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Acrolein detection: potential theranostic utility in multiple sclerosis and spinal cord injury

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Abstract

Oxidative stress has been implicated as a major pathological process underlying CNS disease and trauma. More specifically, acrolein, an unsaturated aldehyde, produced by way of lipid peroxidation, has been shown to play a crucial role in initiating and perpetuating detrimental effects associated with multiple sclerosis and spinal cord injury. In light of these findings, quantification of acrolein levels both systemically and locally could allow for the use of acrolein as a biomarker to aid in diagnosis and guide treatment regimens. The three main approaches currently available are acrolein derivatization followed byLC/GC–MS, application of an acrolein antibody and subsequent immunoblotting, and the 3-hydroxypropylmercapturic acid-based method. Of these three strategies, the 3-hydroxypropylmercapturic acid-based method is the least invasive allowing for rapid translation of acrolein detection into a clinical setting.

Keywords

3-HMPA; acrolein; acrolein–lysine adduct; hydralazine; multiple sclerosis; oxidative stress; spinal cord injury

Pathological role of acrolein in trauma & neurodegenerative diseases

Numerous studies suggest that oxidative stress plays a key role in various CNS diseases and trauma [1–3]. This can partly be attributed to the fact that the CNS is inherently more susceptible to oxidative stress than other tissues of the body for many reasons including low

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levels of intrinsic antioxidants (catalase and GSH-peroxidase), high content of polyunsaturated lipids (membrane and myelin) and high levels of reactive oxygen species (ROS) generated by the fundamental neurochemical processes in resting and pathological conditions [1–6]. Thus, in pathological states, the CNS is particularly vulnerable to oxidative damage mediated by ROS and lipid peroxidation (LPO) products. To date, efforts to curtail oxidative damage in the CNS have predominantly focused on pharmacologically targeting ROS to aid in the suppression of heightened levels of these compounds and ultimately circumvent neurotoxic effects [5,7–9]. However, this approach has demonstrated marginal success in improving behavioral outcome in CNS disease and trauma, prompting efforts to identify an alternative oxidative stress-related target for pharmacological intervention.

Advancements in knowledge of oxidative stress pathways have led to targeting LPO products in lieu of more commonly studied ROS. As such, LPO products are currently being investigated to ascertain whether they can serve as viable pharmaceutical targets to effectively combat oxidative stress. Among the LPO products, acrolein (2-propenal), an α,β-unsaturated aldehyde, has emerged as a key factor in mediating and perpetuating oxidative stress, most notably in CNS pathologies. Acrolein is the most abundant and reactive of LPO-derived aldehydes, present in concentrations 40 times greater than other aldehydes such as 4-hydroxynonenal, and rapidly reacts with key biomolecules including DNA, phospholipids and proteins [10–12]. In addition, the half-life of acrolein is substantially longer than that of ROS, which is in days compared to fractions of a second [10]. Furthermore, acrolein has also been shown to act as a catalyst to generate more oxidative species and, in turn, more acrolein. Therefore, it is likely that acrolein plays a key role in perpetuating oxidative stress. In addition to exposure to acrolein generated through endogenous oxidative LPO processes, acrolein exposure also occurs by way of exogenous sources such as emissions generated by petrol combustion, frying food, cigarette smoking and manufacturing processes [13–17]. The ability of acrolein to be generated both endogenously and exogenously indicates the potential for an additive effect between oxidative stress-related pathologies and environmental acrolein exposures: both synergistically exacerbate oxidative stress.

Acrolein has been shown to play a role in a wide range of nervous system pathologies such as spinal cord injury (SCI), multiple sclerosis (MS), Alzheimer's disease, amyotrophic lateral sclerosis and pain disorders, mainly based on animal models [8,18–21]. There are ample data indicating the highly toxic nature of acrolein to the CNS, capable of eliciting inflammation, demyelination and axonal injury [8,12,22–24]. In particular, animal studies have implicated acrolein as a key pathological factor underlying symptoms of SCI and experimental autoimmune encephalomyelitis (EAE), a murine model of MS [20,25,26]. In the case of SCI, acrolein appears to play a role in secondary or chemically mediated damage that occurs following primary mechanical trauma to the spinal cord tissue and is present in elevated levels in damaged tissue [26]. Also, when exogenous acrolein was directly administered to the spinal cord via micro-injection, or excised spinal cord tissue was exposed *in vitro*, tissue damage, including cell death and demyelination, was observed and was associated with motor and sensory behavioral deficits [8,27–30]. Notably, observed behavioral impairments corresponded to those seen in mechanically injured animals, indicating that acrolein alone is capable of eliciting tissue damage at a severity comparable

to SCI. In addition to the elevated level of acrolein in the damaged spinal cord tissue, the acrolein metabolites have also been reported at increased levels in the urine of an animal model of SCI.

Elevation of the level of acrolein was also detected in EAE, and application of hydralazine, an acrolein scavenger, afforded a neuroprotective effect. Many cellular and molecular changes elicited by the direct application of acrolein in *in vitro* and *in vivo* studies have also been observed in EAE. More specifically, as indicated by morphological studies, acrolein alone is capable of instigating demyelination in the spinal cord and similar effects on myelin integrity were also observed in EAE tissue. In both cases, myelin decompaction and loss resulted in the exposure of juxtaparanodal potassium channels leading to unregulated potassium conduction and aberrant channel expression along the neurons exhibiting myelin injury. Myelin loss and the subsequent dysregulation of ion channel expression leading to the failure of action potential conduction is theorized to be a major factor underlying the symptoms observed in MS patients. Following myelin loss, the axonal membrane becomes increasingly vulnerable to injury by acrolein, ultimately leading to axonal degeneration and permanent conduction deficit. Axonal degeneration observed in animal models likely corresponds to the pathological course seen in later stages of MS where functional recovery following an attack is absent. Furthermore, scavenging acrolein using pharmacological compounds effectively reduced acrolein levels and attenuated behavioral deficit in both SCI and EAE mice [20,31].

At the cellular level, the toxicity of acrolein in trauma and neurodegenerative diseases can be explained through its known mechanisms. For example, the pathological role of acrolein in EAE and SCI can be largely attributed to the ability of acrolein to attack a wide variety of biomolecules including lipids and proteins, both of which are main constituents of myelin sheath and axonal membrane, a damage common in both SCI and MS [20,26]. Recent studies have shown that acrolein may also contribute to myelin destruction through enzymatic mechanisms such as calpain activation [32,33]. Acrolein has also been shown to directly attack neuronal mitochondria, a key cellular organelle injured in both MS and SCI [5,23]. Furthermore, acrolein is also known to damage DNA [10,11,14], a known pathology in SCI [29]. When EAE mice were treated with acrolein scavenger hydralazine, myelin integrity was largely conserved relative to their untreated EAE counterparts, indicating that removal of acrolein offers neuroprotection and, consequently, contributes to the alleviation of structural and behavioral deficits [20]. A similar phenomenon was also observed in SCI rats where acrolein reduction through scavenging provided significant neuroprotection effectively alleviating motor and sensory deficits [26,34].

Due to the proven neurotoxicity, relatively long half-life, and its pathological role in neuronal trauma and degenerative diseases, acrolein has emerged as a pharmacological target to offer an effective treatment strategy. In addition, a potential correlation between the severity of injury and the level of acrolein elevation may indicate that acrolein could serve as a biomarker for diagnosis and even treatment evaluation. Indeed, recent advances in detecting endogenous acrolein levels using non-invasive methods may allow for the clinical use of acrolein as a biomarker to aid in diagnosis and to guide and evaluate clinical anti-acrolein treatment in both MS and SCI victims. Many reviews focus on the pathological role

of acrolein in MS and SCI, and relatively less attention has been paid to its potential usage as a biomarker and on the development of acrolein detection methods. As such, this review will focus on establishing the importance of acrolein detection and its use as a potential biomarker for diagnosis, prognosis and treatment evaluation. Specifically, we will review the methods of detection and their special significance and limitations in the context of nervous system trauma and neurodegenerative diseases.

The development of methods to detect acrolein levels

Research in acrolein-mediated toxic effects and acrolein detection can be dated back to studies conducted in the 1960s, which predominantly focused on the investigation of acrolein as an environmental pollutant [35]. Exposure to exogenous acrolein from emissions of industrial processes or tobacco combustion usually occurs on a larger scale, permitting direct quantification with conventional analytical chemistry techniques such as GC or LC followed by MS and subsequent derivatization steps [22,36,37]. Common derivatization agents, such as 2,4-dinitrophenylhydrazine [36] and cyclohexanedione [22], can effectively extract and stabilize acrolein. Although GC- and LC/MS-based techniques are adequate for acrolein exposure studies from exogenous sources, these methods have limited utility in endogenous acrolein detection. This can be attributed to the highly reactive nature of acrolein that facilitates the formation of covalent bonds with many cellular components, potentially eliciting a significant reduction in derivatization efficiency [11]. In addition to its high reactivity, acrolein is generated endogenously in significantly lower concentrations than when produced exogenously, presenting an additional challenge for GC/LC methods.

In recent years, acrolein has been investigated as not only an environmental pollutant but also an endogenously produced toxic compound and pathological factor in a wide range of neurological disorders [20,26,27]. In order to facilitate quantitative *in vivo* studies that are more comprehensive and capable of investigating the pathological role of acrolein, more sensitive acrolein quantification techniques are needed. Significant improvements in the detection of endogenous acrolein were made following the introduction of antibodies capable of binding specific acrolein–protein adducts, largely through the efforts of Uchida and his colleagues [38]. Consequently, immunoblotting techniques were employed to achieve enhanced sensitivity and specificity for acrolein quantification in biological entities and specific tissues of interest.

Among the acrolein antibody-based techniques, western blotting was the first to be employed in biological research to study the relationship of the nervous system and acrolein with enhanced sensitivity [26]. Acrolein antibodies were designed to bind acrolein–lysine adducts, enabling the quantification of any protein with lysine residues that have complexed with acrolein. Detection of acrolein-bound proteins utilizing these antibodies and western blotting enables the estimation of many proteins that are affected by acrolein, permitting the observation of the interaction of acrolein with many individual proteins. However, quantification of total acrolein-bound protein using western blotting can be inconvenient and marginally accurate when acrolein is present at lower levels. To overcome this, dot blotting, an alternative acrolein antibody-based technique, was introduced [27,29].

In contrast to western blotting, which utilizes a gel for protein separation, dot blotting transfers all the proteins to a single dot that represents the combined intensity of all bands, achieving a profound enhancement of signal intensity [20,29]. This is particularly significant when acrolein concentration is low, such as when measuring acrolein generated by endogenous processes. Additionally, dot blotting also permits detection in larger samples than western blotting and allows for artificial acrolein standards to be prepared and run simultaneously with the experimental samples, increasing the accuracy of quantification of acrolein in dot blotting. These are the important factors highlighting the utility of dot blotting in acrolein quantification and, consequently, dot blotting has been employed in many recent studies. In summary, if the relationships between acrolein and individual proteins are of primary interest, western blotting would be the preferred method. In contrast, when the concentration of acrolein is less and the overall acrolein level is of interest, and not individual acrolein–protein adducts, dot blotting is the appropriate method.

Although acrolein antibody-based techniques offer great sensitivity and have yielded important data, their applications in clinical research remain limited for a number of reasons. First, acrolein antibody-based techniques are highly invasive, significantly limiting their potential as a clinical screening tool. Second, acrolein antibody-based techniques evaluate only local acrolein accumulation rather than systemic acrolein concentrations. Third, although acrolein antibodies only recognize acrolein–lysine adducts, acrolein can also react with other amino acids such as cysteine, histidine and serine [29]. However, the bonds between acrolein and amino acid residues, other than lysine, are significantly weaker and are not as stable as acrolein–lysine adducts. Finally, acrolein antibody-based techniques remain complicated, labor intensive and time consuming, and the results depend highly on the technique and experience of the individual researcher.

To overcome the disadvantages of antibody-based techniques and facilitate translation of acrolein research into a clinical setting, alternative acrolein quantification techniques are currently being developed. In the past several years, many notable new acrolein quantification strategies have been introduced. Among them, the 3-hydroxypropylmercapturic acid (3-HPMA)-based method appears to be the most promising for future use in a clinical environment and has the potential to impact the diagnostic and therapeutic approaches for many pathologies [39,40]. Rather than quantifying acrolein or acrolein adducts, this approach quantifies 3-HPMA, a stable acrolein urinary metabolite, as a surrogate endpoint to deduce the true acrolein level. 3-HPMA is a unique metabolite for acrolein–glutathione adducts and its level correlates highly with acrolein in a 1:1 ratio [41]. One significant advantage of this approach is that 3-HPMA is a urinary metabolite of acrolein, permitting non-invasive estimation of acrolein by collecting urine [40]. Additionally, because urinary metabolites are circulating throughout the body before excretion into urine, 3-HPMA-based method provides a systematic quantification of acrolein concentrations. Notably, the current 3-HPMA quantification technique (LC–MS–MS) is an analytical approach that is significantly simpler compared to acrolein antibody-based techniques and can potentially be automated and miniaturized to facilitate future clinical applications [42].

Despite the aforementioned advantages, detection of 3-HPMA in urine also has limitations. The detection of 3-HPMA is a global estimation of acrolein and, hence, does not reflect the local origin underlying increased acrolein levels. Additionally, the formation of 3-HPMA is dependent on glutathione binding and its detection is more accurate when the level of acrolein is low and glutathione is adequate. However, with an increase in oxidative stress (and consequently, acrolein elevation), the levels of glutathione may not be able to appropriately compensate due to rapid consumption. As a result, diminished amounts of 3-HPMA are generated as the acrolein level continues to increase, resulting in underestimation of acrolein, a phenomenon demonstrated by Shi and colleagues in a rat SCI model [40]. Furthermore, since acrolein can bind molecules other than glutathione and 3-HPMA is a unique metabolite of acrolein–glutathione adducts, the 3-HPMA-based method may underestimate the true systemic acrolein level.

In summary, three main categories of acrolein quantification strategies have been developed so far: acrolein derivatization followed by LC/GC–MS, acrolein antibody-based immunoblotting and the 3-HPMA-based method. Each method has its own set of advantages and disadvantages as summarized in TABLE 1. It is apparent that a single method will not satisfy all the needs of experimental and clinical studies, and the most sensible approach to gain the overall and regional information of acrolein elevation is through the rational and justified combination of detection methods.

The use of acrolein detection methods to assess its pathological role

During the past several decades, researchers across the globe have independently implicated acrolein in a wide range of neurological disorders and trauma, such as Alzheimer’s disease [43], MS [20], SCI [27] and brain injury [44]. Advancements in acrolein detection techniques have enabled more researchers to effectively investigate the role of acrolein in these pathologies. With the emerging critical role of acrolein in trauma and disease and the availability of a variety of detection methods, it is reasonable to speculate that acrolein has the potential to serve as a biomarker for disease diagnosis and the evaluation of therapeutic efficacy.

In SCI, for example, the acrolein level exhibits a strong positive correlation with injury severity in SCI rat models on using both tissue-specific (dot blotting) and systemic (urine 3-HPMA) quantification methods. The different features of these two methods suggest that the dynamics of acrolein increase result from spinal cord trauma. For example, the observed increase of acrolein–lysine adducts in the spinal cord is 4.5 times greater while 3-HPMA increased only 1.8 times compared to control, suggesting that the spinal cord can serve as a major source of acrolein. Such results also suggest the limited diffusion capacity of acrolein into blood stream and considerable dilution prior to quantification of final systemic concentration [40].

In the case of MS, both dot blotting and 3-HPMA-based methods detected an increase in acrolein levels when the symptoms were fully developed in the EAE model. Compared to SCI, the magnitude of increase in acrolein within the spinal cord and its urinary metabolite 3-HPMA was similar in the EAE mouse when behavioral deficit was most severe. This

phenomenon can potentially be attributed to the similarity between local and systemic acrolein concentrations and reflects the characteristic pathology in EAE. Unlike SCI in which sudden increases in acrolein concentration are observed following acute physical trauma, EAE, as is appropriate for an animal model of MS, is a slow-developing progressive neurodegenerative pathology, and consequently, CNS acrolein accumulations occur in a prolonged, gradual fashion allowing local and systemic acrolein levels to become more equilibrated. Additionally, since SCI is a focal injury while EAE is systemic due to its immune-mediated nature, it is likely that acrolein is produced in a more diffusive manner in EAE, further contributing to the observation of more homogeneous acrolein concentrations among different organ systems. In summary, the detection of acrolein both locally and systemically is likely a true representation of acrolein dynamics in EAE. Since the level of acrolein has also been positively correlated with the severity of nervous system pathologies, it is reasonable to speculate that the level of acrolein can be used as a diagnostic tool to suggest not only the existence but also the progression of neurological injury.

Detection of acrolein level to evaluate therapeutic efficacy

To become a viable theranostic biomarker for medical research and drug development, a molecule should not only be able to serve as an indicator for disease severity but also offer insight into the therapeutic potential of drug candidates. Based on the reported data from Shi and his colleagues, acrolein meets such criteria and, therefore, appears to be a strong candidate for a biomarker. Specifically, in the case of SCI, it has been demonstrated that acrolein can be significantly reduced following the application of hydralazine, a known acrolein scavenger, measured locally using antibody immunoblotting to evaluate the spinal cord tissue *ex vivo* and systemically through the quantification of urinary 3-HPMA. Furthermore, a dose-dependent reduction of acrolein with application of the acrolein scavenger, hydralazine, has been shown. These observations strongly suggest that acrolein elevation can be used as an indication for acrolein therapy and the observed change of acrolein in response to acrolein scavenger delivery can be used to evaluate treatment efficacy. In summary, the existing data suggest that acrolein could potentially serve as an effective theranostic biomarker for neurological conditions where acrolein is implicated [40].

Clinical applicability is another crucial consideration that determines the utility of a small molecule as a theranostic biomarker. As mentioned above, the potential application of acrolein antibody-based detection in clinical research remains limited and is plausibly impossible. On the other hand, acrolein estimation through detection of 3-HPMA in the urine is a non-invasive approach, rendering it well-suited for translation to a clinical setting. It is expected that the non-invasive method of detection of 3-HPMA in the urine will play a critical role in future clinical studies aiming to establish the role of acrolein in human patients suffering from various neurological diseases and enable acrolein detection for diagnosis and treatment evaluation.

Conclusion

Existing evidence suggests a key pathological role of acrolein in several neurological diseases and trauma. Detection of acrolein played an indispensable role in the investigations that implicated acrolein as a target both for diagnosis and treatment evaluation. Among the three established acrolein quantification strategies, acrolein antibody strategy is the most suitable to measure local acrolein levels and quantification of urinary 3-HPMA is the best method for assessing global acrolein accumulation. In addition, the invasive nature of acrolein antibody-based detection limits its usage in animal studies while the minimally invasive quality of urinary 3-HPMA quantification seems to hold the greatest promise for clinical applications. It is expected that the 3-HPMA-based method will make a major contribution to future clinical studies, particularly in implicating acrolein in clinical cases of SCI and MS.

While it seems to be promising in potential future clinical applications, the limitations of the 3-HPMA-based method should not be overlooked. Besides those already mentioned, one practical issue is that current 3-HPMA-based method relies heavily on advanced analytical chemistry equipment which, although accurate and efficient, can be cost-inhibitive for regular clinical use. Therefore, future efforts geared toward developing enhanced acrolein quantification strategies that can be used in most clinical scenarios would benefit from focusing on improving the cost–effectiveness without compromising accuracy.

Expert commentary

Acrolein has been implicated in a wide range of neurological disorders and has the potential to become a valuable theranostic target for clinical applications. Powerful acrolein detection methods are essential for clinical acrolein research. Although far from perfect, the newly developed, highly sensitive and non-invasive 3-HPMA-based method offers new advantages, promoting the rapid translation of basic acrolein research into clinical applications. However, the 3-HPMA-based method is, nevertheless, an indirect acrolein detection method and provides only systemic acrolein level assessment. Additionally, generation of 3-HPMA is highly dependent on the local levels of glutathione as 3-HPMA is a unique metabolite of acrolein–glutathione adducts. When glutathione levels are depleted, the 3-HPMA-based method can produce misleading results in terms of quantifying true intrinsic acrolein level. Therefore, traditional acrolein detection methods, such as the immunoblotting-based method, still provide unique advantages and should not be discounted in acrolein research.

Five-year view

In the coming 5 years, we strongly believe that novel acrolein detection methods will continue to evolve, allowing for more reliable and clinically applicable methods for acrolein quantification. The following trends are speculated. Methods based on advanced analytical equipments such as LC-MS-MS will continue to evolve and play an even more important role in acrolein research with standard easy-to-follow protocols developed to flatten the learning curve for clinical researchers. Miniaturization of acrolein analytical equipments will also facilitate translation to a clinical setting. Additionally, notable alternative acrolein

detection methods such as time-resolved luminescence may offer a new avenue to address acrolein detection challenges. There is also potential for improvement with the development of novel acrolein derivatization agents capable of enhancing the sensitivity of acrolein derivatization-based methods. We anticipate that immunoblotting-based methods, as they currently stand, will remain indispensable due to the unique advantages they offer for acrolein detection.

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Key issues

- Acrolein, a byproduct of oxidative stress, is a key mediator of CNS pathologies and is capable of eliciting neuronal membrane damage, demyelination and mitochondrial dysfunction.
- Acrolein was found to be present at increased levels following spinal cord trauma and corresponded with behavioral deficit.
- Experimental autoimmune encephalomyelitis mice exhibited elevated levels of acrolein locally and systemically, which corresponded with the behavioral score.
- Attenuation of acrolein toxicity using hydralazine offered neuroprotection in spinal cord injury and experimental autoimmune encephalomyelitis animals.
- Detection of acrolein levels allows for the potential use of acrolein as a biomarker.
- Acrolein derivatization and subsequent use of LC/GC–MS is a well-characterized method and has been used in many studies.
- Immunoblotting techniques, which utilize an acrolein antibody, offer high sensitivity and allow the investigator to quantify acrolein levels locally; however, due to the highly invasive nature of this technique, clinical applications are limited.
- Quantification of 3-hydroxypropylmercapturic acid, an acrolein metabolite, allows for the measurement of systemic acrolein levels from a urine sample, rendering it highly applicable clinically.

Table 1

Comprehensive view of current acrolein detection methods.

	Acrolein derivatization + LC/GC–MS	Acrolein antibody immunoblotting	3-HPMA method
Target	Derivatized acrolein	Acrolein–protein adducts	Acrolein metabolite: 3-HPMA
Sensitivity	Medium	High	High
Scope	Local/systematic exposure	Local exposure	Systematic exposure
Invasiveness	Medium	High	Minimum
Time to result	<6 h	>12 h	<6 h
Medium		Low	High
Ref.	[22,45,46]	[26,27]	[39,40,47,48]

3-HPMA: 3-Hydroxypropylmercapturic acid.