How abundant are superoxide and hydrogen peroxide in the vasculature lumen, how far can they reach?

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ABSTRACT

Paracrine superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) signaling critically depends on these substances' concentrations, half-lives and transport ranges in extracellular media. Here we estimated these parameters for the lumen of human capillaries, arterioles and arteries using reaction-diffusion-advection models. These models considered O$_2^-$ and H$_2$O$_2$ production by endothelial cells and uptake by erythrocytes and endothelial cells. O$_2^-$ dismutation, O$_2^-$ and H$_2$O$_2$ diffusion and advection by the blood flow. Results show that in this environment O$_2^-$ and H$_2$O$_2$ have half-lives <60 ms and <40 ms, respectively, the former determined by the plasma SOD3 activity, the latter by clearance by endothelial cells and erythrocytes. H$_2$O$_2$ concentrations do not exceed the 10 nM scale. Maximal O$_2^-$ concentrations near vessel walls exceed H$_2$O$_2$'s several-fold when the latter results solely from O$_2^-$ dismutation. Cytosolic dismutation of inflowing O$_2^-$ may thus significantly contribute to H$_2$O$_2$ delivery to cells. O$_2^-$ concentrations near vessel walls decay to 50% of maximum 12 μm downstream from O$_2^-$ production sites. H$_2$O$_2$ concentrations in capillaries decay to 50% of maximum 22 μm downstream from O$_2^-$ (H$_2$O$_2$) production sites. Near arterioles (‘arteries’) walls, they decay by 50% within 6.0 μm (4.0 μm) of H$_2$O$_2$ production sites. However, they reach maximal values 50 μm (24 μm) downstream from O$_2^-$ production sites and decrease by 50% over 650 μm (500 μm). Arterial/olar endothelial cells might thus signal over a mm downstream through O$_2^-$-derived H$_2$O$_2$, though this requires nM-sensitive H$_2$O$_2$ transduction mechanisms.

1. Introduction

Signaling through superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) released by cells to the extracellular medium mediates numerous physiological processes [1–6]. However, whether such signaling is autocrine, near-cell paracrine or volume is under discussion [7–10]. Likewise, the extracellular concentrations involved in physiological signaling remain uncertain, and this knowledge is critical to understand what intracellular mechanisms can viably transduce such signals [11, 12].

Redox signaling plays a prominent role in vascular processes [4,5, 13–16]. For example, H$_2$O$_2$ may act as an agent that promotes endothelial derived hyperpolarization of vascular endothelial and smooth muscle cells by stimulating the elevation of the concentration of Ca$^{2+}$ ions and opening of K$_{Ca}$ channels. This increase in cell polarization is associated to vascular dilation [17]. The present work focuses on signaling through the microvasculature lumen, where the blood flow may help O$_2^-$ and H$_2$O$_2$ reach downstream cells. Mechanical and other types of stimuli to vascular endothelial cells (EC) trigger intracellular phosphorylation cascades that activate NADPH oxidase 2 (NOX2) at the plasma membrane [4]. NOX2 catalyzes one-electron O$_2$ reduction by cytosolic NADPH at a 2 O$_2$/NADPH stoichiometry. The resulting O$_2^-$ is released to the extracellular medium, where it has three main fates. First, it is absorbed by both erythrocytes and ECs through chloride channels [18–20], and then dismutated to O$_2$ and H$_2$O$_2$ via cytosolic superoxide dismutase (SOD1). Second, it is dismutated via extracellular superoxide dismutase (SOD3). Erythrocytes and ECs absorb the resulting H$_2$O$_2$ [21,22], which readily oxidizes the cytosolic peroxiredoxins (Prdx) 1 and 2 (k = 0.13-1.6 × 10$^6$ M$^{-1}$s$^{-1}$ [23–26]), eventually driving
Abbreviations:

BAEC  bovine artery endothelial cells
EC   endothelial cell
NOX  NADPH oxidase
Prdx peroxiredoxin
SOD  superoxide dismutase

redox relays that transduce these signals [27–30]. Third, it reacts extremely fast with nitric oxide (\(*\)NO\)) \((k = 1.9 \times 10^7 \text{ M}^{-1} \text{s}^{-1})\) [31,32]. Consequently, in the presence of typical nM \(*\)NO concentrations in blood plasma [33], \(\text{O}_2^\bullet\) has a half-life <30 ms. The resulting peroxynitrite (ONOO\(^{-}\)) [34] can also cross cell membranes through anion channels and by passive permeation [35–37], and quickly oxidizes Prdx1/2 \((k = 10^7 \text{ M}^{-1} \text{s}^{-1})\) [25,38]). However, most of it reacts with \(\text{O}_2\) \((1.3 \text{ mM in plasma}, k = 5.8 \times 10^4 \text{ M}^{-1} \text{s}^{-1})\) [32,39]) and other blood plasma components. Therefore, \(*\)NO production by the ECs suppresses \(\text{O}_2^\bullet\)-derived \(\text{H}_2\text{O}_2\) production, and the ONOO\(^{-}\) thus formed cannot functionally replace \(\text{H}_2\text{O}_2\) in redox signaling. To a good approximation, only the excess \(\text{O}_2^\bullet\) production over the \(*\)NO production is available for \(\text{H}_2\text{O}_2\)-mediated signaling. Contractile vessels continuously produce \(*\)NO to maintain their vascular tone, which should eliminate most of the \(\text{O}_2^\bullet\). However, in the early moments after a stimulus that increased \(\text{O}_2^\bullet\) production or/and decreased \(*\)NO production, before the vasoconstriction response, an excess \(\text{O}_2^\bullet\) production can coexist with laminar flow.

Whether ECs can also directly release \(\text{H}_2\text{O}_2\) to the vascular lumen remains contentious [15,40]. ECs abundantly express NOX4 [15,40], which catalyzes \(\text{O}_2\) reduction to \(\text{H}_2\text{O}_2\) by NADPH at a rate that is sensitive to physiological \(\text{O}_2\) concentrations [41]. However, most of the NOX4 in ECs localizes to internal membranes, and evidence that some of it localizes to the cell membrane is lacking [40]. Nevertheless, there is evidence that NOX4 levels modulate \(\text{H}_2\text{O}_2\) release by murine lung ECs and by the intact mouse aorta [15], and therefore the possibility that ECs release \(\text{H}_2\text{O}_2\) to the vascular lumen deserves consideration.

Mathematical modeling has previously proved informative about the spatial distribution and dynamics of reactive nitrogen/oxygen species in the vasculature (eg. Refs. [42–48]). Therefore, here we used reaction-diffusion-advection models to estimate how far \(\text{O}_2^\bullet\) and \(\text{H}_2\text{O}_2\) can travel through capillaries, arterioles and arteries, and what concentrations are attainable under conditions where ECs release \(\text{O}_2^\bullet\) in excess of \(*\)NO or release \(\text{H}_2\text{O}_2\). We have also examined what are the main determinants of these distances and concentrations. We discuss these results in the context of other works that address the distribution, concentration and lifetimes of \(\text{H}_2\text{O}_2\) in animal tissues.

2. Models, parameter estimates and methods

2.1. Models and methods

The implemented reaction-advection-diffusion models of the spatio-temporal dynamics of \(\text{O}_2^\bullet\) and \(\text{H}_2\text{O}_2\) concentrations \((C_0, C_H\), respectively\) in the vasculature lumen consider the processes and geometries depicted in Fig. 1. In here, \(C_0\) and \(C_H\) depend on the position along the vessel, \(z\), and on the distance \(r\) to the center of the vessel according to the following equations:

\[
\frac{\partial C_0}{\partial t} = D_0 \left( \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C_0}{\partial r} \right) \right) - \nu(r) \frac{\partial C_0}{\partial z} - 2k_{\text{O}_2} C_0 - k_{\text{O}_2} H(r) C_0 ,
\]

\[
\frac{\partial C_H}{\partial t} = D_H \left( \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C_H}{\partial r} \right) \right) - \nu(r) \frac{\partial C_H}{\partial z} + k_{\text{O}_2} C_0 - k_{\text{H}_2\text{O}_2} H(r) C_H ,
\]

where the symbols have the meanings described in Table 1. We assume that blood is flowing in the laminar regime, with its velocity following Poiseuille’s law, \(v = v_{\text{max}} (R^2 - r^2)\).

We consider that \(\text{O}_2^\bullet\) is released to the lumen by a 20 µm-long ring of ECs (the length of a large EC), which also intake \(\text{O}_2^\bullet\) and \(\text{H}_2\text{O}_2\) as all the other ECs. This is achieved with the following boundary conditions at the vessel wall:

\[
\left. \frac{\partial C_0}{\partial r} \right|_{r=R} = P \delta_t(z) - k_{\text{O}_2} C_0 (R, z),
\]

\[
\left. \frac{\partial C_H}{\partial r} \right|_{r=R} = - k_{\text{H}_2\text{O}_2} C_H (R, z),
\]

where \(\delta_t(z) = 1\) at the \(\text{O}_2^\bullet\)-producing cells and \(\delta_t(z) = 0\) everywhere else along the vessel wall.

In capillaries (Fig. 1A), erythrocytes accumulate in the vicinity of the vessel axis, leaving an erythrocyte-free zone near the vessel wall [59,55]. Accordingly, in a neighborhood of width \(\Delta = 1.65 \mu\text{m}\) of the vessel wall the hematocrit is set to zero, and a “wall” of erythrocytes is located at \(r = R - \Delta\). Here, we implement the following boundary conditions,
Table 1
Symbol meanings and parameter values.

| Vessel | Symbol | Meaning | Value | References |
|--------|--------|---------|-------|------------|
| Arterioles | R | Radius | 25 μm | [54] |
| | v_{max} | Maximum velocity of blood flow | 1.0 × 10^4 μm s\(^{-1}\) | [56] |
| | k_{OH} | Effective rate constant for H\(_2\)O\(_2\) consumption by erythrocytes | 18 s\(^{-1}\) | 0.05 & \(\frac{A_{E}}{V_{E}}\) | [56] |
| | k_{O\bar{H}} | Effective rate constant for O\(_2\)\(^{-}\) consumption by erythrocytes | 2.2 s\(^{-1}\) | 0.05 & \(\frac{A_{E}}{V_{E}}\) | [56] |

which describe the intake of O\(_2\)\(^{-}\) and H\(_2\)O\(_2\) by the erythrocytes:

\[
\frac{dC_{O_{2}^{-}}}{dr}|_{r=R} = \kappa_{O_{2}^{-}} C_{O_{2}^{-}}(R, z),
\]

\[
\frac{dC_{H_{2}O_{2}}}{dr}|_{r=R} = \kappa_{H_{2}O_{2}} C_{H_{2}O_{2}}(R, z).
\]

In turn, for the arteriole (Fig. 1B), the hematocrit function, \(Hc(r) = 1.045 \times \left(1 + \frac{1}{r^{0.5}}\right) - 0.5\), where \(r\) is measured in μm, is fitted to observations reported in 1.4A of reference [55]. For the artery the same function for the hematocrit is used in the 125 μm < \(r\) < 150 μm range, while for 0 < \(r\) < 125 μm we consider a constant value for the hematocrit, \(Hc(r) = 0.5225\). In both these cases, we implement Neumann boundary conditions at the center of the vessel:

\[
\frac{dC_{O_{2}^{-}}}{dr}|_{r=0} = \frac{dC_{H_{2}O_{2}}}{dr}|_{r=0} = 0.
\]

Periodic boundary conditions on \(C_{O_{2}^{-}}\) and \(C_{H_{2}O_{2}}\) are implemented at the extremities of the vessel (\(z = 0\) and \(z = z_{max}\)). Nevertheless, the site of O\(_2\)\(^{-}\)-producing cells and the length of the simulation boxes are chosen such that both \(C_{O_{2}^{-}}\) and \(C_{H_{2}O_{2}}\) are vanishingly small at both extremities.

Henceforth we will denote the just-described models for capillaries and arterioles/arteries by “Model C” and “Model A”, respectively.

The models for the case where ECs release H\(_2\)O\(_2\) instead of O\(_2\)\(^{-}\) are particular cases of the formulations above obtained by neglecting Equation (1) and the boundary conditions for O\(_2\)\(^{-}\), by setting \(k_{OH} = 0\) in Equation (2), and by introducing the H\(_2\)O\(_2\) production term \(\frac{1}{4}P_{H_{2}O_{2}}(z)\) in the corresponding boundary condition. (Note that O\(_2\)\(^{-}\) dismutation generates one H\(_2\)O\(_2\) molecule from two O\(_2\)\(^{-}\) ions, and consequently systems with O\(_2\)\(^{-}\) production equal to \(P\) and with H\(_2\)O\(_2\) production equal to \(\frac{1}{4}P\) generate H\(_2\)O\(_2\) at the same rate if all the O\(_2\)\(^{-}\) is dismutated.)

Equations (1) and (2) were integrated numerically using finite differences in simulation boxes of sizes 33 × 200 for the capillary (\(\Delta r = 0.05\) μm and \(\Delta z = 2\) μm), 20 × 2500 for the arteriole (\(\Delta r = 1.25\) μm and \(\Delta z = 2\) μm), and 120 × 5000 for the artery (\(\Delta r = 1.25\) μm and \(\Delta z = 2\) μm). The integration was carried out until the concentrations reached their stationary values.

The same code, with \(\delta(z) = 1\) throughout the vessel’s length, was used to obtain \(C_{O_{2}^{-}}\) and \(C_{H_{2}O_{2}}\) when all ECs produce either O\(_2\)\(^{-}\) or H\(_2\)O\(_2\).

2.2. Parameter estimates

2.2.1. Superoxide and hydrogen peroxide concentrations and production rates in the microvasculature

In this linear model, the spatial distributions of O\(_2\)\(^{-}\) and H\(_2\)O\(_2\) concentrations are independent of the O\(_2\)\(^{-}\) or H\(_2\)O\(_2\) release rate by human microvascular ECs, but knowing the value of this parameter is essential to estimate local O\(_2\)\(^{-}\) and H\(_2\)O\(_2\) concentrations. Because we are unaware of any experimental determinations of this rate, we assessed its plausible range based on the following findings. Cultured BAEC released (0.64–1.9) × 10\(^3\) H\(_2\)O\(_2\) molecules/s/cell in the physiological range of tissue O\(_2\) concentrations [60,61]. These authors did not determine whether the detected H\(_2\)O\(_2\) resulted from dismutation of released O\(_2\)\(^{-}\) or was directly released by the cells. Therefore, we analyzed two extreme scenarios. In the first scenario, which appears closer to reality, we assumed that all the detected H\(_2\)O\(_2\) resulted from dismutation of released O\(_2\)\(^{-}\) that escaped reaction with eventually produced NO and reabsorption by the cells. This translates into a (1.2–3.8) × 10\(^3\) O\(_2\)\(^{-}\) molecules/s/cell net release rate. Dividing this range’s upper bound by the area of contact of an EC with the vessel lumen in the considered geometry yields the reference value of \(P\) in Table 1. Despite species, cell type and environment differences, basal net O\(_2\)\(^{-}\) release rates by human vascular ECs in vivo can reasonably be expected to be in the same order of magnitude. In turn, some cells release O\(_2\)\(^{-}\) at much higher rates when
2.2.2. Permeability of ECs and erythrocytes to $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$

Under physiological conditions, both $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ are readily consumed in the cytosol of erythrocytes and ECs, the former species by superoxide dismutase and the latter by Prdx. Therefore, the rates of cellular consumption of these species in the blood plasma is limited by these cells’ membrane permeabilities.

Orrico et al. [22] determined a 16. $\mu$m s$^{-1}$ permeability constant of human erythrocytes for $\text{H}_2\text{O}_2$, 2.8-fold higher than a previous more indirect estimate [65]. We estimated a 19. $\mu$m s$^{-1}$ permeability constant from $\text{H}_2\text{O}_2$ consumption rates determined in Ref. [21] for HUVEC cultures exposed to low $\text{H}_2\text{O}_2$ concentrations (details in Supplementary Information section 1, S1).

Erythrocytes and ECs are permeable to $\text{O}_2^\bullet -$ [18–20], but we are unaware of experimental data to estimate the corresponding permeability constants. So, we considered the permeability constants of both erythrocyte and EC membranes for $\text{O}_2^\bullet -$ as 1/10th of that estimated for $\text{H}_2\text{O}_2$ in ECs and assessed the effect of changing this estimate.

3. Results

Below we focus mainly on EC-to-EC signaling, for which the $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ concentrations at the ECs’ surface and their influx rates are the most relevant considerations. The range over which cells can communicate through a diffusible substance depends on how far it is transported within its lifetime and on the concentration threshold for signaling. We will express the former parameter as the distance at which the $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ concentrations decrease to 50% of maximum values.

Orrico et al. [22] reported that treatment of Matrigel-seeded blood-brain barrier microvascular ECs with 1–10 nM $\text{H}_2\text{O}_2$ boluses for 2 h significantly increased EC tube length in a dose-dependent way. This observation suggests that ECs can sense and respond to $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ concentrations in the nM range. However, this inference needs confirmation by experiments including the determination of in situ $\text{H}_2\text{O}_2$ concentrations, as $\text{O}_2^\bullet -$/$\text{H}_2\text{O}_2$ release by the ECs themselves might lead to substantially higher local concentrations than the nominal ones. On the other hand, signaling likely requires local extracellular concentrations to substantially exceed cytosolic ones. This is because virtually all the signal transduction mechanisms for extracellular $\text{H}_2\text{O}_2$ considered to date depend on $\text{H}_2\text{O}_2$ influx into cells [11,66,67], and $\text{H}_2\text{O}_2$ is not known to be actively transported across membranes. As basal cytosolic $\text{H}_2\text{O}_2$ concentrations in human cells are in the 0.1 nM range [68,69], below we consider extracellular $\text{H}_2\text{O}_2$’s concentration threshold for signaling as 1 nM.

3.1. Potential $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ signaling in capillaries is short-range

Numerical integration of Model C reveals that the $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ concentrations at the surface of the ECs are very localized at the vicinity of the $\text{O}_2^\bullet -$-producing cell (green cell in Fig. 2A). The concentrations of $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ steeply decrease in the longitudinal direction away from the $\text{O}_2^\bullet -$ supply zone, reaching 50% of their maximal values within 12 $\mu$m and 22 $\mu$m downstream of the site where the peak concentration is reached, respectively. And the $\text{H}_2\text{O}_2$ concentration falls below the 1 nM signaling threshold within 46. $\mu$m of the peak. In turn, the concentrations of both species remain virtually constant in the radial direction over the short span of the plasma region (Fig. 2B and C). This region is so narrow that molecules of both species cross it within 1 ms, given their diffusion constants. The large contact areas of the plasma with the EC and erythrocyte layers and the narrow width of the plasma layer, lead to the rapid absorption of $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ by both erythrocytes and ECs. The following fractions of $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ that are absorbed by each layer or dismutated (in the case of $\text{O}_2^\bullet -$) at steady state for the reference parameter values in Table 1 can be readily calculated (SI2). Of the $\text{O}_2^\bullet -$ molecules, 80% are dismutated, while the other 12% are absorbed by the ECs and 8% are absorbed by erythrocytes. In turn, of the $\text{H}_2\text{O}_2$ molecules, 64% are absorbed by the ECs, while the other 36% are absorbed by the erythrocytes in the capillary.

The blood flow causes a slight asymmetry of concentration distributions around the $\text{O}_2^\bullet -$ supply zone, and a small 2 $\mu$m gap between the concentration peak of $\text{H}_2\text{O}_2$ and that estimated for $\text{O}_2^\bullet -$ (Fig. 2A). But altogether, these results show that the slow blood flow in capillaries does not substantially advect $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ during their short lifetime, their spread being diffusion-dominated.

3.2. Short-range $\text{O}_2^\bullet -$ and long-range $\text{H}_2\text{O}_2$ transport in arterioles and arteries

Numerical integration of Model A reveals a very different distribution of $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ at the ECs’ surface in arterioles (Fig. 3A). The $\text{O}_2^\bullet -$ concentration attains its maximum in the longitudinal direction within the supply zone (in green) and decays by 50% by 12 $\mu$m downstream. In contrast, the $\text{H}_2\text{O}_2$ concentration has a broad maximum, peaking at 50

![Fig. 2. Spatial distribution of $\text{O}_2^\bullet -$ and $\text{H}_2\text{O}_2$ in capillaries. (A) $\text{O}_2^\bullet -$ (blue) and $\text{H}_2\text{O}_2$ (red) concentrations at the surface of the ECs along the arteriolar length. The background colors represent the average concentration at the surface of each EC represented at the top. The horizontal dashed line marks the assumed signaling threshold. The inset shows the $\text{H}_2\text{O}_2$ and $\text{O}_2^\bullet -$ concentrations in the same scale. (B, C) $\text{O}_2^\bullet -$ (B) and $\text{H}_2\text{O}_2$ (C) concentrations along the radial and longitudinal directions. The position of the $\text{O}_2^\bullet -$-producing ECs is marked in green in all panels. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stimulated: $4 \times 10^6 \text{O}_2^\bullet -$ molecules/s/cell for internal mammary smooth muscle cells stimulated with 100 U/ml IL-1$\beta$ [62], and (1–40) $\times 10^6 \text{O}_2^\bullet -$ molecules/s/cell for polymorphonuclear leukocytes and Kupffer cells [63,64]. In the second scenario we assumed that all the detected $\text{H}_2\text{O}_2$ was directly released.
μm downstream of the $\text{O}_2^\bullet$ maximum, and decays by 50% over 650 μm downstream from the maximum. The concentrations of both species also decrease substantially in the radial direction away from the $\text{O}_2^\bullet$-producing ECs (Fig. 3B and C), with $\text{H}_2\text{O}_2$ penetrating deeper into the vessel.

The very different distribution of $\text{O}_2^\bullet$ and $\text{H}_2\text{O}_2$ at the ECs’ surface has the following explanation. Because the velocity of blood near the vessel’s wall is very low, $\text{O}_2^\bullet$ molecules that stay in this region are not significantly advected down the arteriole before being dismutated or uptaken. $\text{O}_2^\bullet$ molecules that diffuse deeper into the arteriole are further advected by the faster blood flow therein, those returning to near the ECs’ surface explaining the long tail of the $\text{O}_2^\bullet$ concentration profile in Fig. 3A. However, most of that $\text{O}_2^\bullet$ dismutates and yields $\text{H}_2\text{O}_2$ well inside the arteriole’s lumen. From there, the blood flow substantially advects $\text{H}_2\text{O}_2$ while it diffuses back to the ECs’ surface. This causes the 50 μm gap between the concentration peaks of $\text{H}_2\text{O}_2$ and $\text{O}_2^\bullet$, and the shallowness of the longitudinal decline of the $\text{H}_2\text{O}_2$ concentration.

The predominant fates of $\text{O}_2^\bullet$ and $\text{H}_2\text{O}_2$ in arterioles also differ substantially from those in capillaries. Of the $\text{O}_2^\bullet$ molecules, 95% are dismutated, while the other 5% are absorbed by the ECs and erythrocytes. In turn, of the $\text{H}_2\text{O}_2$ molecules, 18% are absorbed by the ECs, while the other 82% are absorbed by the erythrocytes.

In turn, the distribution of $\text{O}_2^\bullet$ and $\text{H}_2\text{O}_2$ at the ECs’ surface in arterioles is remarkably similar to that in arterioles (Fig. 3D). This is because the local environment near these vessels’ walls is quite similar too, characterized by a slow blood flow, a low hematocrit and important contributions of the ECs for $\text{H}_2\text{O}_2$ (and possibly $\text{O}_2^\bullet$) clearance. Although deeper into the artery blood flows much faster than in arterioles, few $\text{O}_2^\bullet$ and $\text{H}_2\text{O}_2$ molecules reach farther than 25 μm from the artery wall (the radius of an arteriole) before dismutating or being absorbed by erythrocytes (Fig. 3E and F). Nevertheless, while the maximal $\text{O}_2^\bullet$ concentration attained near the ECs is quite similar to that attained in arterioles (82% of the latter), that of $\text{H}_2\text{O}_2$ is just 44% of that attained in arterioles. This happens because in the artery fewer of the $\text{H}_2\text{O}_2$ molecules produced by $\text{O}_2^\bullet$ dismutation away from the wall diffuse back. For the same reason, in the artery the $\text{H}_2\text{O}_2$ concentration near the ECs reaches its maximum closer to the $\text{O}_2^\bullet$-producing region (24 μm from the $\text{O}_2^\bullet$ maximum) and shows a slightly steeper decay downstream (50% decay 506 μm downstream from the maximum), despite the faster blood flow.

3.3. Direct $\text{H}_2\text{O}_2$ production by the endothelial cells allows mainly short-range signaling

We examined as well the spatial distribution of $\text{H}_2\text{O}_2$ along the walls of vessels with the same geometry as above, but where the “active” EC ring releases only $\text{H}_2\text{O}_2$ at half the $\text{O}_2^\bullet$ release rates considered in the previous simulations (Fig. 4A,D). (That is, the same $\text{H}_2\text{O}_2$ production rate as if in the previous simulations all the released $\text{O}_2^\bullet$ was dismutated into $\text{H}_2\text{O}_2$.) The results for capillaries show that a higher maximal $\text{H}_2\text{O}_2$ concentration (11 nM vs. 6.3 nM) is attained in this case (Fig. 4B). This is due to the following two factors. First, there is no $\text{O}_2^\bullet$ loss to cellular absorption. Second, $\text{H}_2\text{O}_2$ generation is more concentrated in space, as it is not preceded by $\text{O}_2^\bullet$ diffusion. The latter factor also implies that the $\text{H}_2\text{O}_2$ concentration declines even more steeply along the longitudinal direction, decreasing by 50% over 13 μm and to 1 nM over 34 μm downstream of the maximum. The radial concentration gradient remains negligible (Fig. 4C).

In turn, in arterioles and arteries $\text{H}_2\text{O}_2$ concentrations attained at the ECs’ surface — 1.6 nM and 1.2 nM, respectively — greatly exceed those attained if ECs release only $\text{O}_2^\bullet$ (Fig. 4E,H). However, the concentration decreases very steeply downstream form production zones: in arterioles...
the concentration reaches 1 nM and decreases by 50% from maximal 2.5 μm and 6.0 μm downstream of the maximum, respectively; in arteries these distances are <2 μm and 4 μm, respectively. Nevertheless, the H$_2$O$_2$ distribution shows a heavy downstream tail, due to H$_2$O$_2$ molecules that diffuse further into the vessel, are advected there by the faster blood flow, and then diffuse back to the vessel wall. For this reason, the signaling threshold may be exceeded over a much longer range if H$_2$O$_2$ release substantially increases. The radial concentration gradient near the walls of arterioles and arteries are remarkably similar. These low concentrations are mainly a consequence of the enormous clearance capacity by erythrocytes and ECs. Because erythrocytes in the arterioles consume 77% of the H$_2$O$_2$ when all ECs release H$_2$O$_2$ or O$_2^*$, clearance by erythrocytes alone would suffice to keep H$_2$O$_2$ concentrations at the microvasculature lumen sub-μM for plausible

| Vessel | Species released | Proportionality constant (nM/ molecules s$^{-1}$ μm$^{-2}$) | [H$_2$O$_2$] for P = 6 × 10$^6$ O$_2^*$ molecules s$^{-1}$ μm$^{-2}$ (nM)$^a$ | [H$_2$O$_2$] for P = 6 × 10$^6$ O$_2^*$ molecules s$^{-1}$ μm$^{-2}$ (nM)$^a$ |
|--------|----------------|----------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| Capillaries | O$_2^*$ | 2.5 × 10$^{-2}$ | 15 | 1500 |
| | H$_2$O$_2$ | 5.6 × 10$^{-2}$ | 17 | 1700 |
| Arterioles | O$_2^*$ | 7.6 × 10$^{-3}$ | 4.6 | 460 |
| | H$_2$O$_2$ | 2.0 × 10$^{-2}$ | 6.0 | 600 |
| Arteries | O$_2^*$ | 3.7 × 10$^{-3}$ | 2.2 | 220 |
| | H$_2$O$_2$ | 1.4 × 10$^{-2}$ | 4.2 | 420 |

$^a$ Where ECs are considered to release H$_2$O$_2$, the area-specific H$_2$O$_2$ production rates considered as reference and upper bound are 300 and $3 \times 10^8$ molecules s$^{-1}$ μm$^{-2}$, respectively.

Figs. 2–4 show that under the reference conditions and production geometry H$_2$O$_2$ concentrations at the ECs’ surface peak at 6.5 nM, 0.1 nM and 0.44 nM in capillaries, arterioles and arteries, respectively, in the case where ECs release O$_2^*$; and at 11 nM, 1.6 nM and 1.2 nM, in the case where ECs release H$_2$O$_2$. As per the model equations, these concentrations are directly proportional to the area-specific O$_2^*$ or H$_2$O$_2$ release rates, and they increase with the release area. Thus, to better assess the maximal H$_2$O$_2$ concentrations attainable, we computed the proportionality constants between concentration at the ECs’ surface and area-specific release rate in blood vessels where all ECs release O$_2^*$ or H$_2$O$_2$ (Table 2). Considering these proportionality constants, we then computed the H$_2$O$_2$ concentrations at the ECs’ surface for the reference O$_2^*$ /H$_2$O$_2$ area-specific supply rates, and for an upper limit corresponding to the most O$_2^*$ productive fully activated phagocytic cells —
production rates. The reasons for this large clearance capacity are threefold. First, the very high erythrocyte surface area per unit blood volume (630 m²/L for a 0.45 average hematocrit). Second, the high permeability of erythrocyte membranes to H₂O₂ (Table 1). Third, the large cytosolic H₂O₂ clearance capacity of these cells’ cytosol. The catalase activity alone is sufficient to sustain a large transmembrane concentration gradient even should the Prdx and glutathione pools become fully oxidized, meaning that nearly every H₂O₂ entering the cytosol is immediately consumed. Adding to this, NO released from erythrocytes as a product the strong nitrite reductase activity of hemoglobin [70] may, under some circumstances, help scrub O₂⁻ from the blood plasma thereby also suppressing H₂O₂ generation.

Remarkably, H₂O₂ concentrations near the walls of larger vessels are in the range of those found for arterioles for similar area-specific O₂⁻/H₂O₂ release rates, despite the much lower area/volume ratios of the former vessels (Table 2). This occurs because H₂O₂ clearance is diffusion-limited in the radial length scale of the larger vessels, with characteristic diffusion lengths in the range of the arterioles’ radius.

Relevant for potential communication between ECs and erythrocytes, in arterioles where all the ECs produce O₂⁻ there is a modest radial H₂O₂ concentration gradient: the minimal concentration, at the vessel center, is just 12% lower than the maximal one, attained 2.6 μm from the vessel wall (Fig. S1B). In turn, in arterioles where all the ECs produce H₂O₂, the concentration is maximal adjacent to the vessel’s wall and 48% lower at the vessel’s center (Fig. S1E). In contrast, in the small artery, the H₂O₂ concentration decreases near-exponentially with the distance from the wall, attaining half-maximal values 23 μm (10 μm) away if all ECs release O₂⁻ (H₂O₂) (Figs. S1C and F). This behavior and characteristic distances should be similar in larger vessels. O₂⁻ concentrations at the small artery’s center are nearly four orders of magnitude lower, and those of H₂O₂ nearly three orders of magnitude lower, than near the wall. (But note that the model neglects O₂⁻/H₂O₂ release by plasma enzymes and circulating cells, which may contribute to substantially higher concentrations towards the arteries’ center.) Therefore, H₂O₂ eventually produced from a patch of active ECs at one side of even a small artery does not reach the opposite side.

Fig. 5. Main factors influencing H₂O₂ (red) and O₂⁻ (blue) concentrations at the EC’s surface, in capillaries (A–C) and arterioles (D–F) for 10-fold (thick dark lines), 1-fold (medium lines) and 0.1-fold (thin light lines) the reference values of $k_d$ (A, D), $\kappa_{O, B}$ and $\kappa_{O, E}$ together (B, E) and $\kappa_{H, E}$ (C, F). The insets show the concentrations scaled by the respective maxima in each condition. The horizontal dashed lines in the main plots mark the 1 nM H₂O₂ signaling threshold, those in the insets mark the half-maximal concentrations. The gray bars mark the position of the O₂⁻-producing EC ring. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
3.5. Main factors modulating concentrations and ranges

Besides the production rate, the $\text{O}_2^\cdot$ dismutation pseudo-first-order rate constant ($k_d$) is the parameter with the strongest influence on the maximal $\text{O}_2^\cdot$ concentrations near the walls of both microvessels, and it also substantially influences $\text{H}_2\text{O}_2$ concentrations at this radial location (Fig. 5A,D). In arterioles, it very strongly influences the $\text{H}_2\text{O}_2$ downstream transport range (Fig. 5D inset). Increasing $k_d$ decreases the $\text{O}_2^\cdot$ maximal concentrations and the downstream transport range of both chemical species, whereas it increases $\text{H}_2\text{O}_2$ concentrations. The substantial variation in $\text{H}_2\text{O}_2$ concentration is a consequence of the large sensitivity to $k_d$ of the $\text{O}_2^\cdot$ levels in arterioles far from the $\text{O}_2^\cdot$ production site. The influence of $k_d$ on $\text{O}_2^\cdot$ concentrations at the ECs’ surface in the production site is weaker because the steep $\text{O}_2^\cdot$ gradients create large $\text{O}_2^\cdot$ diffusion fluxes away from this site, such that most $\text{O}_2^\cdot$ diffuses away before it dismutates. The opposite occurs in locations of small $\text{O}_2^\cdot$ gradients (Fig. 5D).

The permeability constant of EC and erythrocyte membranes to $\text{O}_2^\cdot$ is the most uncertain parameter in this model. However, this parameter diffuses away before it dismutates. The opposite occurs in locations of small $\text{O}_2^\cdot$ gradients (Fig. 5D).

The permeability constant of EC membranes to $\text{O}_2^\cdot$ is dismutated.

The permeability constant of EC membranes to $\text{H}_2\text{O}_2$ has no effect on the $\text{O}_2^\cdot$ concentration or transport ranges. However, it strongly influences the maximal $\text{H}_2\text{O}_2$ concentrations at the ECs’ surface in capillaries (Fig. 5C), and also substantially influences the $\text{H}_2\text{O}_2$ transport range in arterioles at the same radial location (Fig. 5F inset). Increasing $\kappa_{\text{H}_2\text{O}_2}$ decreases both $\text{H}_2\text{O}_2$’s maximal concentration and transport range. This happens because for the reference values of the parameters the ECs consume most of the $\text{H}_2\text{O}_2$ near these vessels’ walls.

Results for arteries should closely mirror those presented above for arterioles.

3.6. Is $\text{O}_2^\cdot$ intake a significant $\text{H}_2\text{O}_2$ delivery route to the ECs’ cytosol?

Under the reference conditions, peak $\text{O}_2^\cdot$ concentrations at the ECs’ surface substantially exceed $\text{H}_2\text{O}_2$’s, whether $\text{O}_2^\cdot$ release by the vessels’ wall is considered localized (insets in Figs. 2A and 3A) or uniform (Figs. S1A,B,C). These results suggest that a substantial fraction of the $\text{H}_2\text{O}_2$ supply to the ECs from $\text{O}_2^\cdot$ released to the vessels’ lumen might result from cytosolic dismutation of uptaken $\text{O}_2^\cdot$. Fig. 6 shows the direct $\text{H}_2\text{O}_2$ influx rates into the cytosol and the $\text{H}_2\text{O}_2$ generation rates from uptaken $\text{O}_2^\cdot$ on the assumption that all of the latter is dismutated, for several values of the $\text{O}_2^\cdot$ permeability constants and $k_d$. In capillaries, $\text{O}_2^\cdot$ influx contributes modestly for $\text{H}_2\text{O}_2$ supply to the cytosol, for the reference values of the parameters (compare blue to red mid-thickness lines in Fig. 6A). However, it becomes the predominant pathway near the $\text{O}_2^\cdot$ production zone if extracellular dismutation becomes slower and/or the permeability of EC and erythrocyte membranes for $\text{O}_2^\cdot$ is comparable to that for $\text{H}_2\text{O}_2$ (Fig. 6A and B). In arterioles, $\text{O}_2^\cdot$ influx supplies most of the $\text{H}_2\text{O}_2$ to the cytosol near the $\text{O}_2^\cdot$ production zone even for the reference values of the parameters (Fig. 6C, mid-thickness

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Fig. 6. Hydrogen peroxide supply rate to the cytosol of ECs via direct influx (red) or $\text{O}_2^\cdot$ influx followed by dismutation (blue), in capillaries (A,B) and arterioles (C, D) for 10-fold (thick dark lines), 1-fold (medium lines) and 0.1-fold (thin light lines) the reference values of $k_d$ (A, B) and $\kappa_{\text{H}_2\text{O}_2}$ (C, D). The gray bars mark the position of the $\text{O}_2^\cdot$-producing EC ring. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
lines). This becomes true over several tens of μm downstream if extracellular dismutation becomes slower and/or the permeability of EC and erythrocyte membranes for $O_2^*$ is comparable to that for $H_2O_2$ (Fig. 6C and D).

If all the ECs release $O_2^*$, for the reference parameter values, $O_2^*$ influx contributes 11%, 6.8% and 10% of the $H_2O_2$ supply to the ECs’ cytosol in capillaries, arterioles and arteries, respectively. These relative contributions are substantially lower than in the $O_2^*$-producing region in Fig. 6C and D because in the latter case little $H_2O_2$ reaches this region, due to advection by the blood flow.

4. Discussion

4.1. $H_2O_2$ concentrations in the vasculature lumen are up to the 10 nM range

Physiological $H_2O_2$ concentrations in blood plasma have often been claimed to be in the μM range [43 and references therein]. However, the results above (Figs. 2–4, Table 2) show that for plausible $O_2^*$ or $H_2O_2$ release rates by the EC, the $H_2O_2$ concentrations attained at the EC’s surface do not exceed 17 nM, 6 nM and 4 nM in capillaries, arterioles and arteries, respectively. Achieving μM $H_2O_2$ concentrations in microvessels would require all the ECs releasing $O_2^*$ to the lumen at the maximal $O_2^*$ production rates achievable by the most productive phagocytic cells when maximally activated, which is very implausible.

The following independent experimental evidence further supports physiological $H_2O_2$ concentrations in blood plasma not exceeding the 10’s nM scale. From the permeability constant of the erythrocyte membrane for $H_2O_2$ (Table 1) one derives a 30. s$^{-1}$ apparent rate constant for $H_2O_2$ influx into the cytosol (see SI3). In turn, the erythrocyte’s capacity to reduce the disulfide form of Prdx2 is limited to 1–2 μM s$^{-1}$ [23,65]. Because Prdx2 reduces most of the $H_2O_2$ in human erythrocytes [23,65,71], $H_2O_2$ influx rates in excess of ≈2 μM s$^{-1}$—corresponding to ≈80 nM plasma $H_2O_2$—should extensively oxidize this protein. However, Prdx2 is modestly oxidized in erythrocytes, even under lipopolysaccharide-induced endotoxemia, which strongly stimulates $H_2O_2$ production by circulating phagocytes [72,73]. Therefore, $H_2O_2$ in blood plasma does not reach the 100 nM range systemically. And because $O_2^*$ entering the erythrocyte is dismutated and most of the ONOOH reaching the cytosol also oxidizes Prdx2, the considerations above constrain the total influx of these species into erythrocytes as well.

Several studies and critical reviews of in vitro experiments determining $O_2^*$/$H_2O_2$ production and concentrations in the blood further support the conclusion that physiological systemic plasma $H_2O_2$ concentrations are in the nM range [65,74,75]. Authors claiming otherwise may have underappreciated the $H_2O_2$ clearance capacity of erythrocytes and ECs in vivo, which limits $H_2O_2$’s half-life to <40 ms and thus makes ex vivo methods unsuitable for these experimental determinations (see SI2).

The results above do not exclude that $H_2O_2$ reaches higher concentrations locally and transiently. E.g., wounding may trigger a transient (10-min scale) accumulation of low-μM $H_2O_2$ concentrations within tens of μm of wound margins [1,7]. However, such $H_2O_2$ accumulation is driven by DUOX-mediated $H_2O_2$ production by epithelial cells, not by cells within the vasculature [1]. What the present analysis highlights is the following. Lumenal $H_2O_2$ concentrations in excess of tens of nM are very unlikely to be reached in normal-functioning vasculature as a consequence of endothelial $O_2^*$/$H_2O_2$ production, even locally or transiently. Moreover, maximal $H_2O_2$ concentrations in arterioles and larger vessels should be just up to 35% of those in capillaries for similar $O_2^*$ release rates per μm$^2$ of vessel wall (Table 2, Figs. S1C and F).

These conclusions raise the question of how such low $H_2O_2$ concentration changes can be sensed and transduced. Extracellular $H_2O_2$ concentrations in the nM scale are still substantially higher than cytosolic ones under physiological conditions [68,69], and therefore cause an influx that can significantly increase cytosolic $H_2O_2$ concentrations. At such low influx rates — up to 100’s nM s$^{-1}$ — virtually all $H_2O_2$ is captured by cytosolic Prdx1 and Prdx2, which become oxidized and can relay the oxidation to other proteins [27–30]. Given the large Prdx reduction capacities of some human cells [76,77], these low $H_2O_2$ influx rates may just minimally oxidize the cytosolic Prdx pool. However, scaffold proteins that localize both Prdx and redox targets to the sites of $H_2O_2$ supply [30] may greatly improve the sensitivity of localized redox relays [12]. Other proposed redox regulation mechanisms appear to be too insensitive for this purpose [12].

Considering the low $H_2O_2$ influx rates into ECs predicted in this work and $H_2O_2$ s~0.5 μm diffusion range in the cytosol [12], it is implausible that $H_2O_2$ from the lumen enters ECs and spreads through gap junctions to promote hyperpolarization of neighboring cells and vasodilation [17].

4.2. Is $O_2^*$ uptake a significant $H_2O_2$ delivery route to EC?

Estimated $O_2^*$ concentrations at the surface of ECs substantially exceed $H_2O_2$’s (Figs. 2 and 3). They are mainly determined by the dismutation rate constant, and modestly influenced by the permeability of ECs and erythrocytes (Fig. 5). And they raise the question of whether $O_2^*$ influx might contribute substantially for $H_2O_2$ delivery to ECs. Indeed, even if EC membranes are just 10% as permeable to $O_2^*$ as to $H_2O_2$ and assuming that all the inflowing $O_2^*$ is dismutated, $O_2^*$ influx supplies most of the $H_2O_2$ to the cytosol of ECs within the $O_2^*$ production zone of arterioles (Fig. 6C and D). In capillaries, this pathway has a minor contribution to cytosolic $H_2O_2$ supply under the same conditions, but becomes dominant if ECs’ apical membranes are as permeable to $O_2^*$ as to $H_2O_2$ or if the extracellular SOD activity is substantially lower than the reference values (Fig. 6A and B).

The following experimental observations support the relevance of the $O_2^*$-mediated $H_2O_2$ delivery route. First, SOD1 inhibition attenuates FGF-2- and VEGF-mediated phosphorylation of ERK1/2 in ECs by preventing the formation of sufficient $H_2O_2$ to cause inactivation of protein tyrosine phosphatases [78]. Second, SOD1 can drive the specific oxidation of a variety of thiol proteins, presumably through channeling of the $H_2O_2$ product, and thereby helps adapt cellular metabolism to oxygen availability [79]. Altogether, these computational and experimental results justify paying further attention to the differential responses of vascular ECs to $O_2^*$ vs. $H_2O_2$.

4.3. Potential $O_2^*$ and $H_2O_2$ signaling through the vasculature can be from autocrine to mm-scale

The simulation results in Figs. 2–4 show that $O_2^*$/$H_2O_2$ in the (micro)vasculature lumen can signal over widely different ranges. These ranges are shortest for the species ECs directly release, because blood flows very slowly near the vessels’ walls and ECs rapidly absorb the $H_2O_2$ molecules that stay in this region. On the other hand, release of $O_2^*$ by the ECs introduces a delay in $H_2O_2$ production, which allows this species to reach farther from the signaling cells (Figs. 2A and 3A). In capillaries, this effect just slightly extends the $H_2O_2$ signaling range because the blood flow is too slow to substantially advect the molecules, transport remaining diffusion dominated (Fig. 2A). In all the situations above, the signaling range is limited to the low 10’s of μm, allowing at most communication a few cells across. A very different situation occurs when ECs release $O_2^*$ into the arterioles’ and arteries’ lumen. Here, there is substantial advection as $O_2^*$ diffuses towards the vessel’s center — where blood flows faster — prior to undergoing dismutation, and then the resulting $H_2O_2$ diffuses back to the vessel’s wall. Consequently, $H_2O_2$ attains maximal concentrations 25–50 μm downstream of the $O_2^*$ concentration maximum, which may allow ECs to signal to downstream cells while minimizing autocrine signaling. Moreover, the $H_2O_2$ concentration near the ECs decreases longitudinally over a mm scale, thus potentially allowing communication between distant cells. However, this potential long-distance signaling comes at the cost of the maximal $H_2O_2$ concentrations being very low, such that the considered 1 nM
signaling threshold can only be attained when many cells release $O_2^\bullet$.
Therefore, there is a trade-off in $H_2O_2$ signaling with respect to the mode
of $H_2O_2$ production: direct $H_2O_2$ release allows attaining higher local
concentrations just over a very short range, whereas $O_2^\bullet$ release potentially allows long-range signaling but requires release by a larger
number of cells or release at higher rates/cell for $H_2O_2$ concentrations to
attain the signaling threshold. Nevertheless, in arterioles and arteries
where $H_2O_2$ release by the ECs is exacerbated beyond normal levels the
signaling range may also extend over many 10’s or even 100’s of $\mu$m.
This is because the $H_2O_2$ distribution near the ECs surface shows a heavy
downstream tail, which is due to a minority of $H_2O_2$ that diffuses some
distance away from the vessel wall and then back. But in contrast to
what happens in $O_2^\bullet$-mediated $H_2O_2$ signaling, in this case the pro-
ducing ECs are exposed to substantially higher $H_2O_2$ concentrations than
any other cells.

At the low concentrations involved in signaling through the vessels’
lumen, $H_2O_2$ entering the ECs will be fully consumed by these cells and
will not reach the basal side of the endothelium. However, it may elicit
endothelial responses that affect the surrounding tissue.

Besides absorption by ECs, several other factors can affect $O_2^\bullet$ and
$H_2O_2$’s reach in the microvasculature lumen. SOD3 activity in the blood
plasma is the most prominent of these factors under the reference con-
ditions (Fig. 5). Decreasing SOD3 activity in the blood plasma increases
both $O_2^\bullet$’s concentrations and its half-life, thus extending the range of
signaling (Fig. 5A,D). In turn, $H_2O_2$’s longitudinal reach in capillaries is
mainly determined by the permeability of ECs’ apical membranes
(Fig. 5C,F). As a consequence, there is a trade-off between signaling
sensitivity and spatial range when modulating the permeability of the
ECs’ membrane to $H_2O_2$: increasing this permeability enables the ECs
to better compete with the erythrocytes for $H_2O_2$ and increase the rate of
$H_2O_2$ supply to the cytosol, thus further oxidizing Prdx1 and Prdx2 and
intensifying signaling; but it also decreases the lifetime and signaling
range of extracellular $H_2O_2$.

4.4. $H_2O_2$’s transport range may vary widely depending on tissue
characteristics

How does the transport range of extracellular $H_2O_2$ in the micro-
vasculature lumen compare to those in “solid” tissues? In the absence of
a strong oxidative stress the cytosolic 2-Cys peroxiredoxins sustain a
strong transmembrane concentration $H_2O_2$ gradient [69,80,81]. As a
consequence, the clearance rate of extracellular $H_2O_2$ by the cells is then
determined by their membrane’s permeability. Thus, at sub-$\mu$m extra-
cellular $H_2O_2$ concentrations, the transport range depends primarily on
this permeability and on the volume/area ratio of the extracellular
medium. The effective permeability constants inferred from permeation
studies of erythrocytes and several human cells in culture are in the
10–20 $\mu$m s$^{-1}$ range [22,76]. And a area/volume ratio of $\approx 62\, \mu$m$^{-1}$ is inferred from the observation that in the brain cortex — arguably the best
categorized tissue in this respect — the extracellular medium accounts for 20% of the tissue volume and has a 60 nm average width
[82] (Fig. 4). From these values, one estimates that a $H_2O_2$ molecule has a
$<1$ ms half-life in the extracellular medium, allowing it to diffuse $<3.5$
$\mu$m on average before being absorbed by a cell. In turn, at extracellular
$H_2O_2$ concentrations in the $\mu$M range or higher the cytosolic Prdx pool
becomes completely oxidized. As a consequence, the transmembrane
$H_2O_2$ concentration gradient collapses, and the $H_2O_2$ half-life and
transport range increase and become controlled by the activity of the
remaining intracellular clearance mechanisms.

How do these theoretical conjectures compare to the few known
experimental observations that addressed the transport range of extra-
cellular $H_2O_2$? In the zebrafish tail wounding model, $H_2O_2$ concentra-
tions decrease to 50% of maximal values at the wound borders in $\approx 50$
$\mu$m [7]. In keeping with the conjectures above, these authors observe
that the attained $H_2O_2$ concentrations ($5\, \mu$m) fully oxidize the peroxi-
edoxins of the cells within $30\, \mu$m of the wound margins, which
decreased $H_2O_2$ consumption by these cells and broadened the con-
centration gradient. This phenomenon is very unlikely to occur under
non-inflammatory conditions, though.

A recent study of $H_2O_2$ diffusion and clearance in the brain striatum
of living mice determined an effective diffusion coefficient $D^\bullet = 2.5 \times$
$10^7\, \mu$m$^2$ s$^{-1}$ and a 2.2 s half-life, allowing $H_2O_2$ to diffuse over $180\, \mu$m
in the extracellular space [10]. These surprisingly long half-life and range
may have the following two alternative explanations. First, if the cells in
this tissue have permeabilities comparable to those mentioned above,
the 1 mM $H_2O_2$ bolus administered may have fully oxidized the
cytosolic peroxiredoxin pool and part of the GSH pool in the measure-
ment field. The modest glutathione peroxidase activity in rat brain tis-
uues [83] would be insufficient to sustain a significant $H_2O_2$
transmembrane gradient in this case. Therefore, the determined $H_2O_2$
half-life would then essentially reflect the activity of the remaining
intracellular $H_2O_2$ clearance processes. If this is the correct explanation,
the experimental measurement may have strongly overestimated the
half-life and transport range that applies at physiological extracellular
$H_2O_2$ concentrations. However, this explanation seems inconsistent
with the observation that the first in a series of $H_2O_2$ boluses administered to
the rat brain is already detected by a distant $H_2O_2$-specific microelec-
trode with a time course similar to those of subsequent boluses. The
inconsistency is because according to the proposed explanation the re-
actions with the intracellular redox pools would consume the $H_2O_2$
in the first bolus before it could reach the microelectrode. The alternative
explanation is that the long $H_2O_2$ half-life and transport range are due to
most cells in this tissue being virtually impermeable to $H_2O_2$, perhaps
because they are myelinated. In this case, the determined half-life and
transport range may be representative of the physiological one for the
brain striatum, but does not necessarily apply to other solid tissues.
Further research is thus needed to clarify these important issues.

Meanwhile, computational modeling in the vein of the present work
will remain an important asset to estimate concentrations and distri-
butions, interpret experimental observations, and designing informative
experiments.

5. Concluding remarks

The results in this work show that the blood flow in the vasculature
can transport $H_2O_2$ over a mm-scale, but only when this substance is
generated from dismutation of EC-released $O_2^\bullet$ in arterioles and larger
veins. In virtually all the other conditions studied, $O_2^\bullet$ and $H_2O_2$
transport is limited to $<50\, \mu$m.

$H_2O_2$ concentrations in the blood plasma of normal-functioning
vasculature do not exceed the low tens of nM, which limits the viable
intracellular signal transduction mechanisms

Cellular intake of $O_2^\bullet$ from the blood plasma, followed by cytosolic
dismutation, can be a quantitatively significant $H_2O_2$ signaling route,
depending on the plasma SOD activity and on the EC membrane’s
permeability to $O_2^\bullet$.

Altogether, the theoretical and experimental considerations presen-
ted above suggest that $H_2O_2$’s half-life and transport range strongly
depend on tissue characteristics. Namely, cell membrane permeability,
extracellular space volume/area ratio, intracellular $H_2O_2$ clearance ca-
acity, mode of extracellular $H_2O_2$ production and extracellular fluid
fluxes.

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Competing financial interests

The authors declare no competing financial interests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found at https://doi.org/10.1016/j.redox.2022.102527.

References

[1] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of proteins, Antioxidants Redox Signal. 19 (2012) 523–854.
[2] A. Denicola, B.A. Freeman, M. Trujillo, R. Radi, Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations, Sci. Rep. 9 (2019) 133, https://doi.org/10.1038/s41598-019-04393-z.
[3] N. Makino, K. Nakashi, K. Hashida, Y. Sakakura, A metabolic model describing the H2O2 elimination by mammalian cells including H2O2 permeation through cytoplasmic and peroxisomal membranes: comparison with experimental data, Biochim. Biophys. Acta - Gen. Subj. 1673 (2004) 149–159, https://doi.org/10.1016/j.bbabio.2004.04.001.
[4] F. Orrico, A.C. Lopez, D. Salwiczewy, C. Acosta, I. Rodrigo-Grecco, I. Mourou-Chanteloup, M.A. Ostuni, A. Denicola, L. Thomson, M.N. Möller, The permeability of human red blood cell membranes to hydrogen peroxide is independent of aquaporins, J. Biol. Chem. (2021), 101503, https://doi.org/10.1074/jbc.rj.2021.101503.
[5] A. Donk ¨Ocker, D.R. Trusz, T.S. Fallani, S. V Álvez, J.C. Toledo Junior, O. Augusto, L.E.S. Netto, F.C. Meotti, Urate hydroperoxide oxidizes human peroxiredoxin 1 and peroxiredoxin 2, J. Biol. Chem. 292 (2017) 8705–8715, https://doi.org/10.1074/jbc.M116.767657.
[6] V.J. Thannickal, Hydrogen peroxide is a diffusible paracrine signal for the H2O2 reaction-diffusion in wounded zebrafish larvae, Biophys. J. 112 (2017) 233–236, https://doi.org/10.1016/j.bpj.2017.03.021.
[7] F. Orrico, A. Denicola, M. Geiszt, Spatial and temporal analysis of NADPH oxidase-generated hydrogen peroxide signals by novel fluorescent reporter proteins, Antioxidants Redox Signal. 20 (2013) 899–913, https://doi.org/10.1089/ars.2013.5624.
[8] R.E. Lynch, I. Fridovich, Permeation of the erythrocyte stroma by superoxide, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3566–3570, https://doi.org/10.1073/pnas.95.10.3566.
[9] M.G. Sobotta, W. Liu, S. Stocker, D. Talwar, M. Oehler, T. Ruppert, A.N. Scharf, T. P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signalling, Nat. Chem. Biol. 11 (2014) 64–70, https://doi.org/10.1038/nchembio.1695.
[10] T.N. Vo, J.M. Pueyo, K. Wahi, D. Ezerin, J. Bolduc, J. Messens, Prdx1 interacts with ASK1 upon exposure to H 2 O 2 and independently of a scaffolding protein, Antioxidants 10 (2021) 1060, https://doi.org/10.3390/antioxidants10071060.
[11] S. Portillo-Ledesma, L.M. Randall, D. Parsonage, J.D. Rizza, P.A. Karplus, L. B. Poole, A. Denicola, G. Ferrer-Sueta, Differential kinetics of two-cysteine peroxiredoxin disulfide formation reveal a novel model for peroxide sensing, Biochemistry 57 (2018) 3416–3424, https://doi.org/10.1021/acs.biochem.8b00188.
[12] M.C. Sobotta, W. Liu, S. Stocker, D. Talwar, M. Oehler, T. Ruppert, A.N. Scharf, T. P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signalling, Nat. Chem. Biol. 11 (2014) 64–70, https://doi.org/10.1038/nchembio.1695.
[13] T.N. Vo, J.M. Pueyo, K. Wahi, D. Ezerin, J. Bolduc, J. Messens, Prdx1 interacts with ASK1 upon exposure to H 2 O 2 and independently of a scaffolding protein, Antioxidants 10 (2021) 1060, https://doi.org/10.3390/antioxidants10071060.
[14] M.R. Jarvis, S.M. Hughes, E.C. Ledgerwood, Peroxiredoxin 1 functions as a signal peroxide to receive, transduce, and transmit peroxide signals in mammalian cells, Free Radic. Biol. Med. 53 (2012) 1522–1530, https://doi.org/10.1016/j.freeradbiomed.2012.08.001.
[15] D. Talwar, J. Messens, T.P. Dick, A role for annexin A2 in scaffolding the peroxiredoxin 2–STAT3 redox relay complex, Nat. Commun. 11 (2020) 1–11, https://doi.org/10.1038/s41467-020-18324-9.
[16] C.C. Winterbourn, Reconciling the chemistry and biology of reactive oxygen species, Nat. Chem. Biol. 4 (2008) 278–284, https://doi.org/10.1038/nchembio.1695.
[17] C.C. Winterbourn, Reconciling the chemistry and biology of reactive oxygen species, Nat. Chem. Biol. 4 (2008) 278–284, https://doi.org/10.1038/nchembio.1695.
[18] C. Anasooya Shaji, R.D. Robinson, A. Yeager, M.R. Beeram, M.L. Davis, C.L. Ibel, J.H. Huang, B. Tharakan, The triphasic role of hydrogen peroxide in blood-brain barrier endothelial cells, Sci. Rep. 9 (2019) 133, https://doi.org/10.1038/s41598-019-4469-4.
[19] R. Alhayaza, E. Haque, C. Karbasflashaw, F.W. Selke, M.R. Abid, The relationship between reactive oxygen species and endothelial cell metabolism, Front. Chem. 8 (2020), 592688, https://doi.org/10.3389/fchem.2020.592688.
[20] K. Schroder, M. Zheng, J. Zhao, A. Mithen, S. Elguett, J. Kosowski, K. Krue, P. Luedke, I.R. Michaelis, N. Weissem, S. Dimmer, A.M. Shah, R.P. Brandes, Novo ls is a protective reactive oxygen species generating vascular NADPH oxidase, Circ. Res. 110 (2012) 1217–1225, https://doi.org/10.1161/ CIRCRESAHA.111.2607654.001.
