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RESEARCH ARTICLE

A REVIEW ON THE EFFECT OF SULPHUR MUSTARD ON GENE EXPRESSION ALTERATIONS, FINDING THE MOST IMPORTANT MOLECULAR FUNCTIONS USING GENE SET FUNCTIONAL ENRICHMENT ANALYSIS

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ABSTRACT

Background: Sulphur mustard (SM) is a very reactive agent and causes severe chemical burns. In addition to various cellular and molecular damages, SM affects the expression of several genes. The main aim of this study was to review SM-induced gene expression alterations. Furthermore, using enrichment analysis, we tried to identify the most important molecular functions involved in the occurrence of SM injury, based on the found gene sets.
Methods: With the aim of finding genes associated with SM-induced injuries, the literature was reviewed and data were collected manually. All animal, in vitro and human studies, regardless of ethnicity and sample size, were included in the study. Gene Ontology (GO) database was used for gene set functional enrichment analysis.
Five animal studies, eight in-vitro studies, and six human studies from 1996 to 2016 were thoroughly reviewed and discussed in more detail. The functional enrichment of the animal and in vitro gene sets showed that cytokine system activity and growth factor receptor binding are the most important functions involved in the occurrence of SM injury. The functional enrichment of the human gene set showed that in addition to the cytokine system, disrupting the antioxidant system may also play a major role in causing injuries.
Results: According to our results, the simultaneous administration of antioxidants and anti-inflammatory agents on SM-induced injuries seems to be a significant issue for future studies. **Conclusion:** Our findings largely depend on the previous results and may be affected by novel findings in the future.

INTRODUCTION

Sulfur mustard (SM), commonly known as mustard gas, has been misused as an cytotoxic chemical warfare agent for many years.[1] Pure SM is a viscous colorless and fat-soluble fluid which is easily absorbed through the skin and mucous membranes.[2] Impure forms are usually yellow-brown and have an odor resembling mustard plants or garlic.[3]Depending on environmental conditions, this gas can remains harmful for many years, near the surface of the earth and on the outer layers of the soil.[4] Although SM is prohibited by Chemical Weapons Convention (CWC), and Organization for the Prohibition of Chemical Weapons (OPCW), however, evidence suggests that this agent was used against Iran as a chemical weapon in the Iraq-Iran war.[5, 6] The long-term effects of this agent are still visible in the veterans of this war. SM may affect different body organs through the eyes, skin, respiratory tract and sometimes gastrointestinal tract.[7]

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Cutaneous or respiratory lethal doses of SM are 64 mg/kg and 1500 mg/min/m3, respectively.[8] Clinical cutaneous symptoms induced by SM include itching and burning, redness or painless sunburn, hypo- and hyper pigmentation and large blisters containing serous or seropurulent fluid in both exposed and unexposed areas.[9] Generally, blistering starts on the second day after exposure, and may get worse for two weeks. However, wound healing is extensively slower than for a thermal burn, and usually hospital care is needed. Approximately 20% of the SM could be absorbed through human skin, so that up to 70% of the agent is accumulated in the epidermis layer and the remainder in the dermis and basement membrane.[9] One of the other organs that is rapidly damaged when exposed to SM, is the eye. Aqueous and mucosal nature of cornea and conjunctiva make the eyes vulnerable to SM toxicity. [10] Eye pain and irritation, conjunctivitis, anterior uveitis, photophobia and temporary blindness are symptoms that usually occur in the face of SM. Chronic inflammation and autoimmune reactions are responsible for delayed ocular signs and symptoms.[11] Such

as neovascularization, corneal opacification, ulceration, dystrophy and thinning, and diminished tear meniscus layer.[12] On the other hand, Khateri *et al.* (2003) in a study conducted on 34 000 Iranian victims of chemical warfare reported that 42.5% of the victims are suffering from mild to severe respiratory problems.[13] Respiratory acute symptoms include irritated throat, nasal discharge, sneezing, cough and dyspnea. While, common chronic effects include chronic irritant cough, dyspnea, sputum, esophagogastric reflux, chest pain and chronic bronchitis.[13] These effects are generally associated with problems such as obstructive pulmonary disease, asthma, and narrowing of large respiratory ways.[14] Different cellular and molecular mechanism are involved in damages and inflammation caused by SM. SM potentially alkylates almost every element of the cell, leading to disrupted cellular functions. DNA crosslinks following by alkylation by SM has been identified as the main trigger of cell apoptosis. These crosslinks block DNA replication and arrest cell cycle and may lead to DNA single and double strand breaks. Masta *et al.* (1996) demonstrated that SM alkylates DNA preferentially at 5'-AA, 5'-GG and 5'-GNC sequences on the DNA coding strand and results in the truncated transcription.[15] This study provided the first evidence at the molecular level of SM-induced effects on transcriptional processes.

The severity of the DNA damage may be so high that it will ultimately lead to cell death.[16] SM can alkylate DNA and forms the cross-link, di-(2-guanin-7-yl-ethyl)-sulfide, and two monoadducts, 7-(2-hydroxyethylthioethyl) guanine (HETEG) and 3-(2-hydroxyethylthioethyl) adenine (HETEA).[17] Moreover, Poly (ADP-Ribose) polymerase over-activation in high doses of SM may lead to the formation of nicotinamide adenine dinucleotide and adenine triphosphate (ATP) depletion and subsequently necrotic cell death.[16] Apart from the onset of apoptosis due to DNA damage, different other apoptotic molecular pathways may be induced by SM. Studies have implicated intracellular Ca^{2+} levels in SM toxicity and alterations in Ca^{2+} levels are thought to play a role in apoptosis.[18] Evidence suggests that SM increases intracellular Ca^{2+} concentration due to the induction of oxidative or electrophilic stress following by cellular glutathione levels depletion. Studies have also suggested that SM increases the activity of phospholipase D and this enzyme is believed to elevate intracellular Ca^{2+} levels through the Src/PLCγ pathway.[19] Furthermore, SM exhausts cell energy and leads to generation of reactive oxygen species (ROS).[20] A study on chemical warfare victims of the Iran-Iraq war showed that levels of oxidants in the blood of those exposed to SM are higher than others.[21] This induced oxidative stress may result DNA and chromosome damages, gene expression alteration, genetic mutation and even cell death.[22] It may generate highly reactive electrophilic species following by lipid peroxidation and protein oxidation, which can impair various structural integrities and enzymatic activities.[22] Inflammation is also a common response to SM exposure. SM can trigger different immunologic signaling pathways through activation of various proteins such as activator protein-1 and Nuclear Factor kappa B (NF-κB) which in turn, leads to activation of cyclooxygenase-2 (COX-2) and matrix metalloproteinase. COX-2 and metalloproteinases increase inflammatory mediators and cytokines activity.[23] Over-expression of matrix metalloproteinases 2 and 9 (MMP-2, 9) is known as one of the main mechanisms in the destruction process of transmembrane proteins induces by SM.[23] In the last two decades, studies have tried to examine changes in the expression of genes in exposure to SM. Identification of gene expression changes may be useful to find reliable diagnostic and therapeutic biomarkers in SM injuries. The main aim of this study was to review gene expression alterations in SM victims. Furthermore, we tried to identify the most important molecular functions involved in the occurrence of SM injury based on the found gene set, using enrichment analysis.

MATERIALS AND METHODS

Searching resources and finding genes of interest: With the aim of finding genes expression associated with SM-induced injuries, the literature was extensively reviewed and data were collected manually. Genetic association, and genome-wide association studies (GWASs), systematic reviews and meta-analysis studies were scrutinized using the following keywords in PubMed and Google Scholar databases: “sulphur mustard” OR "SM", AND “gene expression’’, “mRNA level”, ‘‘mRNA microarray”, and “expression alteration”. All animal, in vitro and human studies, regardless of ethnicity and sample size, were included in the study.

Enrichment analysis: Gene set enrichment analysis is a statistical approach to classify genes that are over-represented in a specific gene set. [24] Gene Ontology (GO) database (http://www.geneontology.org /) was used for functional enrichment analysis. The Gene Ontology database is a bioinformatics toolkit which represents the evolving knowledge of how genes encode biological functions at the molecular, cellular and tissue system levels. The project includes over 40,000 biological concepts which are used to "annotate" gene functions based on experiments reported in over 100,000 peer-reviewed scientific papers. [25]

RESULTS

Alterations in genes expression in exposure to SM

Animal studies: In 2000, Carol *et al.* studied in vivo molecular biomarkers of SM-induced mice skin injury within 24 hours.[26] Interleukin 1beta (IL-1beta) mRNA levels were observed to be increased substantially after 3 hours. IL-1beta, granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin 6 (IL-6 mRNA) levels were also reported to be significantly increased at 6-24 hours post-exposure. This immunohistochemical study provided evidence for early elevated in vivo expression of proinflammatory factors following cutaneous SM exposure. In a similar study, in 2002, Sabourin *et al.* investigated in vivo proinflammatory biomarkers of the SM-induced skin injury, using the weanling pig model. [27] They showed that interleukin-1B (IL-1B), interleukin-6 and 8 (IL-6 & 8), and matrix metalloproteinase-9 (MMP-9) mRNA levels were increased following SM exposure. This study suggested that the use of anti-inflammatory drugs in the event of skin exposure to SM could reduce the severity of the injury and accelerate the healing. In 2005, Sabourin *et al.* examined SM-induced changes in gene expression to identify transcriptional alterations associated with SM skin injury, using Atlas Mouse 5K DNA microarrays. [28] They observed that increasing the dose of SM increases the expression of more genes involved in inflammation, apoptosis and cell cycle regulation. They suggested that molecular pathways associated with these three events may be

appropriate targets for developing therapeutic agents for SM skin injury. In 2005, Rogers et al., using cDNA microarrays, measured SM-induced changes in gene expression in mice cutaneous cells exposed to SM. [29] Comparison of skin cells exposed to different concentrations of SM with control cells showed upregulation of 19 genes within apoptosis, transcription, cell cycle regulation, inflammation, and tumor suppression categories. Also, the results of this study showed that increasing the dose of SM leads to a greater increase in the expression of these genes and identified the expression of these gene categories as reliable biomarkers to detect the severity of SM damage. In 2006, Shakarjian *et al.* measured changes in the expression of genes in a time dependent manner for the first time. They evaluated the effects of SM exposure on the expression of MMP-2 and -9 in ear skin from mice.[30] Results showed an increase in overall MMP-9 activity in SM-treated skin, so that after 168 h, the relative levels of its mRNA had been increased up to 27-fold and its protein up to 9-fold, compared with the control skin. These observations suggested the distinct expression of MMP-2 and -9 upon the cutaneous exposure to SM and the involvement of MMP-9 in SM-induced injury. Price et al., in 2009, applied microarray analysis, the new generation of tools to study gene alterations, to evaluate transcriptional alterations in porcine skin due to the repair mechanisms at 7 days following SM injury.[31] The transcriptional profiles demonstrated that SM exposure increases 270 transcripts and decreases in 317 transcripts. They argued that specific genes, including CXCR4, CXCL2, HMOX1, FGFR2, IGF1, PLAU, PF4, SPP1, PLAUR, S100A8, and TNC, may be potential therapeutic targets to promote improvement of SM-injured wound healing. In 2011, Vallet et al., similar to several previous studies, investigated the time-dependent gene expression changes of interleukins-B and -6 (IL-B and -6), tumor necrosis factor alpha (TNF-α), macrophage inflammatory protein 2-alpha (MIP-2α), MIP-1α receptor (MIP-1αR), matrix metalloproteases-9 and -2 (MMP-9 and -2), laminin γ2 monomer (Lamc2) and keratin-1 (K1) in SM saturated vapor-exposed skin. [32] Quantitative expression analysis showed an increased in the expression of IL-6 and-1B, MIP-2α, and MIP-1αR genes as early as 6 h in SM-exposed skins and determined that this increase was continued up to 14 days after exposure. Moreover, K1 mRNA level was identified to be significantly increased 21 days after SM challenge. A summary of animal studies is presented in Table 1.

In vitro cell line studies: In vitro studies on the effect of SM on the gene expression began from the 1990s. In 1996, Detheux *et al.* investigated the expression of plasminogen activator (PLAT), in 3T3 fibroblasts after in vitro exposure to SM.[33] They found that PA protein level was upregulated up to 20-fold after 24 h, independent of the cell density, and persisted for at least 6 days after exposure. They argued that this up-regulation is possibly mediated by intracellular calcium concentrations. In 2002, Zhang *et al.* found that protein kinase B (PKB), a key protein kinase which can inhibit apoptosis, and pyruvate dehydrogenase kinase 1 (PDK1), an upstream effector of Akt, are significantly down-regulated, following SM treatment in Jurkat cells. [34] Concurrently, the down-regulation of anti-apoptotic BCL gene family, and the up-regulation of three death executioner genes, caspase 3, 6, and 8, were observed. The authors suggested that SM attacks the Akt pathway, by direct or indirect inhibition of Akt gene expression. In 2003, Rosenthal *et al.* emphasized the importance of the role of caspases. They found that vesicating doses of SM activate caspases-8 and -9 in a time-dependent fashion and increase the levels of Fas receptor and Fas ligand in human epidermal keratinocytes. [35] These results were consistent with the hypothesis of both cellular and mitochondrial death receptors activation, following by SM exposure. Furthermore, they showed that pretreatment of keratinocytes with Fas-blocking antibodies decreases levels of Fas-associated death domain signaling and markedly decreases caspase-3 activity upon treatment with SM. In order to clarify the molecular pathway associated with increased activity of cytokines, in 2004, Dillman *et al.* investigated the role of p38 MAP kinase (MAPK14) in inflammatory cytokine upregulation following SM exposure.[36] They found increasing phosphorylation of p38 MAP kinase and the upstream kinase MKK3/6 in response to increasing concentrations of SM in cultured human epidermal keratinocytes (HEK) which in turn increases IL-8, IL-6, TNF-α, and IL-1β expression. Their findings showed that p38 MAP kinase plays a central role in SM-induced cytokine expression in HEK and suggested that blocking this pathway may alleviate the cutaneous inflammatory response to SM.

In 2004, Arroyo *et al.* examined the effect of SM on cytokine gene expression in human epidermal keratinocyte (NHEK) cells. [37] They analyzed the transcription of Human tumor necrosis factor-alpha (hTNF-α) gene and found that SM activates hTNF-α gene in cultured NHEK. They concluded that hTNF-α, as a pro-inflammatory mediator, could be a possible biomarker for exposure to SM. Results indicated that the elevated levels of hTNF-α by SM are significantly dependent on the cell densities, therefore, the extent of the damage determines the amount of hTNF-α production following by SM exposure. In 2007, Ishida *et al.* clarified the effect of SM on inducible NO synthase (iNOS) expression in an in vitro wound assay with the aim of designing novel therapies for SM skin injuries. [38] They found that SM exposure strongly decreases iNOS expression. This study suggested that preventing SM-induced reduction of iNOS expression may be a prospective approach to promote healing of SM-induced skin injuries. In 2010, Black *et al.* analyzed the effects of SM on EpiDerm-FT, a full-thickness human skin equivalent, to assess mechanisms mediating these responses.[39] They found a time-dependent increase in expression of proliferating cell nuclear antigen (PCNA), poly-(ADP-ribose) polymerase (PARP), histone H2AX, cyclooxygenase (COX)-2, 5-lipoxygenase, microsomal prostaglandin E2 (PGE2) synthases, leukotriene A4 hydrolase (LTA4H) and glutathione S-transferase II (GST II) in SM-treated skin equivalent. These data demonstrated that SM induces rapid cytotoxicity and inflammation. In 2011, Carmen *et al.* studied responses of normal human keratinocytes to SM, in an dose-dependent manner. [40] They described that exposure to 100 µM SM increases the amounts of IL-8 and TNF-α up to 59-fold and 4-fold, respectively, above control levels. While, exposure of normal human epidermal keratinocytes to 300 µM SM sometimes increases the secretion of IL-1β above baseline level and at other times decreases its secretion. They also found that the secretion of IL-6 varies 8.4-fold, depending on the SM dose. This study suggested that these two pro-inflammatory mediators may play a central role in SM injury and the related changes could be used as potential biomarkers of SM-induced cutaneous injury. A summary of in vitro cell line studies is presented in Table 2.

Human studies: Due to the misuse of SM as a chemical weapon in the Iran-Iraq war, many Iranian veterans suffer from its long-term complications and, therefore, most human studies

Table 1. A summary of animal studies on the effect of SM on changes in gene expression

Study	Year	Gene(s)	Expression
Carol et al.	2000	IL1B, CSF2, and IL6	Up-regulation
Sabourin et al.	2002	IL1B, IL6, CXCL8, and MMP9	Up-regulation
Shakarjian et al.	2006	MMP9	Up-regulation
Price et al.	2009	CXCR4, CXCL2, HMOX1, FGFR2, IGF1, PLAU, PF4, SPP1, PLAUR, S100A8, and TNC	Up-regulation
Vallet et al.	2011	IL6 and-1B, CXCL2, and CCR1	Up-regulation

Table 2. A summary of in vitro studies on the effect of SM on changes in gene expression

Study	Year	Gene(s)	Expression
Detheux et al.	1996	PLAT	Up-regulation
		AKT1 and BCL2	Down-regulation
		CASP3, 6, and 8	Up-regulation
Rosenthal et al.	2003	CASP8 and -9	Up-regulation
Dillman et al.	2004	CXCL8, IL6, TNFA, and IL1B	Up-regulation
Arroyo et al.	2004	TNFA	Up-regulation
Ishida et al.	2007	NOS2	Down-regulation
Black et al.	2010	PCNA, PARP1, H2AFX, PTGS2, 5, PTGES, LTA4H and MGST2	Up-regulation
Carmen et al.	2011	IL8 and TNFA	Up-regulation

Table 3. A summary of human studies on the effect of SM on changes in gene expression

Nourani et al.	2010	HMOX1 and -2	Up-regulation
Nourani et al.	2011	MT1A	Up-regulation
Khaheshi et al.	2011	TGFB1, 2 and R1, R2	Down-regulation
Mirbagheri et al.	2013	SOD1 and SOD2	Up-regulation
Nourani et al.	2014	GSTA1, GSTM1 and GSTP1	Down-regulation
		OXSR1, GPX2, FOXM1	Up-regulation
		MT3 and GSR	Down-regulation

Table 4. Summary of the enrichment analysis studies on the cytokine and antioxidant activity in gene expression

Gene set	Description	P-Value	FDR	Overlapped genes
Animal studies				
GO:0005125	cytokine activity	3.24e-09	8.71e-07	CSF2;CXCL2;IL1B;IL6;CXCL8;PF4;SPP1
GO:0005126	cytokine receptor binding	4.71e-07	6.33e-05	CSF2;CXCL2;IL1B;IL6;CXCL8;PF4
GO:0070851	growth factor receptor binding	0.00048	0.043	CSF2;IL1B;IL6
In vitro studies				
GO:0005126	cytokine receptor binding	1.83e-05	0.0049	IL1B;IL6;CXCL8;CASP3;CASP8
GO:0008234	cysteine-type peptidase activity	8.57e-05	0.011	CASP3;CASP6;CASP8;CASP9
GO:0004175	endopeptidase activity	0.00014	0.013	PLAT;CASP3;CASP6;CASP8;CASP9
Human studies				
GO:0016209	antioxidant activity	2.49e-12	6.72e-10	GPX2;GSR;GSTA1;GSTP1;MT3;SOD1;SOD2
GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	2.31e-05	0.0021	GPX2;GSTA1;GSTP1
GO:0016765	transferase activity, transferring alkyl or aryl (other than methyl) groups	6.26e-05	0.0042	GSTA1;GSTM1;GSTP1
GO:0005126	cytokine receptor binding	0.00026	0.0142	TGFB1;TGFB2;TGFBR1;TGFBR2

have been conducted on gene expression changes in dealing with SM have been conducted in Iran. In 2010, Nourani *et al.* studied the regulation of hemeoxygenases (HO) -1 and -2 in the human airway wall, in an Iranian population suffering from chronic pulmonary lesions due to mustard gas.[41] They indicated the up-regulation of HO-1 expression to about 4 folds in SM-exposed victims in comparison with healthy ones, while, immunohistochemistry results revealed decreased HO-1 protein levels in SM victims. They discussed that HO-1 may plays a central role in cellular protection against reactive oxygen species. In 2011, Nourani *et al.* following their previous study, measured the Metallothioneins-1A (MT-1A) mRNA and protein levels in endobronchial samples from sulfur mustard-exposed patients and control cases.[42] They found an upregulated MT-1A mRNA and protein levels in sulfur mustard-exposed patients and concluded that upregulation to be due to oxidative stress, as an attempt to alleviate this destructive situation by reestablishment of homeostasis. In the same year, Khaheshi *et al.* evaluated transforming growth factor (TGF-βs) and their receptors expressions in 17 SM-exposed victims, 17 chronic dermatitis patients, and 5 normal controls. [43] Their results revealed meaningful decreases in the expression of TGF-β 1, 2 and R1, R2 in victims compared to chronic dermatitis and normal subjects. Therefore, TGF-βs and their receptors appear to be involved in chronic skin inflammations caused by SM. In 2013, Mirbagheri *et al.* evaluated the SOD gene expression in 20 SM-exposed individuals and 10 normal controls. [44] Their results showed that CuZnSOD and MnSOD mRNA levels were elevated about 3 folds in SM victims in comparison with controls. In contrast, immunohistochemical analysis demonstrated down-regulation of CuZnSOD protein levels in SM injured patients. They concluded that SM may reduce the level of SODs proteins and the cell, in order to compensate for it, increases gene transcription. In 2014, the first comprehensive study to determine the expression profile was performed by Najafi *et al.* using cDNA-microarray, on the chronic human mustard lung disease, 25 years after exposure. [45] Results of microarray transcriptional profiling revealed dysregulation of 122 genes in airway tissues of patients which were associated with the extracellular matrix components, stress response, apoptosis, mucus secretion and inflammation. This study was the first high-throughput transcriptomic research applied to SM-

induced chronic airway remodeling. Also, Nourani et al., in the same year, tried to understand the difference in the expression of glutathione S-transferase (GST) gene in airway wall of SM-induced chronic obstructive pulmonary disease (COPD) victims and healthy controls. [46] The expression of GSTA1, GSTM1 and GSTP1 was found to be 2.5, 2.8 and 5.8 folds higher than healthy controls. Therefore, it seems that GST plays a substantial role in cellular defense against SM-induced oxidative stress in airway wall of patients with COPD. Finally, in 2016, Tahmasbpour *et al.* investigated alterations in expression of genes involved in antioxidant defense against stress induced by SM in lung tissue. [47] From the 84 genes functionally involved in cellular oxidative stress response. 47 genes (including oxidative stress responsive-1 (OXSR1), glutathione peroxidase-2 (GPX2), and forkhead box M1 (FOXM1)), were found to be substantially upregulated in patients with mustard-injured lung compared with controls, whereas 7 genes (including metallothionein-3 (MT3) and glutathione reductase (GSR)) were downregulated. They suggested that oxidative stress induced by ROS is the major mechanism for direct effect of SM exposure on respiratory system. A summary of human studies is presented in Table 3.

Enrichment analysis: The functional enrichment of the genes found in animal and in vitro cell line studies showed that cytokine system activity and growth factor receptor binding are the most important functions involved in the occurrence of SM injury. The functional enrichment of the genes found in human studies showed that in addition to the cytokine system, disrupting the antioxidant system can also play a major role in causing injuries (Table 4).

DISCUSSION

Sulphur mustard is a blistering agent, which causes chemical burns and was extensively employed as a chemical warfare agent by Iraq against Iran. [48] This very reactive agent causes permanent alkylation of the DNA strands and prevents cellular division and generally leads to cell apoptosis. [49] Complications caused by SM are usually long-term and involve various organs, including the skin, eyes and respiratory tract.[50] So far, several studies have been conducted on gene expression alterations caused by SM, however, there is still no integrated understanding of this issue. In the present study, we reviewed all of these studies and examined the importance of the found genes in other disorders. Finally, using gene set enrichment analysis, we tried to find the most central functions that are affected by SM. Functional enrichment analysis showed that the cytokine regulatory and antioxidant protective systems are among the most central SM-targeted functions. The importance of these two functions are partially supported by previous studies. For example, several studies have shown that SM can lead to excessive immune responses [26, 51] and it has been shown that anti-inflammatory agents can greatly improve the injuries. [52] On the other hand, production of reactive oxygen species has been proposed to result from oxidative stress due to the depletion of cellular antioxidant levels, including glutathione. [53] In addition, It has been reported that SM can also suppress the activity of enzymes protecting cells against oxidative stress, such as superoxide dismutase, catalase and glutathione peroxidase. [54] Improvement of SM-induced chronic injuries using antioxidants has been suggested in several studies.[55] Of course, it should be noted that oxidative stress and inflammation are mutually related with each other. Chronic inflammation can increase the level of reactive oxygen species[56] and on the other hand, reactive oxygen species, in turn, can induce inflammation. [57] Therefore, the simultaneous administration of antioxidants and anti-inflammatory agents on SM-induced injuries could be a significant issue for future studies. However, it should be noted that studies based on the existing data are largely depended on the previous findings and the publication of novel findings in the future can partly affect their outcomes. However, at present, the only way to study the genetic basis of an injury or disorder in an integrative mode is through the use of computer-based methods, such as enrichment analysis.

Abbreviations

ATP	adenine triphosphate
COPD	chronic obstructive pulmonary disease
COX	cyclooxygenase
COX-2	cyclooxygenase-2
CWC	Chemical Weapons Convention
FOXM1	forkhead box M1
GM-CSF	granulocyte-macrophage colony stimulating factor
GO	Gene Ontology
GPX2	glutathione peroxidase-2
GSR	glutathione reductase
GST	glutathione S-transferase
GST II	glutathione S-transferase II
GWASs	genome-wide association studies
HEK	human epidermal keratinocytes
HO	hemeoxygenases
hTNF-α	Human tumor necrosis factor-alpha
IL-1beta	Interleukin 1beta
IL-6	interleukin 6
iNOS	inducible NO synthase
Lamc2	laminin γ2 monomer
LTA4H	leukotriene A4 hydrolase
MIP-2α	macrophage inflammatory protein 2-alpha
MMP	matrix metalloproteinases
MMP	matrix metalloproteinase
MT-1A	Metallothioneins-1A
MT3	metallothionein-3
NF-κB	Nuclear Factor kappa B
NHEK	human epidermal keratinocyte
OPCW	Organization for the Prohibition of Chemical Weapons
OXSR1	including oxidative stress responsive-1
PCNA	proliferating cell nuclear antigen
PDK1	pyruvate dehydrogenase kinase 1
PGE2	microsomal prostaglandin E2
PKB	protein kinase B
PLAT	plasminogen activator
ROS	reactive oxygen species
SM	Sulfur mustard
TGF-βs	transforming growth factor
TNF-α	tumor necrosis factor alpha

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