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Review article:

ANTIOXIDANT PROPERTIES OF PROPOFOL: WHEN OXIDATIVE STRESS SLEEPS WITH PATIENTS

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ABSTRACT

Propofol is an intravenous sedative-hypnotic agent indicated for induction and maintenance of general anesthesia as well as for sedation of intubated, mechanically ventilated adults in intensive care units (ICU). Propofol is characterized by a phenolic structure similar to that of α -tocopherol, and presents antioxidant properties that have been demonstrated both *in vitro* and *in vivo*. The aim of the present review is to evaluate the antioxidant properties of propofol in various models and whether or not it may be considered an efficient therapeutic tool in counteracting oxidative stress during general anesthesia and sedation in ICU.

Keywords: anesthesia; propofol; oxidative stress; antioxidants; polyphenols

Oxidative stress occurs when there is an imbalance between generation of reactive oxygen species (ROS) and inadequate antioxidant defence systems leading to cell damage either directly or through altering signalling pathways. The consequence of oxidative stress may be oxidative damage of lipids, proteins, and DNA, with subsequent disease development and aging (Finkel et al., 2000). ROS production may result from exogenous factors such as radiation and drug exposure or endogenous factors such as increased mitochondrial respiration and oxidative enzymes in infections and inflammation. Of particular interest to the researchers such as anesthetists, intensive care physicians and surgeons are free radical mediated reperfusion injuries such it occurs

following organ transplantation, and free radical and endotoxin induced acute inflammatory processes such as lung injury, severe sepsis, multi-system organ failure. Free radical scavengers, or anti-oxidants, represent an important component of the body's defence against oxygen-centered free radical mediated injuries, so that boosting of these defences by pharmacological means may emerge as an important therapeutic goal. The roles of vitamins A and E, ascorbic acid, salicylates, barbiturates, superoxide dismutase, polyunsaturated fatty acids and anti-inflammatory agents in ameliorating free radical damage are just but few examples of the importance of antioxidants in protecting tissues against oxidative stress (Aruoma et al., 1991; Buettner 1993; Przyklenk et al., 1989).

The aim of the present review is to evaluate the antioxidant properties of propofol in both *in vivo* and *in vitro* models and whether or not it may be considered an efficient therapeutic tool in counteracting oxidative stress during general anesthesia and sedation in the intensive care units (ICU).

Propofol is an intravenous sedative-hypnotic agent commercially introduced in the United States in 1989 by Zeneca Pharmaceuticals. It was the first of a new class of intravenous anesthetic agents: the alkylphenols. It is indicated for induction and maintenance of general anesthesia as well as for sedation of intubated, mechanically ventilated adults in ICU. Propofol is characterized by a phenolic structure similar to that of α -tocopherol (Figure 1), and presents antioxidant properties that have been demonstrated both *in vitro* and *in vivo*.

Many polyphenols have antioxidant properties (i.e., reductants) and may react directly with reactive chemical species, forming products with much lower reactivity.

In 1989, Weir D. et al (Weir et al., 1989) observed for the first time that propofol attenuated experimental reperfusion injury in the cerebral cortex thus pointing out the antioxidant properties of this compound. Furthermore, Murphy PG et al (Murphy et al., 1992) demonstrated that propofol reacts with peroxynitrite leading to the formation of a more stable propofol-derived phenoxyl radical and has therefore been hypothesized to be a peroxynitrite scavenger. As concern propofol scavenging activity, Green TR et al. (Green et al., 1994) evaluated the specificity of propofol as a scavenger of oxygen and organic radical species showing that propofol preferentially scavenges organoradical species since it was able to reduce significantly riboflavin radicals and the formation of malondialdehyde degradation products

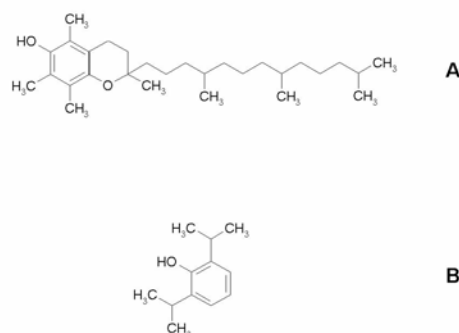


Figure 1: Chemical structure of α -tocopherol (A) and propofol (B) showing their polyphenolic nature.

generated from lipid hydroperoxides of arachidonic acid. These results were further confirmed by following studies showing that propofol is able to inhibit lipid peroxidation in various experimental models (Sayin et al., 2002; Manataki et al., 2001) to protect cells against oxidative stress and to increase the anti-oxidant capacity of plasma in humans (Stratford et al., 1998; Chen et al., 2005; Hans et al., 1997; Sagara et al., 1999). Alternatively, polyphenols may increase the capacity of endogenous antioxidant defences and modulate the cellular redox state. Changes in the cellular redox state, conveying physiologic stimuli through regulation of signalling pathways, may have wide-ranging consequences for cellular growth and differentiation. In addition, it has been well documented that polyphenols modulate protein kinase activities (Agarwal 2000), serve as ligands for transcription factors (Amakura et al., 2003), and modulate protease activities (Moon et al., 2003). The majority of *in vitro* and *in vivo* studies conducted so far have attributed the protective effect of bioactive polyphenols to their chemical reactivity toward free radicals and their capacity to prevent the oxidation of important

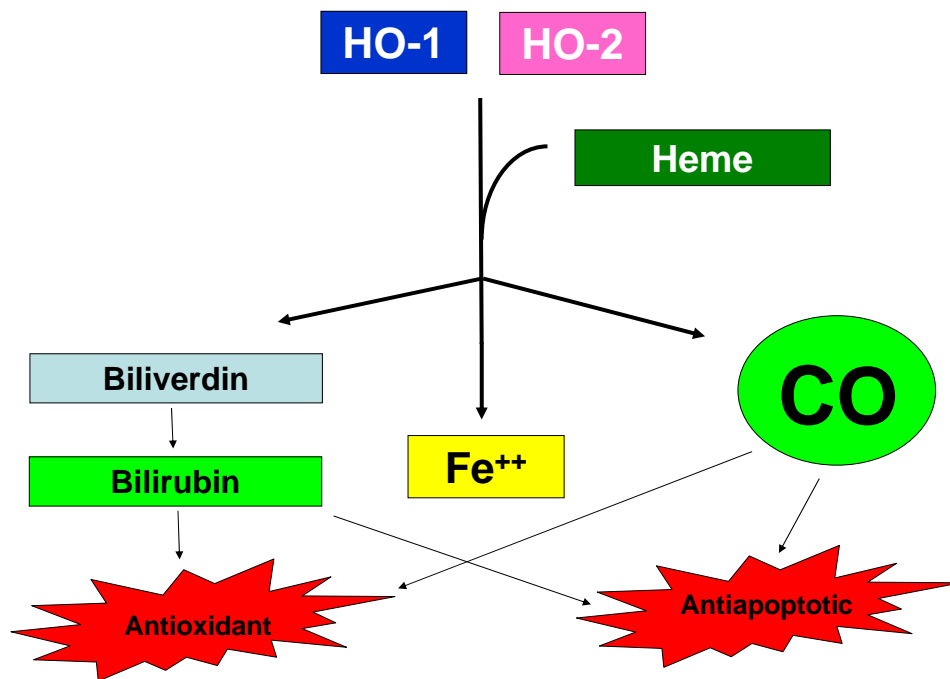


Figure 2: Schematic representation of HO activity.

intracellular components. However, Scapagnini G et al. (Scapagnini et al., 2002) suggested a possible novel aspect in the mode of action of these compounds; that is, the ultimate stimulation of the heme oxygenase-1 (HO-1) pathway is likely to account for the established and powerful antioxidant/anti-inflammatory properties of these polyphenols. In 2004, we studied experimentally and proved scavenger activity and effects on the HO system as an alternative and additional protective mechanism of propofol (Acquaviva et al., 2004). HO isoforms catalyze the conversion of heme to carbon monoxide (CO) and biliverdin/bilirubin with a concurrent release of iron, which can drive the synthesis of ferritin for iron sequestration (Eisenstein et al., 1991; Shibahara et al., 1993) (Figure 2).

HO is the limiting step in heme degradation and, consequently, plays a critical role in regulating the levels of cellular heme available for structural and functional heme-

dependent proteins (Abraham et al., 1988). To date, two HO isoforms have been shown to be catalytically active in heme degradation, and each is encoded by a different gene (McCoubrey, Jr. et al., 1992; Shibahara et al., 1993). HO-1, the inducible isoform, is found ubiquitously in all organs with the exception of the adult brain and is rapidly and transiently expressed by a range of stressful stimuli; in contrast, HO-2 is the constitutive isozyme which, apart from controlling the basal levels of heme in the majority of cells, may also mediate important physiological effects such as vasodilatation and neurotransmission (Abraham et al., 1988). We demonstrated that peroxynitrite cytotoxicity in astrocytes is significantly attenuated by propofol in a dose-dependent manner. Furthermore, we showed that peroxynitrite-mediated DNA damage was attenuated by the addition of propofol in a dose-dependent manner. In this study, all the

above mentioned antioxidant effects of propofol were mitigated by the addition of SnMP, a strong pharmacological inhibitor of HO activity, thus suggesting HO as a protective pathway of propofol (Figure 3).

Our results are consistent with previous clinical studies supporting also a protective effect of propofol against peroxidative injury. Propofol reduced levels of malondialdehyde, a marker of lipid peroxidation, after tourniquet-induced lower limb ischemia-reperfusion injury (Aldemir et al., 2001), and decreased oxidative damage measured in platelets (De La Cruz et al., 1999) from surgical patients. These in vivo results were further confirmed by Sayin MM and Zhang SH et al. (Sayin et al., 2002; Zhang et al., 2004), demonstrating that during coronary artery bypass grafting operations, propofol was more efficient than fentanyl in suppressing lipid peroxidation, F2-isoprostanes, complement C5a and neutrophil adhesion rate.

In addition, propofol mediated HO-1 induction may explain in part some effects of this compound on important heme proteins such as cytochrome P450 (CYP450) and cyclooxygenase. In this regard, Yang LQ et al. (Yang et al., 2003) showed that propofol may be a potential CYP450 inhibitor as it can significantly inhibit 3A4 isoenzyme activity without significantly affecting the protein expression. Furthermore, Ogawa K. et al. (Ogawa et al., 2001) showed that propofol reduces cyclooxygenase activity, as measured by prostaglandin production. Recently, Li Volti G. et al. (Li Volti et al., 2003) showed that pharmacological and retroviral mediated upregulation of HO-1 reduces intracellular heme content and this may account for reduced cyclooxygenase activity. Consistently with our observations, Taille C et al (Taille et al., 2004) elegantly demonstrated that a decrease in heme content due to HO-1 activation limits heme availability for maturation of the gp91(phox) subunit and

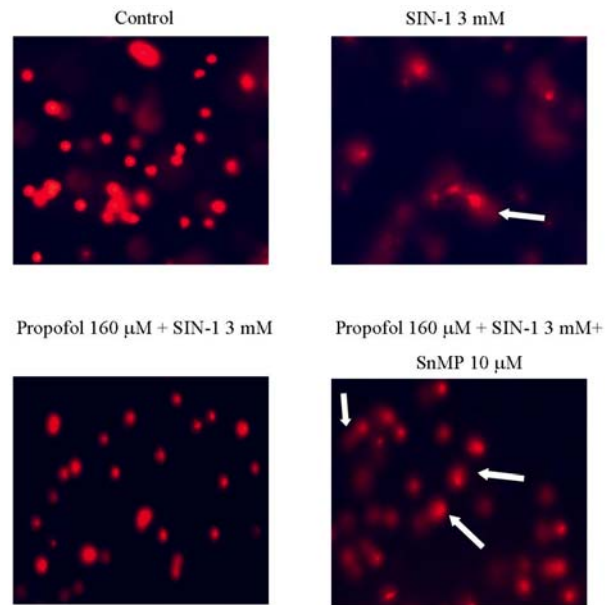


Figure 3: Comet assay of genomic DNA of rat astrocytes. Astrocytes were treated for 24 h with propofol and SIN-1, a peroxy-nitrite donor. Arrows indicate DNA fragmentation.

assembly of the functional NAD(P)H oxidase and thus attenuating free radicals formation and oxidative stress damage. Since heme functions as the prosthetic group in both CYP3A4 and cyclooxygenase, propofol mediated HO-1 induction may be responsible for their reduced activity by lowering intracellular heme content. In addition, since the gene encoding for HO-1 protein contains an NFkB binding site which can be selectively recognized by the ubiquitous transcriptional factor NFkB (Lavrovsky et al., 1993), we tested whether this important transcription factor could play a role in our experimental conditions. We showed that the addition of a synthetic NFkB inhibitor completely reversed propofol mediated HO-1 expression thus suggesting that this nuclear factor is involved in the intracellular cascade following propofol treatment (Acquaviva et al., 2004).

Another important effect of propofol

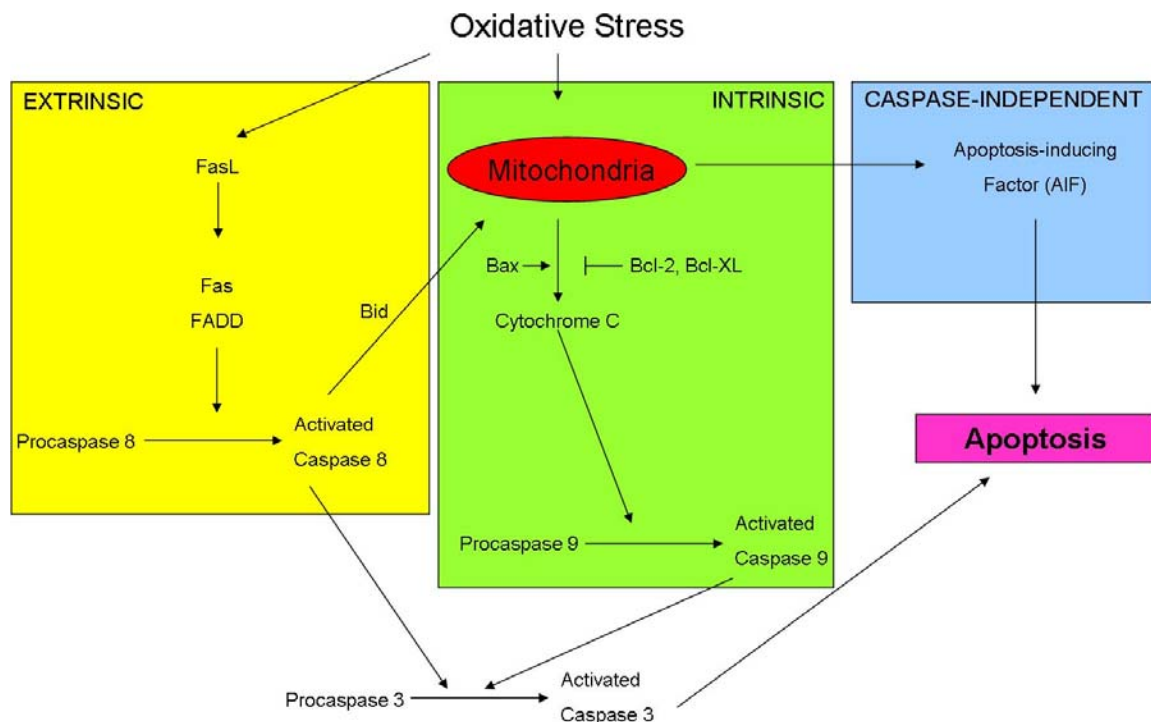


Figure 4: Signaling pathways of ischemia-induced apoptosis, including intrinsic (mitochondria-mediated), extrinsic (receptor-mediated), and caspase-independent pathways. Apoptotic insults lead to cytochrome *c* release from the mitochondria into the cytoplasm, which forms the apoptosome consisting of Apaf-1 and procaspase 9 and then activate caspase 9. This ultimately results in activation of caspase 3. The release of cytochrome *c* is regulated by proapoptotic proteins Bax and antiapoptotic proteins Bcl-2 and Bcl-XL. The death receptor pathway is triggered by the binding of Fas ligand (*FasL*) to Fas, which activates caspase 8 via Fas-associated death domain protein (*FADD*). A number of different cell receptors and their ligands can be involved in this process. Activated caspase 8 then activates caspase 3 and can also interact with the intrinsic pathway by activating proapoptotic protein Bid. A caspase-independent pathway also exists in which apoptotic-inducing factor is released from the mitochondria and directly causes chromatin condensation and DNA cleavage.

which needs also to be described for its clinical relevance is the antiapoptotic effect of this compound.

Apoptosis refers to programmed cell death. This endogenous program is essential in the modeling of organs during fetal life, enabling self-renewing processes, and eliminating infected or injured cells in adults. Superfluous cells die by apoptosis, and their death is initiated by a genetic program. When these dying cells have disintegrated into

apoptotic bodies, they are phagocytosed by macrophages without inflammatory response. Although the mechanism by which apoptosis is triggered is not clear, signaling pathways of ischemia-induced apoptosis may include intrinsic (mitochondria-mediated), extrinsic (receptor-mediated), and caspase-independent pathways (Zheng et al., 2003). The intrinsic pathway is characterized by cytochrome *c* release from mitochondria, which leads to procaspase 9 cleavage and

activation. This ultimately results in activation of effector caspases, including caspase 3 (Fujimura et al., 1998; Fujimura et al., 1999; Green 1998). The extrinsic pathway is characterized by activation of cell death receptors initiated by their ligands [e.g., FasL, tumor necrosis factor- α (TNF- α)], which leads to cleavage of procaspase 8. Cleaved caspase 8 then activates downstream caspases and results in apoptosis (Martin-Villalba et al., 1999; Rosenbaum et al., 2000). By contrast, apoptosis-inducing factor (AIF), which is released from the mitochondria, is also thought to be an important candidate responsible for apoptosis via caspase-independent pathways (Zheng et al., 2003) (Figure 4).

Engelhard K et al (Engelhard et al., 2004) showed that propofol possess antiapoptotic qualities by influencing apoptosis-regulating proteins after cerebral ischemia. The authors

showed that expression of the pro-apoptotic protein Bax was greater in control animals than in propofol anesthetized rats and than in sham-operated animals. Consistently with these results, we showed that propofol treatment was able to attenuate peroxyntirite mediated caspase 3 activation probably via the generation of HO byproduct CO and/or bilirubin.

However, whether the cytoprotective effect of propofol accurately translates to a therapeutic effect in patients remains uncertain.

In conclusion, the data presented above show that propofol posses anti-oxidative and anti-apoptotic properties, and involve important enzymatic systems, such as HO-1, in exerting its effects thus providing an important and powerful strategy for protection during anesthesia (Figure 5).

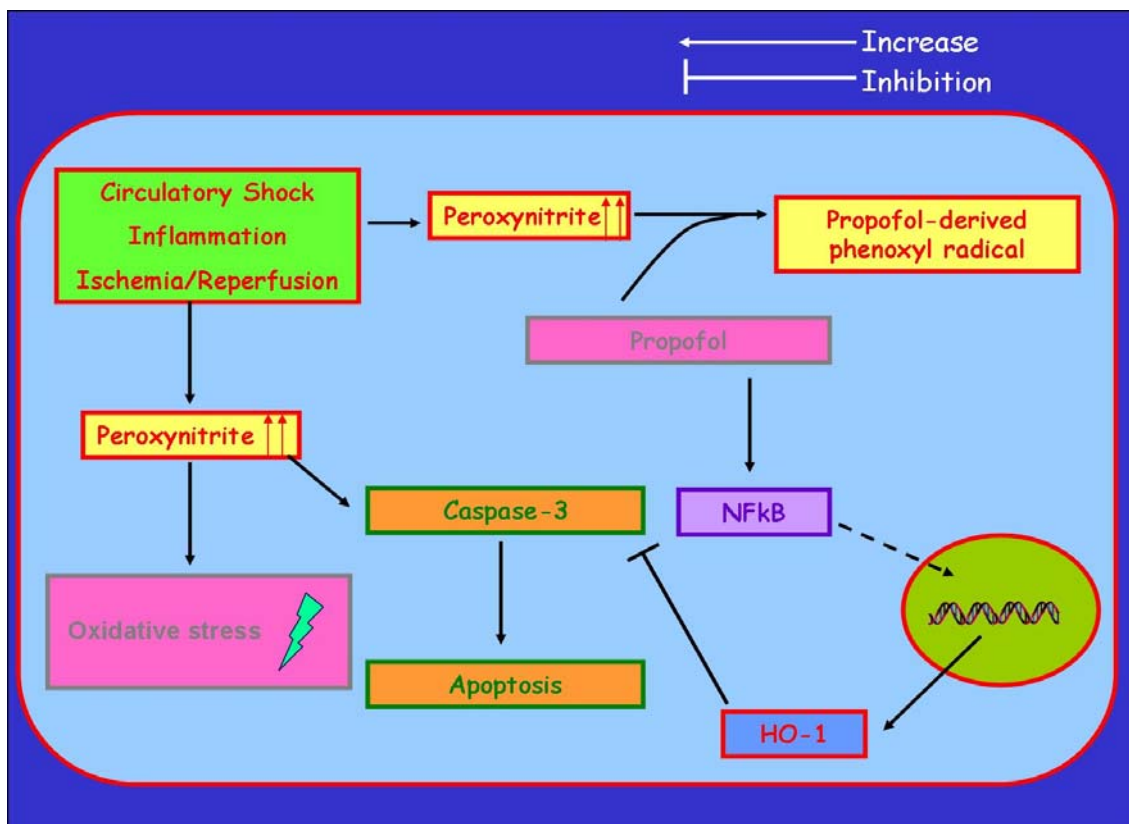


Figure 5: Schematic representation of possible protective mechanism(s) of propofol.

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