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Commentary
Mannitol: Molecule Magnifique or a Case of Radical Misinterpretation?

CHARLES E. GILLBE, FREDERIC J. SAGE and JOHN M.C. GUTTERIDGE*

Department of Anaesthesia & Adult Intensive Care, Royal Brompton Hospital, Sydney Street, London, SW3 6NP, UK

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1. INTRODUCTION

Carbohydrates such as cellulose and sucrose (cane sugar) have been used by man since ancient times, and pre-historic man was well acquainted with honey (a mixture of fructose and glucose). The cultivation of cane sugar as a sweetening agent appears to have originated in north eastern India as early as 300 AD, from whence the practice spread to China, Southern Europe and the Americas. By the middle of the nineteenth century it was established that the sugar present in grapes was identical to one of the sugars of honey and with the sugar present in diabetic urine. This sugar was given the name glucose by Dumas (1838), and dextrose by Kekule (1866). (For a review see Pigman and Horton.[1])

There was no knowledge of chemical structure at this time, and so simple sugars were looked upon as compounds of carbon with water, ie hydrates of carbon ($Cn(H_2O)x$) or carbohydrates. D-Mannitol ($C_6H_{14}O_6$) is a six carbon atom-containing non-branched-chain polyhydric alcohol or hexitol, widespread in plants, and plant exudates, and has been extracted from manna of the manna ash (*Fraxinus ornus*), and marine algae. Commercially it is prepared by the electrolytic reduction of glucose. Mannitol is a white crystalline powder freely soluble in water, used widely in pharmacy as an excipient, that is to dilute liquids or to give form or consistency to solids, and in medicine as an osmotic diuretic.[2] More recently the widespread appreciation that reactive intermediates of oxygen are involved in a variety of tissue damaging processes (reviewed by Gutteridge,[3]) and that mannitol is a scavenger of hydroxyl radicals (·OH) has led to claims, both extravagant and modest, that some of its beneficial clinical effects are due to hydroxyl radical scavenging. Let us examine these claims in detail.

*To Whom correspondence should be addressed

2. WHAT ARE OXYGEN FREE RADICALS AND THEIR SCAVENGERS?

Molecules that react speedily with hydroxyl radicals are known as 'hydroxyl radical scavengers'. The speeds at which hydroxyl radicals react with scavenger molecules are defined in terms of second order rate constants, which are expressed as molar per second ($M^{-1}s^{-1}$). Hydroxyl radicals are so reactive that they damage the first molecule they meet within a few Ångstroms of their formation, and therefore survive for only a few nanoseconds. This type of chemistry is essentially diffusion controlled with second order rate constants of $10^9 - 10^{10}M^{-1}s^{-1}$.[4] The other important variable to consider when assessing the protective ability of a hydroxyl radical scavenger is the actual concentration present in relation to its second order rate constant. In biological systems hydroxyl radicals react far to rapidly to allow them to be directly detected and measured. We therefore seek evidence of their presence by looking for fingerprints of damage characteristic of ·OH attack. For example ·OH attack on the amino acid phenylalanine will give hydroxylation of the aromatic ring in three positions, yielding *ortho*, *meta* and *para* tyrosine isomers. Since only *para* tyrosine is thought to arise in the body by normal enzymic reactions, *ortho* and *meta* tyrosine appear to be useful markers of ·OH radical formation.[5,6] Another highly sensitive technique for indirectly measuring ·OH radicals is to use a chemical 'trap' which stabilises the unpaired electron (spin trapping) on a nitroxide molecule allowing a characteristic signal to be measured by electron paramagnetic resonance (epr) (reviewed in 7). When an ·OH radical attacks another molecule and abstracts an electron from it, an unpaired electron is left behind, and the attacked molecule itself becomes a free radical. When mannitol scavenges ·OH (rate constant $1.8 - 2.7 \times 10^9M^{-1}s^{-1}$)[8,9,10] a mannitol radical is formed (see Figure 1). However, mannitol is no different from any other sugar in this respect, for example glucose has a second order rate constant of $1.0 \times 10^9M^{-1}s^{-1}$.[4] The mannitol radical is of lower reactivity than ·OH, but nevertheless we have little knowledge of its harmful effects in biological systems.

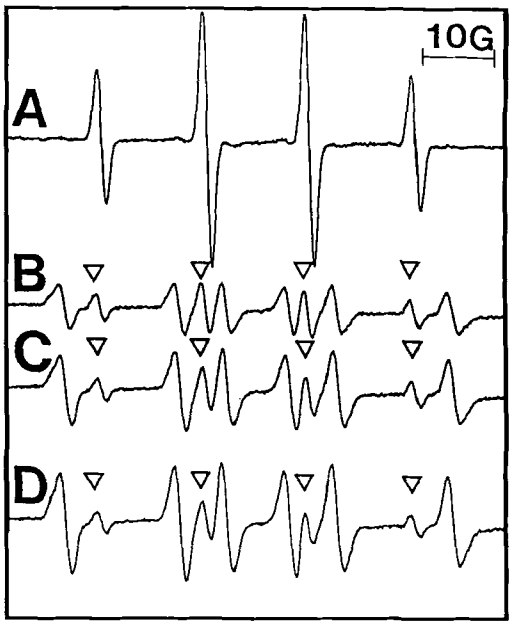

FIGURE 1 EPR spectra during the reaction of xanthine oxidase and hypoxanthine in the presence of EDTA-Fe^{3+}, and DMPO, with and without the addition of mannitol. (J.M.C. Gutteridge, G. Bruggemann and P.B. McCay, unpublished data). A. Reaction system without mannitol. B. Reaction system plus 200 mM mannitol. C. Reaction system plus 400 mM mannitol. D. Reaction system plus 600 mM mannitol. For the DMPO-mannitol adduct, a_N = 15.95 G, $a\frac{H}{B}$ = 22.75 G. For the hydroxyl radical adduct (indicated by inverted triangles over the peaks), $a_N = a\frac{H}{B}$ = 14.92 G.

3. REOXYGENATION INJURY FOLLOWING ISCHAEMIA-REPERFUSION

Depriving tissues of oxygen causes damage to them, and the only successful treatment is to restore blood flow as soon as possible. If ischaemia continues for too long irreversible damage occurs, cells die, and necrosis follows. There are no intervention procedures that restore life to dead cells. The term 'reperfusion injury' was first used by

Hearse and colleagues in 1973[11] to refer to an increase in damage observed when ischaemia is corrected by restoring oxygen supply to an organ. The molecular mechanisms of reoxygenation injury remained unclear until Guarnieri and colleagues[12] observed oxidative damage to lipids and decreases in tissue antioxidants during the reoxygenation of hypoxic rat hearts. Parks, Granger, McCord and their co-workers[13] proposed that the superoxide radical was a key intermediate in reperfusion injury. Their seminal proposal was that the enzyme xanthine dehydrogenase was proteolytically cleaved to xanthine oxidase which then acts on xanthine or hypoxanthine to generate superoxide and hydrogen peroxide. We now know that a variety of biochemical events are triggered during the period of ischaemia which facilitate ROS generation during the re-introduction of oxygen (reviewed by Gutteridge and Halliwell).[14] Reactive oxygen species appear to be generated in almost every organ or tissue model of ischaemia-reperfusion, and many different antioxidants, including mannitol, have been observed to protect against reoxygenation injury.[15]

Cardioplegia solutions are used to protect the heart during ischaemia, and provide a useful vehicle for introducing intervention therapies to protect against reoxygenation injury. Some of the uses of mannitol in cardioplegia and reoxygenation are listed in Table 1.

4. DOES MANNITOL PROTECT BY SCAVENGING HYDROXYL RADICALS?

The answer to the above question is 'we do not know' since it has never been unequivocally demonstrated. Let us, however, examine some of the problems inherent in the concept.

(a) Site-directed ·OH formation

Transition metals, particularly iron, are important in generating ·OH radicals in biological systems. Ferric ions are insoluble at physiological pH values and therefore need to be bound to a ligand in order to remain in solution. It is therefore highly likely that the ligand holding redox active iron will be the first target for any ·OH radicals it participates in generating. Similar considerations of site-directed ·OH formation also apply to the interaction of two endogenous free radicals, namely superoxide and nitric oxide, when they form peroxynitrite, although this reaction is iron independent. It is clear that the chance of competitively scavenging extremely fast site-directed chemical reactions without targeted delivery systems is remote, and at present we have no such delivery systems.

(b) Tissue concentrations of mannitol

Mannitol is not metabolized within the body,[16] and therefore provides a useful nontoxic molecule with which to test the ·OH scavenging hypothesis. Unfortunately, it is not well transported into cells, and tissue concentrations achieved during intervention therapy remain unknown. When considered with point (a) above we have even less knowledge of mannitol concentrations at the target site. Structural components and cell metabolites will always be present at higher concentrations than transported mannitol. Consequently, there is no realistic possibility that mannitol can protect against tissue damage in biological systems by scavenging ·OH.

(c) Limited reactivity of mannitol with oxygen free radicals

As we discussed in section 3, mannitol will react rapidly with ·OH radicals as a function of its second order rate constant and concentration. Mannitol does not react similarly with other reactive oxygen species such as superoxide, nitric oxide, peroxynitrite, alkoxyl or peroxyl radicals[7,17] to an extent that would offer protection in a biological system. For example, mannitol does not protect membranes against the chain reactions of lipid peroxidation.[17]

TABLE 1 Use of Mannitol in cardioplegia solutions

Model	Function/product assessed	Mannitol/Dose g/kg body wt (or other)	Comments	Reference
Human	plasma H_2O_2	0.17 g/kg	Decreased H_2O_2 by mannitol and allopurinol	24
Human	neutrophil stimulation	0.71 g/kg	Mannitol present in cardioplegia and reperfusion solutions. Difference seen only in patients receiving desferrioxamine	25
Human	Haemodynamic and isoenzymes	0.14–0.28 g/kg	No differences observed	26
Human	Biopsies, Chemiluminescence	0.09 g/kg	Reduction of atrial arrhythmias observed with mannitol	27
Human	H_2O_2	0.2 g/kg	Decreased H_2O_2 formation	23
Dog	Ventricular function	3 g/kg	SOD + mannitol in cardioplegia. Pretreatment with allopurinol, showed improved ventricular function.	28
Dog	Protection of mechanical and subcellular function	325 mM	SOD and mannitol improved sarcoplasmic reticulum calcium transport.	29
Dog	Calcium transport	20 mM	Calcium uptake depressed by O_2-generation. Protection by SOD but not with mannitol.	30
Rabbit	Ventricular function myocardial oedema	350mOsm/l	Mannitol improved all functions compared to glucose	31
Rabbit	^{31}P-NMR, contractility ATP recovery	10.7 g/kg	Major protection with SOD, mannitol minor improvements	32
Rabbit	Haemodynamic, oedema	20 mM	Mannitol reperfusion improved myocardial protection	33
Rat	Haemodynamic	100mOsm/l	Mannitol present in the cardioplegia solution. Added peroxidase, glutathione and SOD offered protection.	34
Rat	TBA-reactivity, Creatine kinase	73.3 g/kg	Cumene hydroperoxide stress. DMTU, catalase and allopurinol but not mannitol protected.	35
Rat	TBA-reactivity	5.62 g/kg	SOD, catalase, GSH, histidine vitamin E, mannitol tested. Variable effects observed depending on function measured.	36
Rat	Reperfusion arrhythmias	16.0 g/kg	SOD, GSH and ascorbic acid protective, mannitol and catalase not effective.	37
Rat	Haemodynamic	10 g/kg	Mannitol added to cardioplegia solutions. Protection afforded by desferrioxamine.	38

4.1 ARE THERE ANY OTHER CHEMICAL PROPERTIES OF MANNITOL THAT MIGHT OFFER PROTECTION AGAINST ROS?

Many simple chemical substances are likely to have multiple pharmacological properties. For example, the 'hydroxyl radical scavenger' dimethylsulphoxide (DMSO) ($CH_3{\cdot}SO{\cdot}CH_3$) is known to have over 20 different primary pharmacological actions.[18] It is likely that similar considerations also apply to mannitol although they may be fewer and less profound.

(a) Metal-binding by mannitol

Many carbohydrates are good metal chelating agents, in particular the hexitols; mannitol, sorbitol and dulcitol are good at complexing ferric ions.[19] For example, at pH 12.0 one gram of mannitol can complex 2.5 g of ferric ions.[19] Redox active iron plays a key role in converting poorly reactive oxygen species into highly reactive and damaging species such as ·OH radicals. Mannitol may therefore be able to protect against such damage by taking iron away from a biological molecule at risk of damage and transferring the damage to itself. Iron-binding protection by mannitol in Fenton reactions has been observed both in kinetic studies[20] and in vivo,[21] and may under appropriate conditions be considerably more important than scavenging. Competitive iron binding between the detector molecule (deoxyribose) and a hydroxyl radical scavenger has been proposed by the author[20] as an explanation as to why mannitol is highly protective in ferrous salt driven Fenton chemistry compared to non-sugar scavengers such as ethanol or benzoate. When mannitol is added, it binds iron in the reaction mixture and becomes a major recipient of ·OH radical damage, thereby protecting deoxyribose. Scavengers without superior iron-binding properties do not protect against site-directed Fenton chemistry on the deoxyribose molecule. When EDTA is added, however, all the iron is removed from deoxyribose to the EDTA which redox cycles the iron with release of ·OH radicals into free solution. The effectiveness of all ·OH scavengers in such a system is greatly increased, and is similar to that observed with pulse radiolysis experiments, where ·OH is radiolytically generated in free solution.[20] The ability of EDTA to increase the effectiveness of ·OH scavengers in Fenton reactions is referred to as its 'radiomimetic' effect.[20]

(b) Complexing with hydrogen peroxide

In 1909 the Russian scientist Tanator[22] reported that mannitol forms a stable equimolar compound with hydrogen peroxide. Since this observation was first made the reaction does not appear to have been explored in any detail. Hydrogen peroxide is not a free radical, but in the presence of redox active iron it will generate ·OH radicals. Removal of hydrogen peroxide in biological systems, however, by complexing with mannitol, would have to compete with highly specific enzymes such as catalase and glutathione peroxidase, which seems unlikely. Recently, Yang and co-workers[23] reported, as did England and colleagues in 1986,[24] that mannitol lowered H_2O_2 concentrations in patients undergoing artery bypass graft surgery (Table 1) thereby suggesting mannitol directly reacted with H_2O_2 or decreased its formation.

SUMMARY

Reactive oxygen species are constantly formed in biological systems. When production exceeds antioxidant protection, oxidative stress leading to molecular damage occurs. The most reactive ROS in biological systems is the hydroxyl radical which damages adjacent molecules at diffusion controlled rates. The possibility of preventing such chemistry inside cells with therapeutic doses of mannitol at present seem remote.

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