse, IV) = 0.056 g/kg, and LD50 (rabbit, IV) = 0.047 g/kg. For $CaNa_2EDTA$ the L50 (rat, IV) = 3.0 g/kg. The toxicity is significantly dependent on the salt form used [24].

Disodium edetate is not a first-line agent for any indication; it is approved for use in emergency treatment of severe hypercalcaemia and in the treatment of arrhythmias secondary to digitalis toxicity. In adults with normal renal function, about 50% is excreted in the first hour in the urine and over 95% is excreted with 24 hours. Disodium edetate is almost entirely excreted in the urine unchanged within 24 hours. Therapeutically, a dose of 50 mg/kg Na_2EDTA, as a slow infusion over a 24 hour period, with a maximum daily dose of 3 g, has been used as a treatment for hypercalcemia. For the treatment of lead poisoning, a dose of 60–80 mg/kg of $CaNa_2EDTA$, as a slow infusion in two daily doses, for 5 days has been used[22,25].

Use of the generic term EDTA for all its various salt forms has had fatal consequences. Children and adults have died from overdose of disodium edetate mistakenly administered in place of calcium edetate, or when disodium edetate was used for "chelation therapies" [21]. A Public Health Advisory was issued by the FDA in January 2008 concerning this use and the misleading abbreviation EDTA[22,26].

A recombinant human granulocyte macrophage colony stimulating factor product (Leukine®), was marketed as a lyophilized form without disodium edetate. However, Leukine® was reformulated in 2006 as a liquid containing disodium edetate. An increase in spontaneous reports of adverse events such as syncope and hypotension was noticed, which appeared to coincide with the introduction of the liquid formulation [27]. This increase in adverse event reports was not associated with the lyophilized formulation of Leukine® which did not contain disodium edetate [28]. The liquid Leukine® was voluntarily withdrawn from the market in January 2008 [27]. A new formulation of liquid Leukine® without disodium edetate was approved by the FDA in May 2008[28]. The dose of disodium edetate through Leukine is about 1.9 mg/day for a 2 m^2 patient via the IV or SC routes. Interestingly, in the same time frame, a new IV formulation of fosaprepitant demelgumine (Emend®) was approved with a disodium edetate dose of up to 14.4 mg per injection. Caution is required when using Na_2EDTA in developing new biopharmaceutical products due to these safety concerns. EDTA also has been reported to accelerate protein oxidation in some cases even though the intention was to stabilize proteins in the presence of iron [12,13,29]. Considering the safety and possible stability issues around EDTA, it is important to evaluate the use of alternate chelators.

DTPA is one of the synthetic polyamino polycarboxylic acids that can form stable complexes with a large number of metal ions, such as Cu(II), Ni(II), Co(II), Zn(II), Cd(II), Mn(II) and Ca(II) [30]. Free DTPA contains five carboxylic acid and three amine groups, and five pK_a values of 1.79, 2.56, 4.42, 8.76 and 10.42 have been reported [31]. Similar to EDTA, DTPA chelates iron ions at equimolar ratio [31,32] over pH 2.5–11.0. It has been used in some approved drug