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Tissue-based biosensor for monitoring the antioxidant effect of orally administered drugs in the intestine

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ABSTRACT

For a better understanding of the effect of drugs and their interaction with cells and tissues, there is a need for *in vitro* and *ex vivo* model systems which enables studying these events. There are several *in vitro* methods available to evaluate the antioxidant activity; however, these methods do not factor in the complex *in vivo* physiology. Here we present an intestinal tissue modified oxygen electrode, used for the detection of the antioxidant effect of orally administered drugs in the presence of H_2O_2. Antioxidants are essential in the defense against oxidative stress, more specifically against reactive oxygen species such as H_2O_2. Due to the presence of native catalase in the intestine, with the tissue-based biosensor we were able to detect H_2O_2 in the range between 50 and 500 μM. The reproducibility of the sensor based on the calculated relative standard deviations was 15 ± 6%. We found that the O_2 production by catalase from H_2O_2 was reduced in the presence of a well-known antioxidant, quinol. This indirectly detected antioxidant activity was also observed in the case of orally administered drugs with a reported anti-inflammatory effect such as mesalazine and paracetamol, while no antioxidant activity was recorded with aspirin and metformin.

1. Introduction

Oral drug delivery remains the most preferred route of administration [1,2]. Only during the past years, a myriad of oral drug delivery strategies has been discovered and clinically tried [3]. Oral administration of drugs faces several challenges since the active compounds need to traverse through the gastrointestinal tract, while being exposed to varying pH conditions and enzymes, before being able to be absorbed in the small intestine after crossing the mucous layer and epithelium [4]. Assessing the permeation and interaction of drugs with the intestinal tissue is essential in drug discovery [5]; therefore, evaluating the orally delivered drugs in model *in vitro* and *ex vivo* systems and simulated conditions are essential [6].

Among these approaches, *in vitro* models are ubiquitous due to their ethical and economic viability in comparison with *in vivo* models, especially when large groups of lead candidates are required to be screened. *In vitro* models, particularly cell cultures (e.g., Caco-2 cell monolayers) are widely used for studying orally delivered drugs that are meant to be absorbed in the intestine [7]. One major drawback of these systems is that the intricate array of *in vivo* physiological parameters are either oversimplified or wholly ignored [8]. For instance, epithelial enzymes known to be involved in metabolizing drugs, which are also additionally absorbed [9] are often overlooked in the *in vitro* systems. Although several drug-metabolizing enzymes have been previously investigated [4,10], little is known about the role of native antioxidant enzymes particularly in the site of inflammation in the intestine. In classical assays, the interaction or effect of drugs on enzymes is evaluated using isolated enzymes or *in vitro* cell culture systems [11–13]. Considering the high abundance of catalase in the intestinal mucosa [16,17] and in the intestine [16], it is relevant to evaluate how this enzyme or its substrate interacts with orally delivered drugs. Additionally, it has been also shown the advantages of tissue-based electrochemical biosensors, regarding the prolonged stability of the enzymes in their native environment, better sensitivity, and linear range when compared with the typical isolated enzyme-based biosensors [17,18]. *Ex vivo* methods,

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using isolated small intestinal tissues are widely implemented for drug permeation studies in system such as the Ussing chamber and Franz cells [19–24]. The small intestine, especially jejunum is commonly used for these studies as it is the principal site of absorption for drugs and nutrients in the gastrointestinal tract Commonly, for permeation and interaction studies, porcine intestines are preferred due to their similarities in function and morphology with the human intestines [25,26]. Moreover, porcine intestines are also used as a standard mammalian model to study acute and chronic inflammation [26,27].

In the case of inflammatory bowel diseases such as ulcerative colitis [14], it has been reported that oxidative damage and in particular H_2O_2 having a crucial role. Oxidative stress is caused due to the imbalance between the oxidants (reactive oxygen species (ROS)) and the antioxidant defense system in favor of the oxidants, which can lead to inflammation [28]. Generally, ROS are required for intercellular and extracellular signaling as well as for defense against pathogens [29]. However, in excess, they are extremely reactive and cause damage to proteins, lipids, and nucleic acids, causing oxidative stress and resulting in several disease states [30,31]. To neutralize any adverse effect of the ROS, the intestinal epithelial cells and mucosa are equipped with a very efficient antioxidant system comprising of enzymes such as superoxide dismutase, glutathione peroxidase, and catalase [14]. Among antioxidant enzymes, catalase is highly efficient in neutralizing H_2O_2, one of the stable forms of ROS, by converting it to H_2O and O_2.

Recently, real-time electrochemical monitoring of native catalase in skin has been proposed [32], which was also used to study the reaction of polyphenol-H_2O_2 [33]. In this work, we used porcine small intestine modified oxygen electrode (Fig. 1A) to study the antioxidant effect of orally administered drugs.

2. Materials and methods

2.1. Chemical and materials

Hydrogen peroxide (H_2O_2) (30%), phosphate buffer saline (PBS pH 7.4) in powder, KCl, sodium azide (NaN_3), amitrole (3-amino-1,2,4-triazole), quinol (hydroquinone), mesalazine (5-aminosalicylic acid), paracetamol (acetaminophen), aspirin (acetylsalicylic acid), and metformin (N,N-Dimethylimidodicarbonimidic diamide) were purchased from Sigma Aldrich (St Louis, MO, USA). A custom-made three-electrode system consisting of a 250 μm thick platinum wire as working electrode (WE) melted in glass, an internal Ag/AgCl reference (RE), and internal platinum counter electrode (CE) (Fig. 1A) was purchased from Optronika, UAB (Vilnius, Lithuania). The Teflon membrane of 12.5 μm thickness was obtained from Hansatech Instruments (Norfolk, UK). All solutions were prepared using deionized water from Milli-Q Integral Water Purification System from Merck Millipore (Billerica, MA, USA).

2.2. Intestinal tissue-modified oxygen electrode

The platinum surface of the WE was polished using a 1 μm alumina suspension (Buehler, Lake Bluff, IL, USA) and rinsed with deionized water. The body of the electrode was filled with saturated KCl solution and covered with a Teflon membrane, completing the basic oxygen electrode construction [34]. The oxygen electrode was further covered with tissue which was immobilized using an O-ring and in order to limit the exposure area of the immobilized tissue, the excess tissue in the sides were covered with Parafilm® (Supplementary information, Fig. S1). The jejunum part of small intestine was harvested from two sacrificed pigs (15–16 weeks old, 50–55 kg, Landrace × Yorkshire × Duroc). The tissue was placed on the oxygen electrode in a way that the inner part of the tissue (towards the lumen) was exposed to the buffer solution during measurement, while the outer part was in direct contact with the Teflon membrane of the electrode. The intestinal tissue was cut into small pieces of about 5–6 cm and stored in 15 mL centrifuge tubes aliquots (1 piece of tissues/tube) at − 20 °C. Before experiments, the tissues were thawed, rinsed with PBS and punched with a leather puncher (3 mm ∅). Each aliquot of tissue yielded up to 7 circular tissue punches. If the tissue punches were not used immediately, they were stored at +4 °C in a humid chamber soaked with PBS for no longer than a week. This *ex vivo* study was conducted at the Technical University of Denmark under the license number DK-10-13-OTH-736416.

2.3. Amperometric monitoring of catalase activity in tissue

All amperometric measurements were performed with the three-electrode setup as described above, using a CHI 660C potentiostat/galvanostat (CH Instruments, Austin, TX, USA). The tissue-modified biosensor (Supplementary information, Fig. S1) was submerged into an electrochemical cell containing 10 mL PBS (pH 7.4), with continuous stirring for 30 min to eliminate the loosely bound catalase from the tissue. Before measurement, fresh PBS was placed in the electrochemical cell and chronoamperograms were recorded at a potential of −0.7 V vs. Ag/AgCl/Sat. KCl RE. After baseline current stabilized, typically, a 200 μl of various concentrations of H_2O_2 was added into the electrochemical cell, resulting in a reduction current due to O_2 production catalyzed by the native catalase in the tissue. The current signal was recorded after a stable current was obtained. After the reduction current stabilized, the analyte of interest was added to the electrochemical cell, and the response of biosensor was measured after stabilization. If not otherwise specified, the concentration of the tested drugs was three times higher than the concentration of H_2O_2. For all measurements, the electrochemical cell was continuously intermixed with a magnetic stirrer and was carried out at room temperature.

2.4. Data analysis

The data was recorded using the software supplied by CH Instruments. All experiments were performed in triplicates, if not otherwise specified. In assays aimed for quantitative measurements, the same tissue or tissues from the same animal were used for a set of experiments. For better graphical representation, the obtained raw data were treated using Savitzky-Golay method with 30 points averaging. Origin 2018b (OriginLab Corp, Northampton, USA) was used to analyze and display the data. When applicable, the chronoamperograms were baseline shifted for better visualization. Normalization of the current was performed by considering the current response for H_2O_2 to be 100%. The inhibition current for tested drugs is expressed with respect to the normalized current response for H_2O_2.

3. Results and discussion

3.1. Working principle of the biosensor and detection of H_2O_2

The working principle of the sensor is depicted in Fig. 1B. In the presence of native catalase in the intestine, H_2O_2 is converted to O_2 and H_2O. The O_2 will diffuse through the Teflon membrane and will be reduced on the Pt WE at − 0.7 V vs. Ag/AgCl/Sat. KCl RE. As shown in Fig. 2A (red and grey curve), after addition of H_2O_2 there is an apparent decrease in current as a result of the O_2 produced by catalase while there is no signal in the control measurement, when PBS is added to the electrochemical cell (Fig. 2A, black curve). We

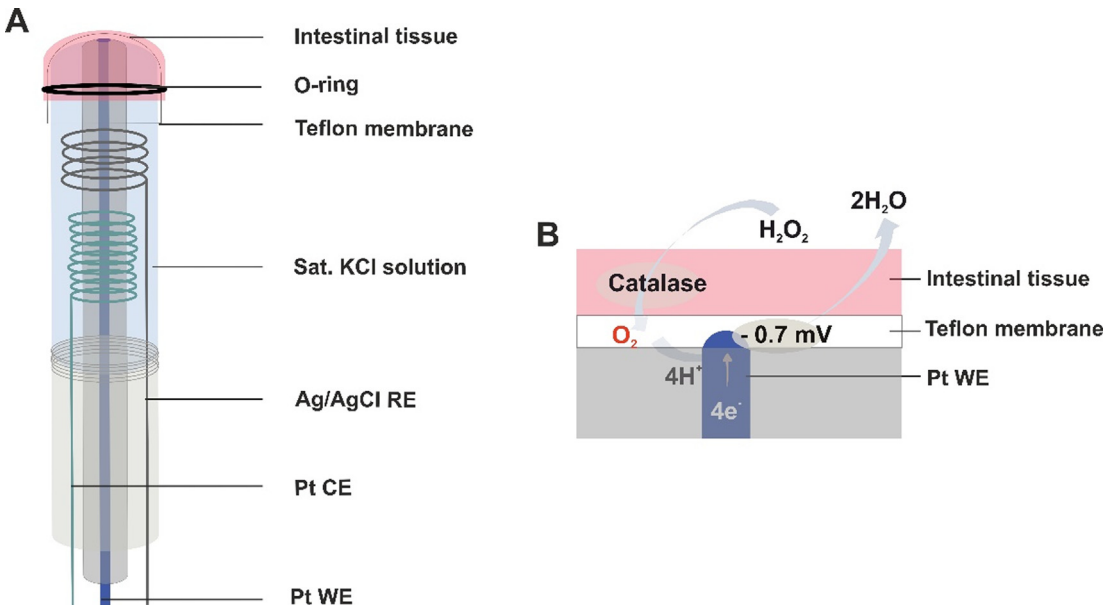

Fig. 1. A) Schematic representation of the tissue-modified oxygen electrode; **B)** Working principle of the tissue-modified oxygen electrode.

also measured the current response from dissolved O_2 in the PBS with a bare and tissue-modified oxygen electrode. As expected, the recorded reduction current was lower in the presence of the tissue due to the diffusion limitation of O_2 (Supplementary information, Fig. S2A).

To corroborate the role of catalase in the production of O_2 from H_2O_2, we evaluated the effect of enzyme inhibitors. Fig. 2A shows the signal recorded after the addition of an unspecific catalase inhibitor, sodium azide (grey curve) and amitrole (red curve) the specific inhibitor of catalase [35]. We observed that sodium azide inhibits the native catalase in the intestine, an effect earlier reported by Nocchi et al. [32] using native catalase in skin. When comparing the inhibitors, we observed that in the case of amitrole, the 100% catalase inhibition was slower than for sodium azide (Fig. 2A, red curve). However, catalase was reversibly inhibited by sodium azide and irreversibly by its specific inhibitor amitrole (Supplementary information, Fig. S2B). The data presented in Fig. 2B, confirms that the change in current recorded after addition of H_2O_2 is due to O_2 reduction on the Pt WE, where the O_2 was produced as a result of catalase activity.

We found that when working with freshly immobilized intestinal tissue without rinsing, it was challenging to obtain a stable reduction current after the addition of H_2O_2 (Fig. 2B, black curve). The unstable baseline could be attributed to the fast consumption of H_2O_2 due to the high abundance of catalase produced by the large population of bacteria present in the mucosal layer [36]. On the other hand, when the tissue was thoroughly rinsed after immobilization, a stable current could be recorded (Fig. 2B, red curve). To evaluate if the unstable baseline in the case of the unrinsed tissue is related to the excess of catalase in the mucosa, we measured the O_2 produced with a bare oxygen electrode in the presence of H_2O_2 and added mucosa, removed during the rinsing of the intestine. As shown in Fig. 2B, grey curve), there is a substantial amount of O_2 produced in the presence of 200 μM H_2O_2 present in the PBS, when the loose mucosa (1 mL) from the rinsing process was added to the electrochemical cell containing unmodified oxygen electrode. This excessive O_2 production confirms the presence of abundant catalase in the mucosa layer, and therefore for further experiments the tissues were rinsed prior measurement.

Fig. 2C shows the linear dependence between the H_2O_2 concentration and current recorded with the tissue-modified oxygen electrode. For studies evaluating the antioxidant activity of drugs, 200 μM H_2O_2 was chosen to mimic oxidative stress conditions. The concentration was selected considering the relatively low concentration of H_2O_2, leading to a substantial reduction current while a stable signal can be obtained in less than 15 min (Fig. 2B, red curve). Furthermore, using a relatively low concentration of H_2O_2 is essential to prevent catalase inactivation [37]. To evaluate the stability of the native catalase, we recorded the current response of 200 μM H_2O_2 using the same batch of tissue over a period of three days. We found that the relative standard variation between the obtained signals (using the same electrode) was around 9% based on at least three measurements within the same day, while the variation was 10% for measurements performed across three days (using three different electrodes, with tissue from the same batch). The obtained data and a relatively low variation within the same day indicates that the biosensor can be reused several times and the same batch of tissue can be used up to three days.

3.2. Effect of drugs on O_2 production catalyzed by catalase

Many phenolic compounds are known to have antioxidant activity [38], mostly related to their ROS scavenging capacity due to their chemical structure [39]. It has been shown that certain orally delivered drugs with phenolic structure and anti-inflammatory properties, such as mesalazine, paracetamol and aspirin display antioxidant activity *in vitro* [40], although their therapeutic effect is not connected to this antioxidant behavior. Mesalazine is administered to reduce inflammation and therefore to ameliorate the symptoms of patient suffering from ulcerative colitis [41]. There are several formulations of mesalazine available; however the oral form is extensively used for patients at advanced disease state [42,43]. The mechanism of action of mesalazine is not fully known, but its anti-inflammatory effect has been associated with its capacity to indirectly reduce production of prostaglandins which results in decreased inflammation [41]. Besides this effect, it has also been shown that mesalazine has antioxidant properties using animal models and isolated microvillus membrane vesicles [12,44]. In the animal models, Managlia et al., showed that the mesalazine reduced ROS levels over 40% [12]. As shown in Fig. 3A, using the intestinal tissue biosensor we measured a decrease in O_2 production after addition of mesalazine (red curve).

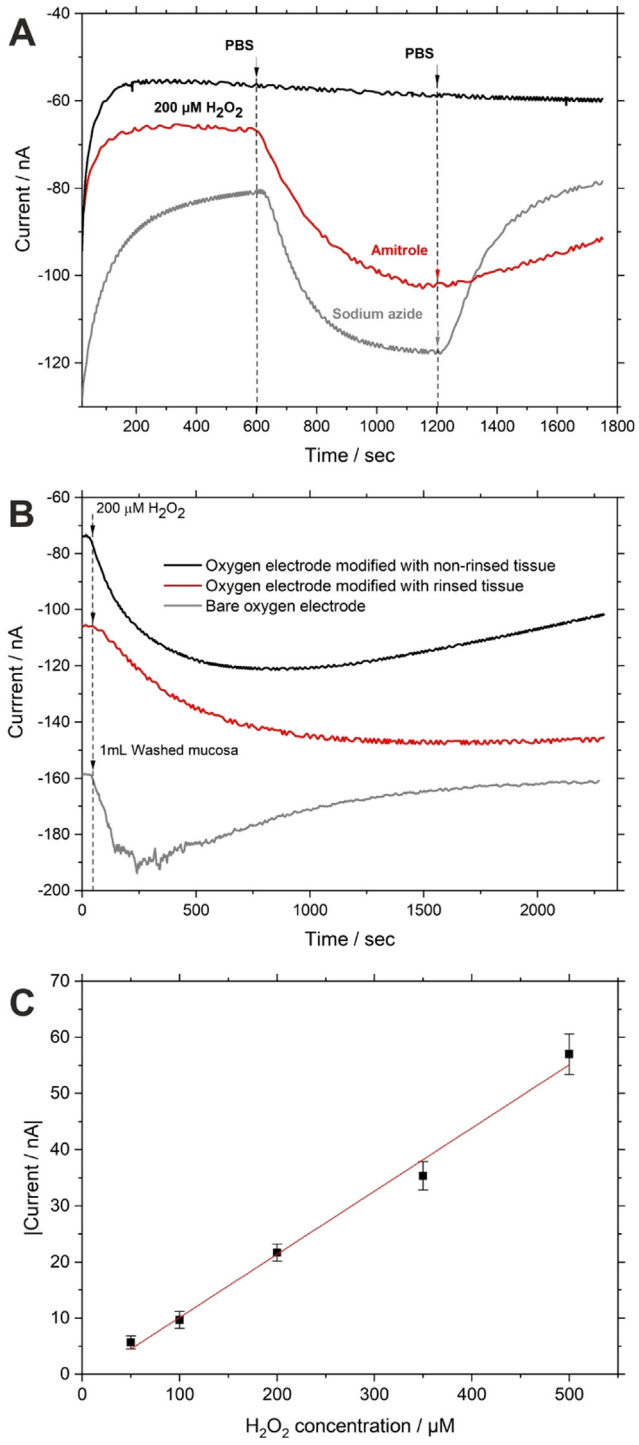

Fig. 2. A) Representative figure showing the current recorded before and after the addition of H_2O_2 (red and grey curve), the signal recorded in the control experiment (black curve), and current measured in the presence of catalase inhibitions, amitrole (red curve) and sodium azide (grey curve); **B)** Current response after the addition of H_2O_2 recorded with the tissue-modified electrode without rinsing (black curve), after rinsing (red curve) and the signal measured with bare oxygen electrode in the presence of H_2O_2 in solution after the addition of mucosa from rinsing (grey curve). **C)** H_2O_2 calibration curve (R^2 = 0.989) obtained with the tissue biosensor, from different aliquots of tissues from the same animal. Error bars represents the standard deviations, n = 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We observed that the inhibitory effect of mesalazine was similar to the signal recorded after addition of quinol (Fig. 3A, blue curve), a well-known antioxidant with phenolic structure [45]. Whereas, addition of PBS did not cause any inhibition in O_2 production (Fig. 3A, black curve).

Paracetamol is another phenolic derivate with reported anti-inflammatory effect [46,47]. Although its antioxidant effect has also been reported using cellular models [13,48,49], paracetamol is mostly known for its oxidative stress inducing properties outside its therapeutic window [50]. It has been previously shown that the oxidative and antioxidant effect of this compound is concentration dependent [51]. For instance, Dinis et al. demonstrated using a cell-free assay system that paracetamol can directly scavenge ROS with a concentration range of 2–10 µM [40]. As seen in Fig. 3B, we recorded a substantial inhibition of O_2 production (blue curve) after the addition of paracetamol, which indicates antioxidant like activity of this drug. For further experiments, the ratio (1:3) between H_2O_2 and tested drugs was chosen based on the data presented in Supplementary information, Fig. S3.

We further evaluated this effect, considering that the inhibition of the O_2 produced from H_2O_2 could be a result of either (i) direct inhibition of catalase activity or (ii) reaction of paracetamol with H_2O_2. When H_2O_2 was added after 30 min incubation with paracetamol (still present in the electrochemical cell), the resultant O_2 production was similar to a typical response for 200 µM H_2O_2 (Fig. 3B, black curve). However, when H_2O_2 was introduced to the electrochemical cell together with paracetamol, we noticed decrease in the reduction current, indicating lower O_2 production (Fig. 3B, red curve) compared to the case when H_2O_2 alone was added (Fig. 3B, blue curve). These findings suggest that paracetamol does not directly inhibit catalase and that the antioxidant like behavior is due to its ROS scavenging activity, which was also reported by Dinis et al. [40]. Additionally, when we recorded the signal from the sensor, while sequentially adding H_2O_2 - paracetamol - H_2O_2– PBS to the electrochemical cell, we found that the second addition of H_2O_2 resulted in a comparable O_2 reduction current as the first one (Supplementary information, Fig. S4). The comparable O_2 reduction current reinforces that paracetamol does not affect catalase activity. Fig. 4 shows the relative inhibition of O_2 production recorded with the tissue-modified oxygen sensor with different drugs, and using PBS as control. Among the tested drugs, paracetamol has the highest antioxidant effect due to high inhibition current of 55% followed by mesalazine.

Our findings concur with the study performed by Dinis et al., where they revealed the antioxidant activity in cell-free assay. We also evaluated the effect of aspirin, yet another orally delivered drug with phenolic structure with well-known anti-inflammatory and antipyretic property [52,53]. Antioxidant activity of aspirin has been demonstrated previously in both *in vitro* and *ex vivo* [54,55], however, in our study we have not recorded any such activity, which could be due to low concentration of aspirin used in our experiments. For instance, Dinis et al. used aspirin 50x times higher in concentration than paracetamol to show antioxidant effect in the cell-free assay system.

Additionally, we evaluated metformin, which is an anti-hyperglycemic agent primarily used in type-2 diabetes treatment. However it is also known to scavenge intracellular ROS [56], increase catalase activity in diabetic rats [57], and recently it has been shown to also have anti-inflammatory effect [57]. Bonnefont-Rousselot et al. [58] studied the ROS scavenging ability of metformin using different ROS species and showed that

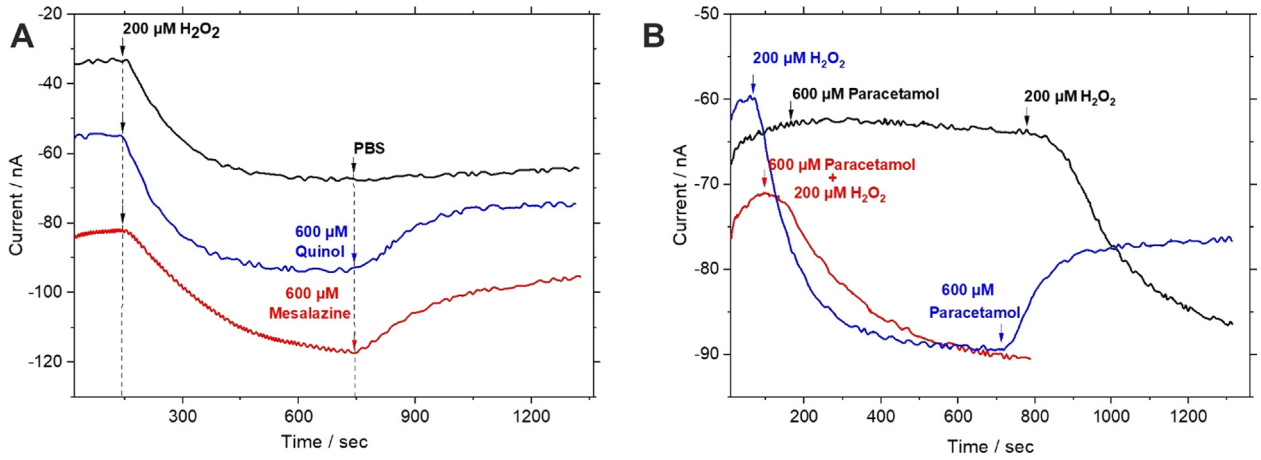

Fig. 3. A) Effect of mesalazine and quinol on the O_2 produced from H_2O_2 catalyzed by catalase in the intestinal tissue; **B)** Evaluation of antioxidant behavior of paracetamol.

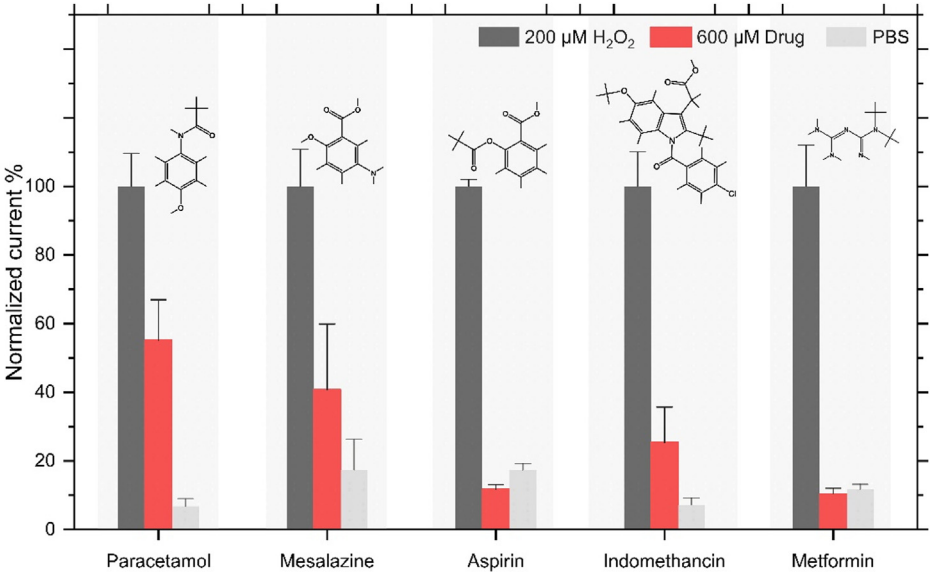

Fig. 4. Normalized current values recorded from tissue-modified oxygen electrode for consecutive additions of H_2O_2, test drug and PBS as control, respectively (n = 3). Tissues from different aliquots were used for each drug, and all aliquots were from the same animal. The antioxidant effect can be evaluated from the current inhibition %. The inset shows the chemical structures of test drugs.

metformin particularly does not react with H_2O_2. In our study (Fig. 4), we observed that metformin does not reduce the O_2 production catalyzed by catalase, which could be related to the observation that metformin does not react with H_2O_2 [58] and therefore does not show any antioxidant-like effect.

4. Conclusion

In this study, we proposed an intestinal tissue-modified oxygen electrode, as a tool to measure the antioxidant activity of compounds in an isolated intestinal tissue. We were able to monitor the H_2O_2 scavenging properties of a known antioxidant, quinol, by measuring the inhibition of the O_2 produced by catalase in the presence of H_2O_2. Using this method, we measured the antioxidant activity of mesalazine and paracetamol. This effect could be explained by the presence of phenolic ring, since phenolic compounds are known to have ROS scavenging properties. Although in therapy these drugs are not primarily used for their antioxidant properties, they could potentially be used for their antioxidant properties based on their chemical structures. The lack of any antioxidant effect recorded in the case of metformin could be explained from reports indicating that metformin does not react with H_2O_2. Based on our finding, we can conclude that the presented *ex vivo* sensor would be suitable for testing newly developed drugs that could have radical scavenging properties or can affect catalase activity. In addition, it could be used for mechanistic investigation as well as for studying the dose dependent antioxidant activity of oral drugs.

CRediT authorship contribution statement

Sriram Thoppe Rajendran: Investigation, Methodology, Validation, Writing - original draft. **Kinga Huszno:** Investigation, Methodology. **Grzegorz Dębowski:** Investigation, Methodology. **Javier Sotres:** Resources. **Tautgirdas Ruzgas:** Conceptualization, Methodology, Resources, Writing - review & editing. **Anja Boisen:** Funding acquisition, Writing - review & editing. **Kinga Zór:** Conceptualization, Methodology, Supervision, Project administration, Writing - original draft.

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.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2020.107720.

References

[1] C. Mazzoni, F. Tentor, S.A. Strindberg, L.H. Nielsen, S.S. Keller, T.S. Alstrøm, C. Gundlach, A. Müllertz, P. Marizza, A. Boisen, From concept to in vivo testing: Microcontainers for oral drug delivery, J. Control. Release 268 (2017) 343–351, https://doi.org/10.1016/J.JCONREL.2017.10.013.

[2] G. Gaucher, P. Satturwar, M.-C. Jones, A. Furtos, J.-C. Leroux, Polymeric micelles for oral drug delivery, Eur. J. Pharm. Biopharm. 76 (2010) 147–158, https://doi.org/10.1016/J.EJPB.2010.06.007.

[3] S. Mitragotri, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: Formulation and delivery strategies, Nat. Rev. Drug Discov. 13 (2014) 655–672, https://doi.org/10.1038/nrd4363.

[4] E. Sjögren, B. Abrahamsson, P. Augustijns, D. Becker, M.B. Bolger, M. Brewster, J. Brouwers, T. Flanagan, M. Harwood, C. Heinen, R. Holm, H.-P. Juretschke, M. Kubbinga, A. Lindahl, V. Lukacova, U. Münster, S. Neuhoff, M.A. Nguyen, A. van Peer, C. Reppas, A.R. Hodjegan, C. Tannergren, W. Weitschies, C. Wilson, P. Zane, H. Lennernäs, P. Langguth, In vivo methods for drug absorption – Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects, Eur. J. Pharm. Sci. 57 (2014) 99–151, https://doi.org/10.1016/J.EJPS.2014.02.010.

[5] I.T. Consortium, K.M. Giacomini, S.-M. Huang, D.J. Tweedie, L.Z. Benet, K.L.R. Brouwer, X. Chu, A. Dahlin, R. Evers, V. Fischer, K.M. Hillgren, K.A. Hoffmaster, T. Ishikawa, D. Keppler, R.B. Kim, C.A. Lee, M. Niemi, J.W. Polli, Y. Sugiyama, P. W. Swaan, J.A. Ware, S.H. Wright, S.W. Yee, M.J. Zamek-Gliszczynski, L. Zhang, Membrane transporters in drug development, Nat. Rev. Drug Discov. 9 (2010) 215–236, https://doi.org/10.1038/nrd3028.

[6] M. Kasendra, A. Tovaglieri, A. Sontheimer-Phelps, S. Jalili-Firoozinezhad, A. Bein, A. Chalkiadaki, W. Scholl, C. Zhang, H. Rickner, C.A. Richmond, H. Li, D.T. Breault, D.E. Ingber, Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids, Sci. Rep. 8 (2018) 2871, https://doi.org/10.1038/s41598-018-21201-7.

[7] K.E. Samy, E.S. Levy, K. Phong, B. Demaree, A.R. Abate, T.A. Desai, Human intestinal spheroids cultured using Sacrificial Micromolding as a model system for studying drug transport, Sci. Rep. 9 (2019) 9936, https://doi.org/10.1038/s41598-019-46408-0.

[8] R.R. Castillo, M. Colilla, M. Vallet-Regí, Advances in mesoporous silica-based nanocarriers for co-delivery and combination therapy against cancer, Expert Opin. Drug Deliv. 14 (2017) 229–243, https://doi.org/10.1080/17425247.2016.1211637.

[9] B. Sarmento, F. Andrade, S.B. da Silva, F. Rodrigues, J. das Neves, D. Ferreira, Cell-based in vitro models for predicting drug permeability, Expert Opin. Drug Metab. Toxicol. 8 (2012) 607–621, https://doi.org/10.1517/17425255.2012.673586.

[10] S.A. Peters, C.R. Jones, A.-L. Ungell, O.J.D. Hatley, Predicting drug extraction in the human gut wall: assessing contributions from drug metabolizing enzymes and transporter proteins using preclinical models, Clin. Pharmacokinet. 55 (2016) 673–696, https://doi.org/10.1007/s40262-015-0351-6.

[11] C. Awortwe, P.S. Fasinu, B. Rosenkranz, Application of Caco-2 cell line in herb-drug interaction studies: current approaches and challenges, J. Pharm. Pharm. Sci. 17 (2014) 1–19 (accessed October 3, 2019) http://www.ncbi.nlm.nih.gov/pubmed/24735758.

[12] E. Managlia, R.B. Katzman, J.B. Brown, T.A. Barrett, Antioxidant properties of mesalamine in colitis inhibit phosphoinositide 3-kinase signaling in progenitor cells, Inflamm. Bowel Dis. 19 (2013) 2051–2060, https://doi.org/10.1097/MIB.0b013e318297d741.

[13] D. Tripathy, P. Grammas, Acetaminophen protects brain endothelial cells against oxidative stress, Microvasc. Res. 77 (2009) 289–296, https://doi.org/10.1016/j.mvr.2009.02.002.

[14] A. Bhattacharyya, R. Chattopadhyay, S. Mitra, S.E. Crowe, Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases, Physiol. Rev. 94 (2014) 329–354, https://doi.org/10.1152/physrev.00040.2012.

[15] V. Bhor, N. Raghuram, S. Sivakami, Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozotocin-induced diabetic rats, Int. J. Biochem. Cell Biol. 36 (2004) 89–97, https://doi.org/10.1016/S1357-2725(03)00142-0.

[16] A. Tomusiak-Plebanek, P. Heczko, B. Skowron, A. Baranowska, K. Okoń, P.J. Thor, M. Strus, Lactobacilli with superoxide dismutase-like or catalase activity are more effective in alleviating inflammation in an inflammatory bowel disease mouse model, Drug Des. Devel. Ther. 12 (2018) 3221–3233, https://doi.org/10.2147/DDDT.S164559.

[17] D.C. Wijesuriya, G.A. Rechnitz, Biosensors based on plant and animal tissues, Biosens. Bioelectron. 8 (1993) 155–160, https://doi.org/10.1016/0956-5663(93)85027-L.

[18] M. Mascini, M. Iannello, G. Palleschi, A liver tissue-based electrochemical sensor for hydrogen peroxide, Anal. Chim. Acta 138 (1982) 65–69, https://doi.org/10.1016/S0003-2670(01)85287-9.

[19] A.-L. Ungell, S. Nylander, S. Bergstrand, Å. Sjöberg, H. Lennernäs, Membrane transport of drugs in different regions of the intestinal tract of the rat, J. Pharm. Sci. 87 (1998) 360–366, https://doi.org/10.1021/js970218s.

[20] S. Wu-Pong, V. Livesay, B. Dvorchik, W.H. Barr, Oligonucleotide transport in rat and human intestine Ussing chamber models, Biopharm. Drug Dispos. 20 (1999) 411–416, https://doi.org/10.1002/1099-081X(199912)20:9<411::AID-BDD208>3.0.CO;2-4.

[21] B.J.P. Nejdfors, M. Ekelund, Mucosal in vitro permeability in the intestinal tract of the Pig, the Rat, and Man: species- and region-related differences, Scand. J. Gastroenterol. 35 (2000) 501–507, https://doi.org/10.1080/003655200750023769.

[22] I.S. Haslam, D.A. O'Reilly, D.J. Sherlock, A. Kauser, C. Womack, T. Coleman, Pancreatoduodenectomy as a source of human small intestine for Ussing chamber investigations and comparative studies with rat tissue, Biopharm. Drug Dispos. 32 (2011) 210–221, https://doi.org/10.1002/bdd.751.

[23] E. Pretorius, P.J.D. Bouic, Permeation of four oral drugs through human intestinal mucosa, AAPS PharmSciTech. 10 (2009) 270–275, https://doi.org/10.1208/s12249-009-9207-4.

[24] H. Lennernäs, Animal data: The contributions of the Ussing Chamber and perfusion systems to predicting human oral drug delivery in vivo☆, Adv. Drug Deliv. Rev. 59 (2007) 1103–1120, https://doi.org/10.1016/j.addr.2007.06.016.

[25] D. Hubbard, H. Ghandehari, D.J. Brayden, D.J. Brayden, Transepithelial transport of PAMAM dendrimers across isolated rat jejunal mucosae in ussing chambers, Biomacromolecules 15 (2014) 2889–2895, https://doi.org/10.1021/bm5004465.

[26] E.M. Walters, E. Wolf, J.J. Whyte, J. Mao, S. Renner, H. Nagashima, E. Kobayashi, J. Zhao, K.D. Wells, J.K. Critser, L.K. Riley, R.S. Prather, Completion of the swine genome will simplify the production of swine as a large animal biomedical model, BMC Med. Genomics 5 (2012) 55, https://doi.org/10.1186/1755-8794-5-55.

[27] J.A. Jiminez, T.C. Uwiera, G. Douglas Inglis, R.R.E. Uwiera, Animal models to study acute and chronic intestinal inflammation in mammals, Gut Pathog. 7 (2015) 29, https://doi.org/10.1186/s13099-015-0076-y.

[28] J. Pravda, Radical induction theory of ulcerative colitis, World J. Gastroenterol. 11 (2005) 2371–2384, https://doi.org/10.3748/wjg.v11.i16.2371.

[29] Y.S. Bae, H. Oh, S.G. Rhee, Y. Do Yoo, Regulation of reactive oxygen species generation in cell signaling, Mol. Cells 32 (2011) 491–509, https://doi.org/10.1007/s10059-011-0276-3.

[30] P.D. Ray, B.-W. Huang, Y. Tsuji, Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling, Cell. Signal. 24 (2012) 981–990, https://doi.org/10.1016/j.cellsig.2012.01.008.

[31] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid, Alexandria J. Med. (2017), https://doi.org/10.1016/J.AJME.2017.09.001.

[32] S. Nocchi, S. Björklund, B. Svensson, J. Engblom, T. Ruzgas, Electrochemical monitoring of native catalase activity in skin using skin covered oxygen electrode, Biosens. Bioelectron. 93 (2017) 9–13, https://doi.org/10.1016/j.bios.2017.01.001.

[33] M. Eskandari, J. Rembiesa, L. Startaitė, A. Holefors, A. Valančiūtė, F. Faridbod, M.R. Ganjali, J. Engblom, T. Ruzgas, Polyphenol-hydrogen peroxide reactions in skin: In vitro model relevant to study ROS reactions at inflammation, Anal. Chim. Acta 1075 (2019) 91–97, https://doi.org/10.1016/j.aca.2019.05.032.

[34] L.C. Clark, C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, Ann. N. Y. Acad. Sci. 102 (1962) 29–45, https://doi.org/10.1111/j.1749-6632.1962.tb13623.x.

[35] M. Ueda, H. Kinoshita, T. Yoshida, N. Kamasawa, M. Osumi, A. Tanaka, Effect of catalase-specific inhibitor 3-amino-1,2,4-triazole on yeast peroxisomal catalase in vivo, FEMS Microbiol. Lett. 219 (2003) 93–98, https://doi.org/10.1016/S0378-1097(02)01201-6.

[36] M. Gyuraszova, A. Kovalcikova, R. Gardlik, Association between oxidative status and the composition of intestinal microbiota along the gastrointestinal tract, Med. Hypotheses 103 (2017) 81–85, https://doi.org/10.1016/j.mehy.2017.04.011.
[37] O.M. Lardinois, M.M. Mestdagh, P.G. Rouxhet, Reversible inhibition and irreversible inactivation of catalase in presence of hydrogen peroxide, Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1295 (1996) 222–238, https://doi.org/10.1016/0167-4838(96)00043-X.
[38] C. Rice-Evans, N. Miller, G. Paganga, Antioxidant properties of phenolic compounds, Trends Plant Sci. 2 (1997) 152–159, https://doi.org/10.1016/S1360-1385(97)01018-2.
[39] L. Fu, B.-T. Xu, X.-R. Xu, R.-Y. Gan, Y. Zhang, E.-Q. Xia, H.-B. Li, Antioxidant capacities and total phenolic contents of 62 fruits, Food Chem. 129 (2011) 345–350, https://doi.org/10.1016/j.foodchem.2011.04.079.
[40] T.C.P. Dinis, V.M.C. Madeira, L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch. Biochem. Biophys. 315 (1994) 161–169, https://doi.org/10.1006/abbi.1994.1485.
[41] R. Karagozian, R. Burakoff, The role of mesalamine in the treatment of ulcerative colitis, Ther. Clin. Risk Manag. 3 (2007) 893–903. https://www.ncbi.nlm.nih.gov/pubmed/18473013.
[42] M. Ham, A.C. Moss, Mesalamine in the treatment and maintenance of remission of ulcerative colitis, Expert Rev. Clin. Pharmacol. 5 (2012) 113–123, https://doi.org/10.1586/ecp.12.2.
[43] M.A. Peppercorn, Sulfasalazine, Ann. Intern. Med. 101 (1984) 377, https://doi.org/10.7326/0003-4819-101-3-377.
[44] D.C. Pearson, D. Jourd'heuil, J.B. Meddings, The anti-oxidant properties of 5-aminosalicylic acid, Free Radic, Biol. Med. 21 (1996) 367–373, https://doi.org/10.1016/0891-5849(96)00031-7.
[45] F. Shahidi, P.K. Janitha, P.D. Wanasundara, Phenolic antioxidants, Crit. Rev. Food Sci. Nutr. 32 (1992) 67–103, https://doi.org/10.1080/10408399209527581.
[46] P. Løkken, P. Skjelbred, Analgesic and anti-inflammatory effects of paracetamol evaluated by bilateral oral surgery, Br. J. Clin. Pharmacol. 10 (Suppl 2) (1980) 253S–260S, https://doi.org/10.1111/j.1365-2125.1980.tb01808.x.
[47] Potential anti-inflammatory role of paracetamol in knee OA, Nat. Clin. Pract. Rheumatol. 3 (2007) 67–67. https://doi.org/10.1038/ncprheum0391.
[48] R.N. DuBois, K.E. Hill, R.F. Burk, Antioxidant effect of acetaminophen in rat liver, Biochem. Pharmacol. 32 (1983) 2621–2622, https://doi.org/10.1016/0006-2952(83)90034-5.
[49] G.F. Merrill, E. Goldberg, G.F. Merrill, E. Goldberg, Antioxidant properties of acetaminophen and cardioprotection, Basic Res. Cardiol. 96 (2001) 423–430 (accessed November 1, 2018) http://production.datastore.cvt.dk/filestore?oid=548206838c7966ac1d019ca8&targetid=548206838c7966ac1d019caa.
[50] C.I. Ghanem, M.J. Pérez, J.E. Manautou, A.D. Mottino, Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity, Pharmacol. Res. 109 (2016) 119–131, https://doi.org/10.1016/j.phrs.2016.02.020.
[51] E.R. Blough, M. Wu, Acetaminophen: beyond pain and Fever-relieving, Front. Pharmacol. 2 (2011) 72, https://doi.org/10.3389/fphar.2011.00072.
[52] R. Amann, B.A. Peskar, Anti-inflammatory effects of aspirin and sodium salicylate, Eur. J. Pharmacol. 447 (2002) 1–9, https://doi.org/10.1016/S0014-2999(02)01828-9.
[53] J.J. Loux, P.D. DePalma, S.L. Yankell, Antipyretic testing of aspirin in rats, Toxicol. Appl. Pharmacol. 22 (1972) 672–675, https://doi.org/10.1016/0041-008X(72)90295-5.
[54] N. Grosser, A. Abate, S. Oberle, H.J. Vreman, P.A. Dennery, J.C. Becker, T. Pohle, D.S. Seidman, H. Schröder, Heme oxygenase-1 induction may explain the antioxidant profile of aspirin, Biochem. Biophys. Res. Commun. (2003), https://doi.org/10.1016/S0006-291X(03)01504-3.
[55] A. Guerrero, J. González-Correa, M. Arrebola, J. Muñoz-Marín, F. Sánchez De La Cuesta, J. De La Cruz, Antioxidant effects of a single dose of acetylsalicylic acid and salicylic acid in rat brain slices subjected to oxygen-glucose deprivation in relation with its antiplatelet effect, Neurosci. Lett. 358 (2004) 153–156, https://doi.org/10.1016/J.NEULET.2004.01.036.
[56] N. Ouslimani, J. Peynet, D. Bonnefont-Rousselot, P. Thérond, A. Legrand, J.L. Beaudeux, Metformin decreases intracellular production of reactive oxygen species in aortic endothelial cells, Metabolism. (2005), https://doi.org/10.1016/j.metabol.2005.01.029.
[57] A.R. Cameron, V.L. Morrison, D. Levin, M. Mohan, C. Forteath, C. Beall, A.D. McNeilly, D.J.K. Balfour, T. Savinko, A.K.F. Wong, B. Viollet, K. Sakamoto, S.C. Fagerholm, M. Foretz, C.C. Lang, G. Rena, Anti-inflammatory effects of metformin irrespective of diabetes status, Circ. Res. 119 (2016) 652–665, https://doi.org/10.1161/CIRCRESAHA.116.308445.
[58] D. Bonnefont-Rousselot, B. Raji, S. Walrand, M. Gardès-Albert, D. Jore, J.A. Legrand, J. Peynet, M.P. Vasson, An intracellular modulation of free radical production could contribute to the beneficial effects of metformin towards oxidative stress, Metabolism 52 (2003) 586–589, https://doi.org/10.1053/meta.2003.50093.