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Oxidation-labile linkers for controlled drug delivery

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ABSTRACT

The continuous symbiosis throughout chemical biology and drug discovery has led to the design of innovative bifunctional molecules for targeted and controlled drug delivery. Among the different tools, protein-drug and peptide-drug conjugates are trend approaches to achieve targeted delivery, selectivity and efficacy. To meet the main goals of these bioconjugates, the selection of the appropriate payloads and linkers is crucial, as they must provide *in vivo* stability, while they may also help to achieve the therapeutic target and action. In neurodegenerative diseases or some cancer types, where oxidative stress plays an important role, linkers sensitive to oxidative conditions may be able to release the drug once the conjugate achieves the target. Considering specially this specific application, this mini-review covers the most relevant publications on oxidation-labile linkers.

Introduction

Bioconjugates containing an antibody, nanobody, protein or peptide and a specific drug are an ongoing trend within chemical biology.[1,2,3] Chemoselective modification of proteins and peptides with a bioactive compound is useful to improve the selectivity of its biological action, by directing the drug to a specific site, and promoting its delivery.[4,5] The construction of the right structure to allow the transport and the passage through biological barriers is crucial. Therefore, the linker that brings the different parts of the conjugate together is a very important piece of this puzzle. This linker must be appropriate to the expected use of the conjugate, and it should not interfere with either the biomolecule or the drug function, being at the same time biocompatible and non-toxic.[6]

The development of antibody-drug conjugates (ADCs) is being explored mainly in oncology, where the delivery of a cytotoxic drug to specific cells may surpass important side effects of the therapeutics on healthy cells.[7,8] Another field in which these approaches may be interesting is neurodegeneration. Neurodegenerative diseases are complex pathologies, highly prevalent all over the world, causing a big impact on the global health.[9] Most of these diseases remain incurable, and many of the approved therapies can only palliate the symptoms, failing to reverse the progression of the disease.[10] As example, exogenous delivery of antioxidants to the brain holds promise to alleviate oxidative stress to regain the redox balance.[11] However, one of the principal handicaps to the effectiveness of the therapeutics is the presence of the blood–brain barrier (BBB). This physiological barrier prevents the passage of xenobiotics into the central nervous system (CNS). Even if this mechanism is essential to protect the brain from toxic compounds, it also affects the ability of some drugs to reach the targets.[12] However, there are substances that may achieve the CNS via specific transporters, among which some peptides and proteins can be found.[13] This has been the inspiration for the design of new conjugates capable to reach the brain, avoiding physiologic membranes and efflux pumps.[14] Innovative therapeutic approaches for these complex and multifactorial diseases are urgent, and bioconjugation is opening new doors.

Neurodegenerative disorders present some special conditions that may be unique and useful for the design of new strategies to controlled drug release.[15,16] The increased oxidative stress observed within the CNS when neurodegenerative diseases are established is inspiring the design of new linkers able to delivery drugs in the presence of reactive oxygen species (ROS).[17] High levels of ROS have also been found in different cancers.[18] Suitable linkers for this class of conjugates should be able to take advantage of this property to be cleaved in these conditions while being stable in the peripheral circulation (Fig. 1).

This mini-review aims to analyze the different options currently available for the design and synthesis of chemical linkers that can be cleaved in mild oxidative environments. Bibliographic sources as SciFinder, Mendley and PubMed have been consulted in order to highlight the most relevant research articles and reviews within the field. The criteria for the selection of relevant materials have focused on the amount of data from each author and the impact of the publication within the field. Due to the wide range of the topic, a wide variety of

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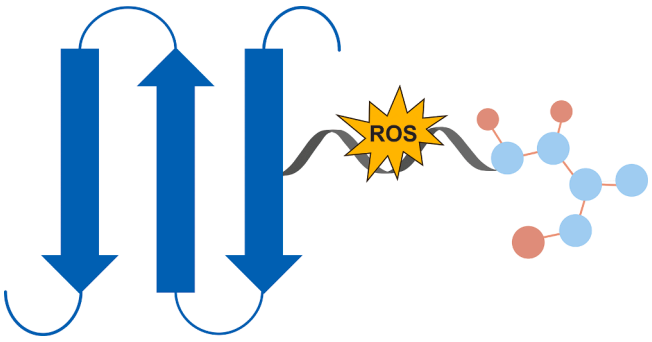

Fig. 1. Schematic representation of ROS-responsive drug delivery systems made up of a biomolecule (protein, antibody, nanobody or peptide), a ROS sensitive linker and the drug of interest.

journals from different fields has been examined, giving a more holistic view of the subject of study. Although efforts have been made to include as much relevant published information as possible, this BMCL Digest tries to provide a quick and concise overview of the subject. In this sense, the families of the most relevant linkers are organized considering their chemical structure. This division allows the discussion of six different categories (aryl boronates, aryl hydrazides, diazoborines, thioacetals and thioketals, thiazolidinones, vinyldithioethers, Fig. 2) that are going to be discussed in terms of their feasibility to be implemented in drug conjugation techniques.

Aryl boronates, comprising both phenylboronic acids and phenylboronic esters, are susceptible to oxidative cleavage in the presence of hydrogen peroxide (H_2O_2, Fig. 2A).[19] The activation mode involves a nucleophilic attack by H_2O_2 to the electron-deficient boron atom of the aryl boronate. Subsequently, these species undergo a Baeyer-Villiger oxidation-like transformation that can help the release of a conjugated drug.[20] These boron-containing linkers allow the possibility of conjugating drugs bearing a free hydroxyl group. The selected biomolecule may be feasible to attach, given the electrophilicity of the boronate[21] and the variety of nucleophilic groups present in amino acids. This would lead to the formation of a stable biomolecule-drug conjugate that could be activated once exposed to a high level of H_2O_2, which is likely to happen in cells affected by oxidative stress.

Despite the limited scope of this kind of linkers, they are a potentially valuable option to explore when a suitable functional group is present in the drug of interest. They are easily accessed synthetically, and the conditions required for the cleavage can be specific enough to get them directly to the area where the therapeutic action is needed. Further studies are needed to confirm this hypothesis.

Aryl hydrazides have been long known to be cleaved by oxidants to produce arenes and nitrogen *via* dehydrogenation, as shown in Fig. 2B.[22] This reactivity was exploited to facilitate solid-phase synthesis,[23] with optimization studies carried out to evaluate the specific oxidative conditions required to break the linker.

Adapting these findings about the oxidative cleavage of aryl hydrazides to drug conjugation can be feasible, considering that the byproducts of the reaction are nitrogen gas, biologically inert and easily eliminated *via* exhalation, and a carboxylic acid derivative, as the major nucleophile in the human body is water, while the drug with a terminal

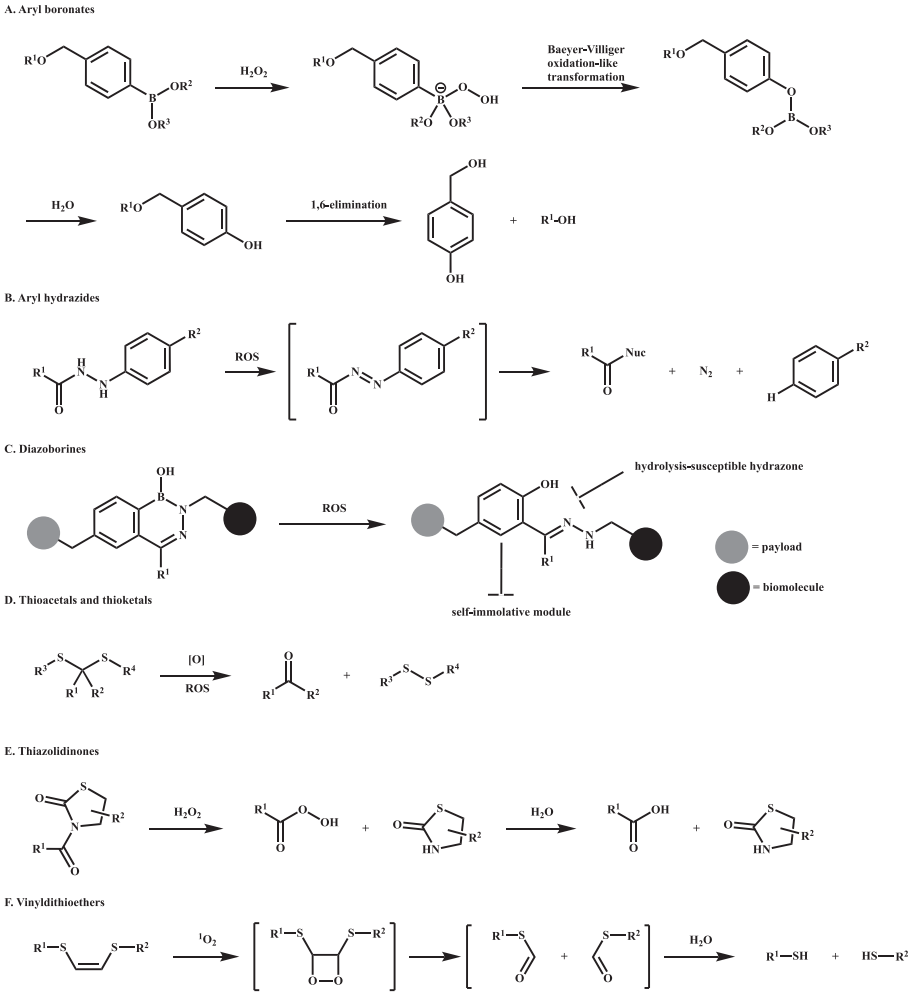

Fig. 2. Examples of the cleavage of the most relevant linkers sensitive to oxidation stimuli. A. Oxidative cleavage of a phenyl boronate linker in the presence of H_2O_2. B. Oxidative cleavage of an aryl hydrazide via dehydrogenation. C. Oxidative cleavage of a diazoborine via a hydrolysis-susceptible hydrazone. D. Oxidative cleavage of thioacetal/thioketal in the presence of ROS. E. Oxidative cleavage of a thiazolidinone linker in the presence of H_2O_2. F. Oxidative cleavage of a vinyldithioether linker in the presence of $^1O_2$.

aryl group is released.[24] This feature can be considered to design the corresponding aryl hydrazide-based linkers to release a particular amino acid after cleavage. This can be easily synthetically achieved and would lead to the *in vivo* release of the drug along with non-toxic chemical species.[25].

The high concentration of ROS in a human brain affected by oxidative stress due to a neurodegenerative disease, for example, can generate the appropriate conditions for the cleavage. While further exploration must be made, testing this possibility, along with the stability of the conjugates in peripheral circulation, could result in the implementation of aryl hydrazides as potential oxidation-labile linkers for drug conjugation to be explored for CNS diseases.[26] As a drawback, this strategy would only be possible in the case of drugs that present terminal aryl groups, as this motif is required to perform the reaction.

A synthetic ROS-responsive homogeneous ADC highly selective and cytotoxic to B-cell lymphoma (CLBL-1 cell line, $IC_{50} = 54.1$ nM) has been recently described for the first time (Fig. 2C).[27] The synthesis of this ADC, a turning point within the field, has been possible due to the discovery that diazaborines are a very effective ROS-responsive unit that are also very stable in physiologic conditions (studied in buffer) and in plasma. The oxidation mechanism of these linkers proved to be similar to the aromatic boronic acids.[28] Diazaborines very fast formation rate and modularity enabled the construction of different ROS-responsive linkers featuring self-immolative modules, bioorthogonal functions, and bioconjugation handles.

These conjugates are a new class of therapeutics that combine the lethality of potent cytotoxic drugs with the targeting ability of antibodies to selectively deliver drugs to cancer cells in which high levels of ROS may be involved. This approach can also be applied to neurodegenerative disorders, using the right biomolecule able to cross the BBB and the ideal payload.

As already discussed in a review focused on drug delivery systems, sulfur-containing motifs can be used as oxidation-labile linkers.[29] These linkers can be subclassified in terms of the reactive functional group in thioacetals/thioketals, thiazolidinones and vinylthioethers.

Thioacetals and thioketals are sulfur analogs of the more common oxygen-containing acetals and ketals. These sulfur species are known to be stable in both acidic and basic conditions, but they are nonetheless able to be cleaved in oxidative conditions, as shown in Fig. 2D.[30] This cleavage of a potential thioketal-based linker would release a ketone and a disulfide when exposed to a high concentration of ROS. Thus, it can be feasible to use this kind of linkers to attach a ketone-containing drug to the correspondent biomolecule. Care should be taken with the disulfide byproduct, as these species can lead to secondary effects, such as bad odor.[31] Nonetheless, it can be speculated that these secondary effects can be mitigated by modifying the structure of the thioketal to ensure that the byproduct formed is not harmful.

A thiazolidinone is a heterocycle containing sulfur and nitrogen that can act as a leaving group after nucleophilic attack by H_2O_2,[32] which is present in living cells as a ROS (Fig. 2E).[20] The reactivity inherent to this chemical moiety enables the design of thiazolidinone-linked conjugates to release drugs containing a carboxylic functional group, a strategy that has already been used for prodrug purposes.[33].

Carboxylic acid-containing compounds are usually too polar to get into cells, so they tend to be masked with an ester group to improve their diffusion properties with prodrug approaches. [34] Considering this strategy as a reference, using thiazolidinone linkers could enable drug delivery strategies to transport the relevant compounds directly into the cells affected by oxidative stress, while releasing their cargo after exposure to ROS. Therefore, this kind of linkers, while specific to carboxyl-containing drugs, can be expected to show promising applications in the therapeutic field, while further studies are needed to confirm the hypothesis.

A vinyldithioether is an olefin-containing structure that can undergo a [2 + 2] cycloaddition with singlet oxygen ($^1O_2$) when exposed to ultraviolet (UV) light. This reaction leads to the formation of unstable dioxetanes, that get fragmented under physiological conditions to form two carbonyl groups that subsequently get cleaved to yield the two corresponding thiol derivatives, as shown in Fig. 2F.[35].

The main drawback of this type of linkers is that they need external UV light for their activation, although this does not prevent them from finding their use in cancer therapy. This is consistent with the applications that have been reported in the literature.[36] Despite its limitations, the chemical nature of this linker arises some advantages, such as the possibility of attaching it directly to a cysteine residue in a potential biomolecule. Furthermore, it has proven to be sufficiently stable in biological systems.[37] These features make vinyldithioethers a suitable option to be considered in particular contexts for drug conjugation, while the conditions required for their activation discard them as universal linkers.

Other types of moieties able to be cleaved by ROS have been reported in the literature in addition to those already presented in this mini-review. Among them, selenium and tellurium linkers must be highlighted due to their novelty and versatility. The species containing these atoms in their structure get cleaved in oxidative conditions if they are carefully designed to form unstable intermediates that react spontaneously to release the drug. This strategy is described in more depth in the original reference.[38] This approach may be an alternative for metabolic chemical reactions occurring in an oxidative environment in cells and tissues.

The ability to use fewer common atoms for medicinal chemistry purposes make these strategies have a great scientific interest beyond their applications as oxidation-labile linkers, such as the study of cage complexes in bioinorganic chemistry, and progress in these approaches is expected.

Conclusions

The continuous progress of chemical biology and medicinal chemistry enabled the possibility of approaching new strategies beyond the traditional small molecules design. One of them is the development of drug conjugates to improve the pharmacokinetic profile of drugs and increase their selectivity and efficacy. These strategies can be particularly fitted for those diseases where oxidative stress plays an important role, such as neurodegenerative diseases or cancer, as conjugates can be designed to release the drug in these specific conditions. Together with drug delivery systems, ROS labile linkers may find interesting applications in prodrug and fluorescent probes design. For these purposes, the linker employed must be responsive to ROS and be cleaved in their presence. The system may bring the conjugate to the affected cells and be activated at a specific location. There are several options available to perform this drug conjugation techniques, as this mini-review has aimed to cover. Among them, aryl hydrazides, sulfur-containing linkers and aryl boronates have been reported as the most common and reliable ones, while different options are found related to the use of selenium or tellurium. There are more chemical species that can be considered for this matter, having this review been focused on those that have been more studied. This is a topic that can be expected to be further explored in the near future with the rise of heterobifunctional molecules in drug development requiring advances in the arsenal of ROS-cleavable linkers.

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.

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Author contribution

All authors have given approval to the final version of the manuscript.

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