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Cold Injury Prevention and Management in High Altitude Extreme Environments: Pharmacological and Therapeutical Interventions

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ABSTRACT

Cold injury refers to local or systemic body response that occurs due to massive loss of body heat when the body is exposed to extremely cold temperatures. The current modalities for the prevention and management of cold injury(ies) are very limited due to the paucity of availability of targeted therapeutics. Pathophysiological cascades in cold injury include: (a) desensitisation of sensory neurons can be manifest as a result of altered pathophysiological functions viz., Ca^{2+} imaging, calcitonin gene-related peptide release, expressions of inflammatory mediators (PGE_2: prostaglandin E_2, NGF: nerve growth factors), (b) inflammatory markers viz.; interleukins (IL-1β, IL-6, and IL-10), tumor necrosis factor-alpha (TNF-α), and CD62E/endothelial-leukocyte adhesion molecule 1 (E-selectin); (c) oxidative stress markers associated with cold injury measured through serum level of protein carbonyl, 4-hydroxy-2-nonenal (4-HNE), superoxide dismutase (SODs), advanced oxidative protein products (AOPP) and nitrotyrosine; (d) endothelial damage: nitric oxide (NO), prostacyclin (PGI_2), reactive oxygen species (ROS), Von-Willebrand factor (VWF), CD31/PECAM-1 (platelet/endothelial cell adhesion molecule 1), CD36/SR-B3 (scavenger receptor class B member 3) and tissue-type plasminogen activator (TTPA). In this review paper, we elaborate on the current state-of-the-art pharmacological interventions for cold injury that may be beneficial in developing novel and targeted therapeutics for the prevention, management, and treatment of cold injury.

Keywords: High altitude; Cold injury; Pharmacological interventions; Endothelial damages; Oxidative stress; Inflammatory mediators

1. INTRODUCTION

1.1 The Challenge of Sustaining Operational Efficiency in Extreme Environments

High altitude refers to areas with heights over 2500 m ft above mean sea level and it can range up to extremes of height around 5500 m ft as seen in Siachen Glacier and other regions of Leh-Ladakh. With an increase in altitude, weather conditions deteriorate in terms of hypoxia, extreme subzero temperature, blizzards, and lack of vegetation and food supply. People living in high-altitude areas are known as high landers and those who come from plains are non-high landers. Although high landers are well acclimatised to the extreme environment, still they may face great difficulty in day-to-day activities. When Armed Forces personnel are deployed in these harsh environments they face extreme challenges starting from dyspnea on minimal exertion, chill blains, sunburns, and insomnia to life-threatening conditions like pulmonary edema, myocardial infarction, cerebral edema, snow blindness, also limb-threatening injuries like chilblains and frostbite, trench foot leading to decreased function of involved limb(s) and in extreme cases even amputation. These limb and life-threatening injuries/ conditions can be prevented by proper acclimatisation at lower altitudes and gradual ascent with ample time of stay at various altitudes with gradual increment in level of activities. While inducting a non-highlander to these extreme altitudes, one has to undergo a total of 14 days of acclimatisation (Acclimatisation protocol given by DIPAS: 6 days at stage 1 (9000-12000 ft), 4 days at stage 2 (12000-15000 ft), and 4 days at stage 3 (15000-18000 ft). A total number of nights stayed at lower altitude are more important than days as the parasympathetic system is activated and the sympathetic response of the body is blunted during sleep so individuals are prone to adverse outcomes. These life-threatening adverse events cannot be averted as they happen due to the physiological response of the body in susceptible individuals, however limb-threatening injuries can be prevented with the use of protective garments, heating appliances, and prompt detection of superficial cold injuries by following a daily routine of hand and foot parade and seeking medical attention[1-2].

The Indian Armed Forces operate in inhospitable environments that include bone-chilling winters of Northern glaciers, barren mountains of Ladakh, and hilly areas of North East. In the high altitude areas, night temperature plummets down to subzero temp in summer and between

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-35 ℃ and -40 ℃ in winter. On top of this, snow-laden roads and terrain pose difficulty in the movement of vehicles, troops, and supply of necessary materials and goods; the non-availability of adequate professional health setup further aggravates the agony of soldiers posted in these areas.

The troops posted at forward locations have to fight on a dual front - first with the enemy and second against cold weather, snowstorms ranging up to 100 mph, blizzards, avalanches, and massive crevasses. Often the casualties caused by weather are much more than the casualties due to military operations. Apart from this, troops also face nutrition-related problems (loss of appetite, weight loss, sleep disorders memory loss, etc.) at high altitude[33].

Cold weather and its related illness are a cause of major concern for the soldiers posted at high altitudes. The incidences of cold-related illness like hypothermia, high altitude pulmonary edema (HAPO), and high altitude cerebral edema (HACO) have reduced due to the availability of better shelters, extreme winter clothing, acclimatisation protocols in vogue and pre-induction medical examination of troops. However, local injuries due to cold weather like frostbite, chill blains, and trench foot are still a cause of concern as troops are constantly exposed to harsh climates while patrolling, during guard duties, and exercises. Most military equipment like rifles, guns, mortars, and machinery are manually operated in the cold environment and any bare-hand contact can lead to severe cold injuries like frostbite and tissue necrosis.

Furthermore, relative lack of oxygen (hypobaric hypoxia) at these high altitudes and lack of vegetation leads to decreased exercise capacity and easy fatigability along with hypoxia-induced anorexia, resulting in weight loss, decrease in efficiency, and physical work capacity. During peak extreme winters, when roads are blocked and weather conditions do not permit aerial delivery of fresh ration, troops have to rely on dry ration and tinned items for days to weeks occasionally resulting in overall poor nutrition and health.

Another aspect is the decreased availability of fresh water- soldiers have to melt snow for water consumption and this scarcity of fresh water leads to decreased overall personal hygiene, summed with perspiration during patrolling or activities involving physical exertion, which if not attended to timely can result in frostbite; besides fungal infections like tenia are also a cause of concern.

Cold injury-related conditions such as chilblains and frostbite develop during physical exertion and daily activities like mountain climbing and patrolling with heavy military equipment, which leads to excessive sweating in extremities and continued contact with wet protective gear results in freezing of skin in contact which leads to local tissue damage, tissue necrosis, and cell death to the affected area[1].

Chilblains also known as pernio is a type of localised inflammation lesions caused by exposure to cold wind above its freezing point continuously to the open skin surface leading to redness of skin and loss of sensitisation, which ultimately develops into a chilblain-like condition. Clinically, the chilblain condition is characterised by primary erythema to the local skin surface, oedematous macules, papules, and plaque[3]. However, chilblains are categorised into non-freezing cold injury where no severe damage to extremities take place.

Frostbite is another limb-threatening freezing cold injury that needs early detection and prompt treatment. It has 4 grades; grade 1 is similar to mild chilblain where patients develop swollen digits with mild itching and sometimes overlying skin is shiny, grade II when skin becomes red and blisters develop containing clear to milky fluid. grade III, when these blister rupture and ulcers develop with some necrosis of overlying skin, grade IV when extensive necrosis of subcutaneous tissues happens along with gangrene, resulting in amputation of limb[4-5]. Different grades/spectrums of frostbite are depicted in Table 1 and a human image of grades I & II is depicted in Fig. 1.

Limb-threatening injuries develop when ambient temperature drops down to less than 0 ℃ leading to freezing and ice crystal formation of subcutaneous tissues, activation of inflammatory cascade and subsequent vascular endothelium dysfunction, vasoconstriction, ischemia of distal digits followed by necrosis and amputation if sustained exposure is present or no medical intervention is done. There is no such test/biomarker *per se* that can predict the development of cold injuries; diagnosis is based only on a history of exposure to a cold environment along with symptoms and signs presented by the patient. Timely detection and treatment of these injuries by individuals and health care professionals is of utmost importance, when detected early in grades I and II can result and complete recovery with no disability.

However, to date, there is no specific intervention that has proven beneficial in cold-related freezing injury (ies). Modalities like rewarming, especially passive rewarming have shown some benefit in reversing blood flow in cold affected tissues of grade I & II injuries, although active and enthusiastic rewarming with warm water and direct heat has overall adverse outcomes.

Thrombolytic therapy with rtPA as studied[12] has shown lower amputation rates if performed within 24 hours of first exposure to cold. The remote locales, particularly where individuals are posted in tough hilly snow-capped mountains, the availability of intensive medical setup and therapeutics such as rtPA is logistically impossible and cannot be undertaken due to the complexity in administration, and the associated prohibitive cost of rtPA administration.

Other modalities like hyperbaric oxygen therapy, vasodilators, etc. have not shown significant improvement in terms of decreasing morbidity and amputation rates.

2. FACTORS AFFECTING COLD INJURY

It has been observed that from the last decade onwards the number of cold injury cases has reduced,

Spectrum of frostbite[4-7]:

S No.	Spectrum of frostbite	Clinical manifestation
	A. SUPERFICIAL FROSTBITE	
1.	First-degree frostbite	Similar to mild chilblain with hyperthermia, mild itching, mild edema, no sign of blistering or peeling of the skin, and no necrosis.
2.	Second-degree frostbite	Blistering containing clear or milky fluids and desquamation, full thickened skin freezing, substantial edema, erythema.
	B. DEEP-VEIN FROSTBITE	
3.	Third-degree frostbite	Necrosis of subcutaneous skin and tissue, ulceration, hemorrhagic blisters with some necrosis
4.	Fourth-degree frostbite	Deep red and mottled appearance with eventual gangrene, extensive necrosis, and minimal edema, includes destruction of connective tissue and bone.

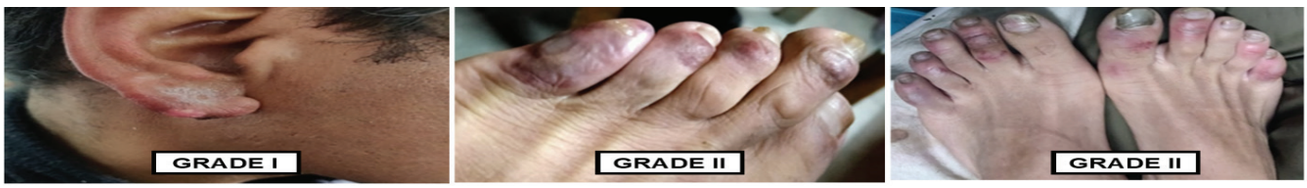

Figure 1. Representative images on cold injuries (grade I & II). ***Image credit: Capt. Kishan Kumar Roy, RMO (1 Mahar, 192 BDG, Batalik Sector).***

and the predisposing factors for cold injury have become countable, thus protection from these predisposing factors helps in combating the situation like frostbites[8-9]. Some of the most prominent factors which affect cold injury are depicted in Table 2.

3. PATHOPHYSIOLOGY OF COLD INJURY(IES)

Cold injury occurs due to exposure to a freezing environment, duration of exposure and rapidity of cooling determines the overall outcome of injury. Rapid cooling with prolonged exposure has more detrimental outcomes than slow and limited exposure.

The following mechanisms which lead to tissue necrosis in cold injuries are described below:

1) Direct damage to tissue due to ice crystal formation and further inflammation
2) Indirect damage of cell/tissue due to dehydration, vasoconstriction, thrombosis, and tissue ischemia
3) Damage of cell/tissue during rewarming/ reperfusion

Initial exposure to a cold environment leads to reflex vasoconstriction to save the body's heat with intermittent episodes of vasodilation to avoid tissue ischemia, however, with continued exposure vasodilation is lost and tissue ischemia develops. Exposure to extreme cold leads to extracellular ice-crystal formation and sustained exposure results in intracellular ice-crystal formation as well. These ice crystals lead to direct cell membrane damage resulting in loss of movement of electrolytes across the cell membrane hence disrupting transmembrane electrolyte gradient leading to electrolyte and pH disturbance, finally leading to oedema and cell death. Subsequently intravascular ice-crystal formation results in direct endothelium damage, dehydration, vascular stasis, thrombosis and tissue ischemia, and cellular death[10-11].

Table 2. Factors affecting cold injury

Risk factor for superficial cold injury
• History of using intoxicants (tobacco, alcohol, and other abusive drugs), about 30 to 50% risk factor. • Environment-related factors such as inappropriate clothing and chill wind conditions. • Peripheral vascular diseases such as Raynaud's disease and Buerger's disease. • Stress and Age factor
Risk factor for deep and amputated cold injury
• Failure for sudden hospitalisation in case of exposure to first-degree or second-degree frostbites. • Use of prolonged medication such as alkaloids, Barbiturates, aminophylline and caffeine, etc. • Psychiatric illnesses, approx. 10 to 20 % contribution to cold injury.

Re-warming of exposed areas does not significantly increase the blood flow due to microvascular thrombosis and venous stasis due to dehydration and can also exacerbate ongoing cellular damage due to the generation of reactive oxygen species (ROS). Ischemia leads to decreased availability of O_2 to cells resulting in a reduced state of the inner mitochondrial membrane and increased free radical formation by the UBQ-Cytochrome B region of the electron transport chain.

Furthermore, ischemia at the cellular level leads to anaerobic respiration at the cellular level resulting in the generation of metabolites of free fatty acid metabolism, like arachidonic acid[13] which on rewarming are metabolised via cyclooxygenase *(*COX) and lipoxygenase (LOX) pathway resulting in generation of prostaglandins, thromboxane A2 (TXA2) and oxygen free radicals, thus accelerating the process of tissue damage, and persisting damages leads to necrosis, gangrene and amputation. A schematic representation of the mechanism involved in cold injury is depicted in Fig. 2.

3.1 Pathophysiological Cascades in Cold Injury

3.1.1 Pharmacological De-sensitisation of Sensory Neurons

During extreme cold exposure, cutaneous vasoconstriction acts as a primary defense mechanism. To the superficial region, the very first exposure to an extreme cold wave causes cold-induced vasodilation (CIVD), which modulates the effect of vasoconstriction via the release of calcium ion [Ca^{2+}], via induction of phosphorylated myosin light chain (p-MLC, which is also known as 'hunting effect' first elucidated by Lewis (1930)[18]. Pharmacological cascades involving physiological reflexes in CIVD are still unclear, while research suggests that selected TRP (transient receptor potential) such as TRPA1 (Transient Receptor Potential Ankyrin-1) may be involved in regulating primary defensive responses such as CIVD.

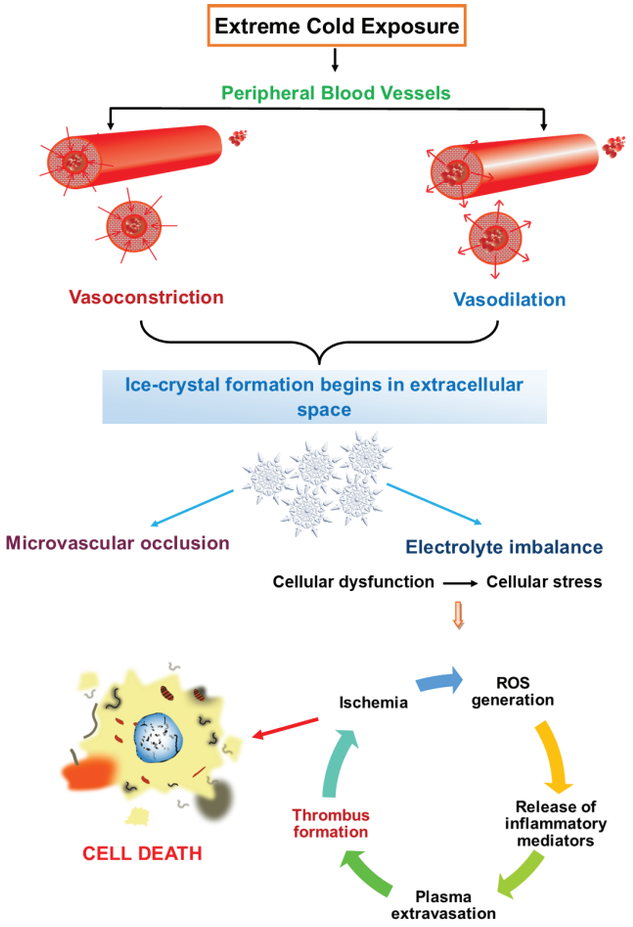

Figure 2. Schematic elaboration of the mechanism involved in cold injury.

The dynamics of the nervous system exhibit remarkable plasticity, manifested by the balance between functional sensitisation and desensitisation of membrane receptors. It is well documented that the main physiological function of TRP channels is to mediate the responses of cells and sensory neurons to chemical and physical stimuli[15-16]. Thus, regulation of the TRP channel by responses like sensitisation and desensitisation contributes to nociceptive processing. During continuous exposure to cold, differential sensitisation of these channels especially in Transient receptor potential cation channel subfamily A member 1 and transient receptor potential cation channel subfamily V member 1 (TRPA1 & TRPV1) influenced by the distinct expression of inflammatory mediators such as prostaglandins E2 (PGE2) nerve growth factor (NGF) and bradykinin (BK), and by kinases viz., protein kinase A & C has been well demonstrated by Patil, *et al.*, 2020[17].

3.1.2 Inflammatory Mediators Involved in Cold Injury

It is important to note that inflammatory responses after cold injury may not be always harmful, they may act as a beneficial positive response such as substantial upregulation of immune cells, activated by microglia and removal of debris activated by macrophages. Many cytokines and chemokines, including interleukins are involved in guiding of immune cells to provide rapid healing and re-epithelisation.

While interleukins such as IL-1β, IL-6, and IL-10 are shown to contribute to aggregation injury as well as neuroinflammation. Tissue necrosis factor-alpha (TNF-α) is also produced at the site of cold injured tissue in response to various pathological processes such as infectious agents and trauma caused by cold waves. E-selectin is another important marker of cold injury, highly expressed in vascular endothelium. The differential polyadenylation of E-selectin provides information regarding the phase of injury on their molecular basis[19].

3.3.3 Oxidative Stress Levels

Extreme cold as a stressor contributes to the generation of oxidative stress, which is implicated in cellular activities involved in inflammatory responses, apoptosis, and tissue necrosis. Research suggests that the nuclear factor erythroid-derived-2 like-2 (Nrf2) factor plays a protective role against oxidative stress-induced cellular damage by regulating the expression of antioxidant proteins[20].

Oxidative stress markers such as serum level of protein carbonyl, 4-hydroxy-2-nonenal (4-HNE), superoxide dismutase (SODs), advanced oxidative protein products (AOPP), and nitrotyrosine are useful in the diagnosis of early stages of cold injury.

3.1.4 Endothelial Cell Damages

When blood flow in microcirculation stops flowing during the initial phase of cold injury, re-warming may play a beneficial effect on restoring the blood flow. However, persistent cold exposure causes microvasculature endothelial cell damage, which causes "gaps' between endothelial lining some of which contain leukocytes and platelets. Prominent markers of endothelial cell damage can be assessed through nitric oxide (NO), prostacyclin (PGI_2), reactive oxygen species (ROS), Von-Willebrand factor (VWF), CD31/PECAM-1 (platelet/endothelial cell adhesion molecule 1), CD36/SR-B3 (scavenger receptor class B member 3) and tissue-type plasminogen activator (TTPA). Furthermore, the pathophysiological cascade leads to the formation of prostaglandins F2α (PGF2α) and further thromboxane (TXA2), if there is no therapeutic intervention provided, which in an aggravated state can subsequently lead to extensive irreparable tissue damage and even necessitate amputation. The events involved in the pathophysiology of cold injury are depicted in Fig. 3.

targeting disease pathophysiology. In the case of dermal delivery systems, hydrogel, colloidal patches, liposomes, inserts, polymeric nanoparticles, metallic nanoparticles, nanocrystals, etc. have been introduced as novel drug delivery systems. However, despite immense advances, the lack of supporting evidence and understanding of the proper mechanism often retards the process of developing modern therapeutics[35].

The outcome of pharmacological interventions in cold injury is greatly limited due to its being dependent on the duration and severity of cold injuries and the local availability of therapeutics. After, extreme exposure to severe cold conditions, it is advised to follow the guidelines provided by government and health care institutions for prevention and early management of cold injury. This includes (i) immediate transfer of the patient to the protected environment, (ii) removal of wet clothing, (iii) covering the patient with warm blankets, and (iv) infusion of i.v. fluids. The affected area should not be re-warmed if the exposure to cold is for more than 2 hrs because this may lead to ischemia and thrombosis due to alternative freezing and thawing processes.

The administration of ibuprofen (12 mg/kg body weight, b.i.d.) is recommended due to its anti-prostaglandin and analgesic effect, principally aiming to inhibit the thrombogenic activity of prostaglandins F2α and thromboxane A2.

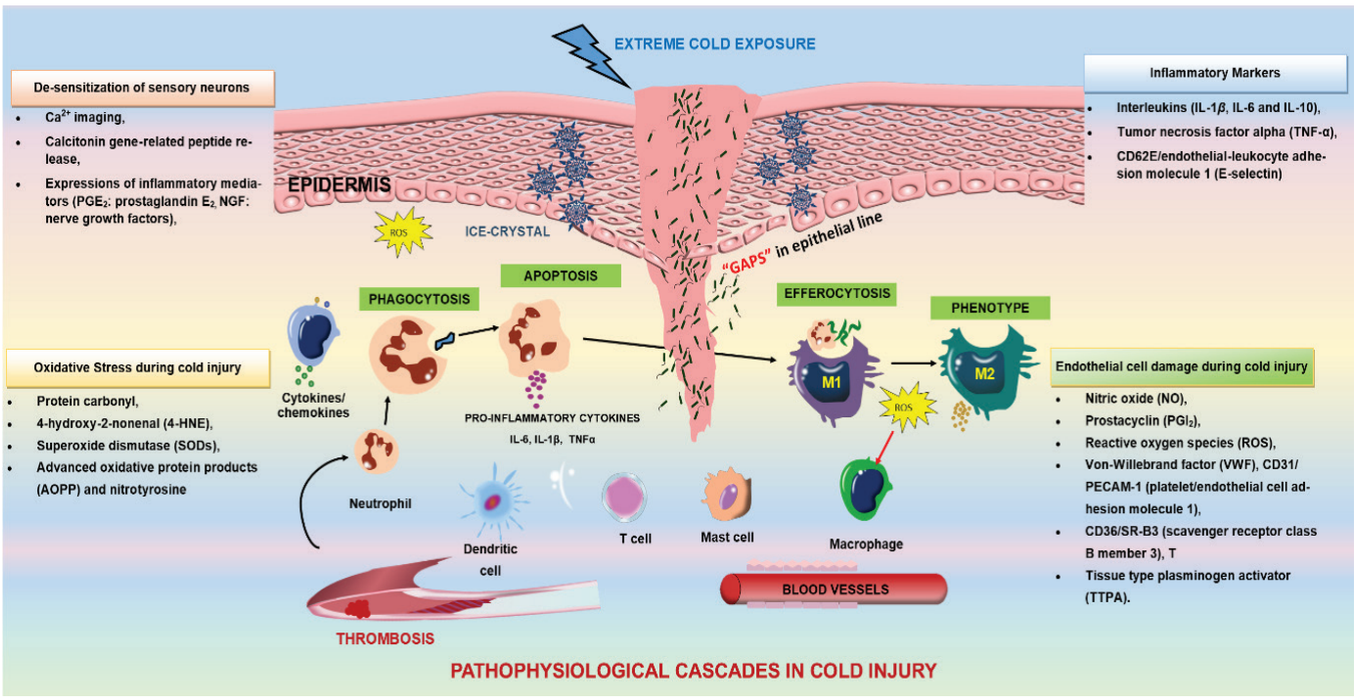

Figure 3. Schematic representation of pathophysiological cascades in cold injury showing prominent plasma markers to examine phase of cold injury.

4. MODERN PHARMACOLOGICAL INTERVENTIONS FOR PREVENTION, MANAGEMENT, AND TREATMENT OF COLD INJURY

Recent advances in drug delivery systems have substantially increased the capability to target diseases as a wide range of novel approaches are now available for

In case of hospitalisation, the primary aim should be to achieve the patient's normal body temperature and once it exceeds 34 °C, the physician should begin the local treatment for cold injury. Some topical formulations such as *Aloe vera-based* cream exhibit great anti-thrombogenic activity, which acts as potent inhibitors of thromboxane

A2 and can be administered topically every 6 hrs. on the locally injured area[21]. The *Aloe vera*-based ALOCAL cream developed by the Defence Institute of Physiology and Allied Sciences (DIPAS) has been extremely useful in reducing the incidences of frostbite in forward areas.

Following are some of the experimental approaches employed as pharmacological interventions for the management of cold injury:

Hyperbaric Oxygen Therapy (HBOT): For the treatment of frostbite, hyperbaric oxygen therapy has been tested as a therapeutic modality either as an individual treatment or in combination with vasodilatory, anticoagulating, and hemorheological agents such as pentoxifylline[32]. The modality is reasonable, safe, and well-tolerated by patients. A shred of strong evidence to follow this therapy for cold injury has not been recommended anywhere yet as the evidence is limited to a few case studies only. However, the few successful cases of individually treated patients with hyperbaric oxygen therapy (HBOT) have shown favorable clinical outcomes. A recent case report presented by Davis, *et al*., 2022[36] demonstrated that delayed HBOT in a severe frostbite case (where numerous digits sustained grade II-III cold injury and the right fifth digit which was having grade III injury) experienced good functional recovery, and showed patient-centered outcome[22].

Sympathectomy: Practicing surgical sympathectomy requires a good understanding of the mechanism cascades of cold injury. It is suggested that to achieve better results surgical sympathectomy must be performed within 2 hrs of injury[23].

Thrombolytics: Treating thrombolytics such as rTPA (recombinant tissue plasminogen activators) is a great choice for severe forms of cold injury. Bruen, *et al.*, 2007[24] showed a reduction in digital amputation up to ~41 % when compared with those groups of patients who had not received TPA. Thrombolytics are contraindicated in pregnancy, thrombocytopenia, craniocerebral trauma, and thrombocytopenia[34].

Vasodilators: In severe forms of cold injury, vasodilators may serve as a choice of therapeutics. Some of the potent vasodilators such as synthetic prostacyclin analog iloprost (analog of prostacyclin PGI2), prostaglandin E1 (PGE1), nitroglycerin, nifedipine, pentoxifylline (Miller and Koltai, 1995), etc. are used in the treatment of cold injuries[25-26].

Newer therapeutics for the treatment of cold injury: Newer pharmacological interventions include the development of formulations based on novel drug delivery technologies which have shown high effectiveness when compared to conventional delivery systems. For successful therapy many researchers have developed different formulations showing greater efficacy in different experimental models, examples of which include: design and development of novel herbosomal PEG-poloxamer topical formulation (n-HPTF) for treatment of cold injury[27]; ointment containing essential oils which shows efficacy in frostbitten wound[28]; a nano-spray gel (NSG) containing heparin and ibuprofen liposomes for effective management of frostbite[29]; A nanostructured lipid of *Ganoderma* triterpenoids used for the treatment of cold injury[30]; a topical lotion of poly-l-arginine (PAL) developed to treat the frostbite condition[31] etc.

5. CONCLUSION

The exact pathophysiology of cold injuries is quite complex and multifactorial (due to the interplay of various mechanisms at a single point in time) as compared to cascades of other traumatic and non-traumatic wounds. Various approaches for the treatment of cold injuries have been undertaken in the past but none have shown favorable outcomes. Moreover, incorrect pharmacological interventions may worsen the situation, therefore, correct and targeted therapy is essential to decrease overall morbidity and disability associated with cold injuries. However, the choices of treatment depend upon the patient's clinical situation, duration of exposure, grade of tissue trauma/tissue loss, and availability of prompt and specialised medical and surgical care. Not many options are available if cold injuries are severe and the only option left is surgical intervention and amputation of the affected limb(s). However primary prevention and early treatment of initial stages using local topical-based formulations and novel drug delivery systems which have better bioavailability and efficacy can serve as a good modality for the effective management of severe cold injuries like frostbite.

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REFERENCES

1. Tomas, F. Hornbein. & Robert, B. Schoene eds. High altitude: An exploration of human adaptation. *Marcel Dekker*, Inc., 2001, **161**, 25-41. doi: 10.3109/9780203908044.
2. Ranjan, P.; Pawar, A.; Naik, A.K.; Bhardwaj, S. & Sharma, P. Cold injuries in the glacial regions of India. *J. Mar. Med. Soc.,* 2021, **23**, 24-8. doi 10.4103/jams.jmms_51_20.
3. Lt. Col. Singh, G.K.; Brig. Datta, A.; Brig. Grewal, B.S.; Col. Suresh, M.S. & Brig. Vaishampavan, S.S. Pattern of chilblains in a high altitude region of Ladakh,

India. *Med. J. Armed Forces.* India, 2015, **71,** 265-269. doi: 10.1016/j.mjafi.2013.01.011.
4. Brig. Nagpal, B.M. & Surg. Lt. Cdr. Sharma, R. Cold Injuries: The Chill Within. *Med. Armed Forces. J. India.,* 2004, **60**, 165-171. doi: 10.1016/S0377-1237(04)80111-4.
5. Moran, D.S.; Heled, Y.; Shani, Y. & Epstein, Y. Hypothermia and local cold injuries in combat and non-combat situations--the Israeli experience. *Aviat. Space Environ. Med.,* 2003, 74**(8),** 890. PMID: 12650278
6. Wittmers, L.E. Jr. Pathophysiology of cold exposure. *Minn. Med.,* 2001, **84**(11)**,** 30-6. PMID: 11816961
7. Biem, J.; Koehncke, N.; Classen, D. & Dosman, J. Out of the cold: Management of hypothermia and frostbite. *Can. Med. Assoc. J.,* 2003, **168**(3), 305-11. PMID: 12566336
8. Harirchi, I.; Arvin, A.; Vash, J.H. & Zafarmand, V. Frosbite: Incidence and predisposing factors in mountaineers. *Br. J. Sports. Med*., 2005, **39**, 898-901. doi: 10.1136/bjsm.2004.016097
9. Rintamäki, H. Prediction and prevention of frostbite. *Int. J. Cir. Health*., 2000, **59**, 92-8. PMID: 10998828
10. Wagner, C. & Pannucci, C.J. Thrombolytic therapy in the acute management of frostbite injuries. *Air. Med. J.,* 2011, **30**(1), 39-44. doi: 10.1016/j.amj.2010.08.006
11. Twomey, J.A.; Peltier, G.L. & Zera, R.T. An open-label study to evaluate the safety and efficacy of tissue plasminogen activator in treatment of severe frostbite. *J. Trauma.,* 2005, **59**, 1350-1355. doi: 10.1097/01.ta.0000195517.50778.2e.
12. Bruen, K.J.; Ballard, J.R.; Morris, S.E.; Cochran, A.; Edelman, L.S. & Saffle, J.R. Reduction of the incidence of amputation in frostbite injury with thrombolytic therapy. *Arch. Surg.,* 2007, **142**(6), 546-51. Discussion 551-3. doi: 10.1001/archsurg.142.6.546.
13. Kalogeris, T.; Baines, C.P.; Krenz, M. & Korthuis, R.J. Cell biology of ischemia/reperfusion injury. *Int. Rev. Cell. Mol. Biol.,* 2012, **298**, 229-317. doi: 10.1016/B978-0-12-394309-5.00006-7.
14. Folio, L.R.; Arkin, K. & Butler, W.P. Frostbite in a mountain climber treated with hyperbaric oxygen: Case report. *Mil. Med.,* 2007, **17**(5), 560-563. doi: 10.7205/MILMED.172.5.560.
15. Clapham, D.E. TRP channels as cellular sensors. *Nature*, 2003, **426**, 517–524. doi: 10.1038/nature02196.
16. Numazaki, M. & Tominaga, M. Nociception and TRP channels. *Curr. Drug Targets CNS Neural Disord.,* 2004, **3**, 479–485. doi: 10.2174/1568007043336789.
17. Patil, M.J.; Salas, M.; Bialuhin, S.; Boyd, J.T.; Jeske, N.A. & Akopian, A.N. Sensitisation of small-diameter sensory neurons is controlled by TRPV1 and TRPA1 association. *FASEB J*., 2020, **34**(1), 287-302. doi: 10.1096/fj.201902026R.
18. Lewis, T. The blood vessels of the human skin. *Br. Med. J.,* 1926, **2**(3418), 61-2. doi: 10.1136/bmj.2.3418.61.
19. Gupta, G.S. Selectins and Associated Adhesion Proteins in Inflammatory disorders. *Ani. Lectins: Form, Fun. Clin. Appl.,* 2012, **20**, 991–1026. doi: 10.1007/978-3-7091-1065-2_44.
20. Cong, P.; Liu, Y.; Liu, N.; Zhang, Y.; Tong, C.; Shi, L.; Liu, X.; Shi, X.; Liu, Y.; Tong, Z. & Hou, M. Cold exposure induced oxidative stress and apoptosis in the myocardium by inhibiting the Nrf2-Keap1 signaling pathway. *BMC Cardiovasc. Disord.,* 2018, **15**, 18(1), 36. doi: 10.1186/s12872-018-0748-x.
21. Twomey, J.A.; Peltier, G.L. & Zera, R.T. An open-label study to evaluate the safety and efficacy of tissue plasminogen activator in treatment of severe frostbite. *J. Trauma*., 2005, **59**(6), 1350-1354. doi: 10.1097/01.ta.0000195517.50778.2e.
22. Kemper, T.C.; De Jong, V. M.; Anema, H. A.; van-den, Brink, A. & van Hulst, R.A. Frostbite of both first digits of the foot treated with delayed hyperbaric oxygen: A case report and review of literature. *Undersea. Hyperb. Med. J.,* 2014, **41**, 65–70. PMID: 24649719.
23. Bouwman, D.L.; Morrison, S.; Lucas, C.E. & Ledgerwood, A.M. Early sympathetic block ade for frostbite–is it of value? *J. Trauma*., 1980, **20**, 744-9. PMID: 7411663
24. Bruen, K.J.; Ballard, J.R.; Morris, S.E.; Cochran, A.; Edelman, L.S. & Saffle, J.R. Reduction of the incidence of amputation in frostbite injury with thrombolytic therapy. *Arch. Surg*., 2007, **142,** 546–551. doi: 10.1001/archsurg.142.6.546.
25. Yeager, R.A.; Campion, T.W.; Kerr, J.C. & Hobson, R.W. 2nd Lynch TG: Treatment of frostbite with intra-arterial prostaglandin E1. *Am. Surg*., 1983, **49**, 665–7. PMID: 6546186
26. Groechenig, E. Treatment of frostbite with iloprost. *Lancet*., 1994, **344,** 1152-3. doi: 10.1016/s0140-6736(94)90657-2.
27. Yadav, R.B.; Pathak, D.P.; Varshney, R. & Arora, R. Design and optimisation of a novel herbosomal-loaded PEG-poloxamer topical formulation for the treatment of cold injuries: a quality-by-design approach. *Drug Del. Trans. Res*., 2022. **12**(11), 2793-2823. doi: 10.1007/s13346-022-01140-7.
28. Joshi, K.; Goyary, D.; Mazumder, B.; Kishor, S.; Dey, P.; Bhutia, Y.D.; Karmakar, S.; Chattopadhyay, P. & Dwivedi, S.K. Development and standardisation of a method for inflicting frostbite injury in rats and formulation of essential oil in treatment of frostbite. *Def. Life Sci. J*., 2020, **5**, 103-108. doi: 10.14429/dlsj.5.15606.
29. Vaghasiya, K.; Sharma, A.; Kumar, K.; Ray, E.; Adlakha, S.; Katare, O.P.; Hota, S.K. & Verma, R.K. Heparin-encapsulated metered-dose topical "nano-spray gel" liposomal formulation ensures rapid on-site management of frostbite injury by inflammatory cytokines scavenging. *ACS Biomater. Sci. Eng.,* 2019, **5**, 6617–6631.

doi: 10.1021/acsbiomaterials.9b01486.
30. Shen, C.Y.; Dai, L.; Shen, B.D.; Zhou, X.; Bai, J.X.; Xu, H.; Lv, Q.Y.; Han, J. & Yuan, H. L. Nanostructured lipid carrier based topical gel of Ganoderma Triterpenoids for frostbite treatment. *Chin. J. Nat. Med*., 2015, **13**, 454-460. doi: 10.1016/S1875-5364(15)30039-X.
31. Auerbach, L.J.; DeClerk, B.K.; Garrison Fathman, C.; Gurtner, G.C. & Auerbach, P.S. Poly-l-Arginine topical lotion tested in a mouse model for frostbite injury. *Wilderness Environ. Med*., 2014, **25**, 160–165. doi: 10.1016/j.wem.2014.01.006.
32. Purkayastha, S.S.; Bhaumik, G.; Chauhan, S.K.; Banerjee, P.K. & Selvamurthy, W. Immediate treatment of frostbite using rapid rewarming in tea decoction followed by combined therapy of pentoxifylline, aspirin & vitamin C. *Indian J. Med. Res.,* 2002, **116**, 29-34. PMID: 12514975.
33. Van Dongen, T.T.C.F.; Berendsen, R.R.; de Jong, F.J.M.; Endert, E.L.; van Hulst, R.A. & Hoencamp, R. Frostbite: a systemic review on freezing cold injuries in a military environment. *BMJ Mil. Health.,* 2023, **7**, e002171. doi: 10.1136/military-2022-002171.
34. Murphy, J.; Endorf, F.W.; Winters, M.K.; Rogers, C.; Walter, E.; Neumann, N.; Weber, L.; Lacey, A.M.; Punjabi, G. & Nygaard, R.M. Bleeding complications in pateints with severe frostbite injury. *J. Burn Care Res.,* 2023, **44**(4), 745-750. doi: 10.1093/jbcr/irac180.
35. Zaramo, T.Z.; Green, J.K. & Janis, J.E. Practical review of the current management of frostbite injuries. *Plast. Reconstr. Surg. Glob. Open.,* 2022, **10**(10)*,* e4618. doi: 10.1097/GOX.0000000000004618.
36. Davis, A., Sinopoli, B., Mann, N., Stenbit, A.E. A photographic case of frostbite treated with delayed hyperbaric oxygen therapy. *High Alt. Med. Biol.,* 2022, 194-197. doi: 10.1089/ham.2021.0047.

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