Mechanism action of homocysteine and its thiolactone in haemostasis system

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Summary

In the article, the actions of homocysteine (Hcy) and its metabolite - cyclic thioester – homocysteine thiolactone (HTL) on complex process of haemostasis, which regulates the flowing properties of blood, are described. Possible interaction of Hcy and HTL with endothelial cells, blood platelets, plasmatic fibrinogen and plasminogen, as the important major components of haemostasis are also discussed. The modification of haemostatic proteins (N-homocysteinylated or S-homocysteinylated proteins) induced by Hcy or its thiolactone, and links of homocysteine or homocysteine thiolactone to \*NO metabolism seem to be the main reason of biotoxicity of homocysteine in cardiovascular diseases.

Key words: homocysteine, homocysteine thiolactone, haemostasis
Introduction

Haemostasis is a complex process that regulates in vivo the flowing properties of blood. Classical primary and secondary haemostasis only comprised the sequential formation of the white and red thrombus, but there is growing awareness that haemostasis is intimately coupled to fibrinolytic processes, inflammatory reactions as well as to initiation of angiogenesis and wound healing. Basically, three components need to interact to assure effective primary and secondary haemostasis together with the normal sequelae, fibrinolysis and tissue repair: (1) the vascular wall (smooth muscle, matrix and connective tissue, endothelial cells), (2) formed elements of the blood (blood platelets, granulocytes, monocytes, lymphocytes), and (3) the plasmatic clotting and fibrinolytic systems (Becker et al. 2000). Haemostatic abnormalities can lead to excessive bleeding, thrombosis or other cardiovascular diseases. Recently, it have shown that homocysteine (Hcy), which is an intermediate formed during the catabolism of the essential dietary amino acid methionine induces changes in haemostasis, including blood clotting and fibrinolysis (Perla-Kajan et al. 2007). Elevated level of Hcy may disrupt functions of the vascular endothelium, changing the character of its surface from anticoagulant to procoagulant (Jacobsen 1998, Perla-Kajan et al. 2007). This review describes the chemical structure and biological activities of homocysteine and its cyclic thioester – homocysteine thiolactone (HTL) and their effects on haemostasis process.

Chemical structure and function of homocysteine and its metabolites

Homocysteine is involved in conversions of methionine (Met) and cysteine (Cys). The immediate precursor of Hcy is S-adenosylhomocysteine (SAH), which is hydrolyzed by SAH hydrolase to homocysteine and adenosine. In the next step homocysteine is remethylated to
methionine by methionine synthase or betaine:homocysteine methyltransferase. Moreover, Hcy enters the transulfuration pathway and is converted into cystathionine β–synthase and cystathionine γ–lyase. This process is present only in the liver, kidney, pancreas and small intestine (Brosnan et al. 2004, Perla-Kajan et al. 2007). Homocysteine may also enter the first step of protein synthesis. Because structural similarity of Hcy to Met, homocysteine may be recognized and activated by methionyl-tRNA synthetase. However, error-editing activity of methionyl-tRNA synthetase does not allow homocysteine to be incorporated into proteins. As a product of the editing reaction homocysteine thiolactone is formed (Jakubowski and Fersht 1981, Jakubowski 2003, Jakubowski 2004, Perla-Kajan et al. 2007). The chemical structure of Hcy and its thiolactone is presented on Figure 1. Homocysteine metabolism depends on the level of vitamins (folic acid, vitamin B₆ and B₁₂) as cofactors for the enzymes involved in Hcy turnover. In human blood homocysteine may exist in free or protein bound forms as N-homocysteinylated (N-Hcy-protein) or S-homocysteinylated proteins (S-Hcy-protein), that were described as N-Hcy-hemoglobin, N-(Hcy-S-S-Cys)-albumin, S-Hcy-albumin, and cysteinylhomocysteine (Cys-Hcy)) (Jakubowski 2002, Chwatko and Jakubowski 2005A Chwatko and Jakubowski 2005B, Jakubowski 2005, Jakubowski 2006, Perla-Kajan et al. 2007). Mechanism of N-homocysteinylation involves acylation of Lys ε-amino group by the activated carboxyl group of HTL (Jakubowski 2003, Jakubowski 2004, Jakubowski 2005, Jakubowski 2006), whereas S-homocysteinylation is induced by Hcy (Fig. 1). In human plasma some various haemostatic proteins are S–homocysteinylated, for example coagulation factor Va (Undas et al. 2001). N-homocysteinylated proteins in human plasma represent from 0.3 to 23 % of total homocysteine. Small amounts of N-Hcy-proteins are also found in different haemostatic proteins (antitrypsin, fibrinogen) (Jakubowski 2002). These modifications may lead to impairment of protein functions. Approximately 80% of plasma homocysteine is protein bound, and only a small amount exists as a free reduced
homocysteine. The majority of the unbound portion of Hcy is oxidized to form dimers (homocystine) or combined with cysteine to form mixed disulphides (Jakubowski 2002, Chwatko and Jakubowski 2005A, Chwatko and Jakubowski 2005B, Jakubowski 2005, Jakubowski 2006, Perla-Kajan et al. 2007). Plasma Hcy level is determined by multiple factors, like genetic, demographic, acquired, and lifestyle determinants. Total plasma Hcy concentration for healthy adults is in the range of 5-15 µM. Patients with mild hyperhomocysteinemia have 15-25 µM Hcy (Perla-Kajan et al. 2007). In urine after oxidation of Hcy is referred to as homocystinuria. Thus, for abnormal metabolism of Hcy, the blood can be analyzed. Mild hyperhomocysteinemia is an independent risk factor for atherosclerotic disease, deep vein thrombosis and thromboembolism. Elevated Hcy levels promote thrombosis, although the mechanism by which Hcy exerts its prothrombotic effect remains unclear. Moreover, homocysteine metabolites (thiolactone) and protein homocysteinylation formed in plasma, are implicated in different cardiovascular diseases (Thambyrajah et al. 2000, Yang et al. 2006).

The effect of homocysteine and its thiolactone on endothelial cells

At cellular level pathological role of homocysteine seems to be associated with an alteration of endothelial cells, which play an important role in haemostasis. Endothelial cells are very sensitive even to a mild increase of Hcy concentration. This sensitivity may be explained by the fact, that human endothelial cells do not express active form of cystathionine β-synthase and consequently can not initiate homocysteine catabolism through transsulfuration pathway (Jacobsen 1998). Elevated level of Hcy may modulate functions of the vascular endothelium, changing the character of its surface from anticoagulant to procoagulant (Jacobsen 1998). Endothelial anticoagulant pathway is based on heparin-like
glycosaminogycan-antithrombin III interaction. Results of Rodgers et al. (1986) showed that after incubation of endothelium with Hcy activity of coagulation factor V increases. Coagulation factor V in Hcy-modified endothelium is cleaved in fragments different than those obtained after factor V cleavage by thrombin or coagulation factor Xa (Rodgers et al. 1986). Moreover, in hyperhomocysteinemia the prothrombotic tendency may be related to impaired inactivation of S-homocysteinylated coagulation factor Va by activated protein C (Undas et al. 2001). However, Hcy and its thiolactone have no effect on factor V activation by thrombin. It was showed that factor V incubated with [35S]homocysteine (10-450 µM) incorporated label within 5 min, which was found only in those fragments that contained free sulphydryl groups: the light chain (Cys-1960, Cys-2113), the B region (Cys-1085), and the 26/28-kDa (residues 507-709) activated protein C cleavage products of the heavy chain (Cys-539, Cys-585). On the other hand, Lentz et al. (2002) suggest that activation of protein C by thrombin and inactivation of factor Va by activated protein C are not impaired during moderate hyperhomocysteinemia in vivo in monkeys. Another studies showed the effect of Hcy on activity of thrombomodulin, which is involved in blood clotting.

It has been demonstrated that not only Hcy, but also HTL may modulate properties and functions of endothelial cells. Results of Raposo et al. (2004) indicate that both compounds – Hcy and its thiolactone inhibit activity of lysyl oxidase (an enzyme involved in extracellular matrix maturation) in vascular endothelial cells. Report of Jakubowski (2000) showed that in endothelial cells protein N-homocysteinylation exists, and this process depends on the concentration of Hcy. Modification of endothelial cells proteins may cause different pathophysiological consequences, such as modulation of haemostasis system, which may contribute to cardiovascular diseases.
Nitric oxide (\(\bullet\)NO) plays an important role in a number of physiological processes. It is well known, that vascular endothelial cells produce \(\bullet\)NO. However, other cells, which are involved in haemostasis - blood platelets may also synthesize \(\bullet\)NO and platelet \(\bullet\)NO synthase (NOS) has been described and identified during the mid 90s (Muruganadam and Mutus 1994). Two main NOS isoforms have been isolated: calcium independent inducible NOS (iNOS) and a calcium/calmodulin dependent endothelium type NOS (eNOS) (Mehta et al. 1995). \(\bullet\)NO is released by NOS-es action from the guanidino moiety of L-arginine, yielding NO and cytruline. \(\bullet\)NO is very simple free radical which play important regulatory role in many tissues, but originally \(\bullet\)NO was identified as a factor influenced mainly human vascular system. \(\bullet\)NO has been implicated in a number of cardiovascular diseases and every risk factor for these appears to be associated with a reduction in endothelial generation of \(\bullet\)NO.

Evidences of some cooperation between homocysteine and \(\bullet\)NO in vascular system pathogenesis originate from physiological, cellular and genetic reports. Patients from population with \textit{NOS3} 894TT genotype (mutation in gene for nitric oxide synthase) have tendency to higher Hcy concentration in the blood. Mentioned tendency was especially distinct in subpopulation of \textit{NOS3} 894TT patients who had low foliate levels. However, there was no strictly correlation between \textit{NOS3} 894TT genotype and Hcy plasma levels (Brown \textit{et al.} 2003). The question is how Hcy can modulate \(\bullet\)NO synthase. First thought is that Hcy may influence on NOS activity. Transformation of radiolabelled arginine to cytruline catalyze by nitric oxide synthase is unchanged after renal arterial endothelium incubation with Hcy (40 \(\mu\)M) in comparison with control cells. Also after incubation of endothelial cells with Hcy (10, 20 and 50 \(\mu\)M during 24 hours) NOS activity was untouched (Fatini \textit{et al.} 2005). Despite the fact that NOS activity seemed to be normal also during
hyperhomocysteinemia in vitro, level of $\cdot$NO under such conditions is definitely lower and this effect is directly proportional to Hcy plasma level (Becker et al. 2005). One of possible way of NOS activity regulation by Hcy is action through direct dimethylarginine dimethylaminohydrolase (DDAH) binding via disulfide bounds and further asymmetric dimethylarginine (ADMA) accumulation, what disrupts NOS activity (Stühlinger et al. 2001). Similar observations confirming ADMA accumulation during hiperhomocysteinemia has been done in animal models (Akhand et al. 1999). The same authors have observed in vitro that ADMA secretion by human endothelial cells after stimulation by Hcy (24 hours) can be stopped by using S-adenosylhomocysteine – methylation inhibitor.

The main molecules responsible for decrease of $\cdot$NO level in hyperhomocysteinemic patients are probably reactive oxygen species (ROS). Hcy can enhance ROS production (Li et al. 2002, Fischer et al. 2003, Fatini et al. 2005) – the generation of superoxide anion ($O_2^{-*}$) in reaction catalyzed by NADPH oxidase (Becker et al. 2005). Results of Fischer et al. (2003) showed the increased of protein nitration (induced by peroxynitrite, which is produced in the reaction of $\cdot$NO with $O_2^{-*}$).

The effect of homocysteine and its thiolactone on blood platelets

Blood platelets are multiresponding cells, both with respect to the number of agonists and number of responses. They can be activated by different compounds including coagulation factors (thrombin), hormones (epinephrine, vasopresin), low-molecular-weight substances (serotonin, adenosine diphosphate (ADP)), lipid derivatives (platelet aggregating factor (PAF), thromboxane $A_2$ (TXA$_2$)), and other protein substances (collagen or immune complexes). The responses of platelet to agonists, which is named platelet activation, include mainly adhesion (to foreign surfaces such as collagen or glass), shape change, aggregation
and secretion of active compounds from three different storage granules (dense granules, \( \alpha \)-granules and lysosomes), shedding of microvesicles, formation of platelet procoagulant activity and retraction of fibrin clots (Wu 1996, Levy-Toledano 1999, Ryningen and Holmsen 1999). Increased platelet activation with hyperaggregability is one of the risk factors in pathogenesis of different cardiovascular disease. Blood platelets obtained from patients with peripheral occlusive arterial disease, with associated hyperhomocysteinemia, are far more reactive and sensitive for agonists, but also far less sensitive for inhibitors (Riba et al. 2004). In diabetic patients a high level of Hcy levels is associated with more potent platelets aggregation (Rajkumar et al. 1999). Signorello et al. (2007) observed that Hcy induces oxidative stress, inhibits nitric oxide formation in platelets from type 2 diabetic patients, and may promote platelet hyperactivity and various cardiovascular diseases. Moreover, Hcy stimulates the calcium mobilization in platelets from 2 diabetic patients (Alexandru et al. 2007). The study of Mohan et al. (2008) suggest that platelet activation and hypercoagulability occur after exposure to Hcy, especially in patients with critical limb ischemia. Hcy concentrations of approximately 50 \( \mu \)M appear to be the level at which these changes occur in vitro, and this effect on platelets appears to be indirect. In animal model with hyperhomocysteinemia (induced by diet poor of folic acid), the aggregation of platelets stimulated by ADP or thrombin is higher then in control animals (Durand et al. 1996). The direct action of Hcy on blood platelets is unknown and sometimes controversial. Some studies demonstrated that homocysteine promotes arachidonic acid release, thromboxane A\(_2\) formation (Signorello et al. 2002 and 2007) and protein tyrosine phosphorylation in blood platelets (Leoncini et al. 2006). Results of Undas et al. (2007) demonstrated that elevated total Hcy is associated with increased platelet activation at the site of microvascular injury. McDonald et al. (1964) showed increased platelet adhesion in homocysteinuric patients. However, there is no evidence of a direct in vitro effect of homocysteine on platelet adhesion
(Uhlemann et al. 1976) or aggregation (Stamler et al. 1993). Our preliminary studies have reported that reduced form of Hcy slightly stimulated platelet aggregation induced by a physiological agonist - thrombin (Olas et al. (in press)). We also observed that HTL alone do not induce platelet aggregation, but HTL, like Hcy increased platelet aggregation induced by thrombin (Olas et al. (in press)). Platelets aggregation induced by HLT is followed by thromboxane A\textsubscript{2} synthesis and secretion (McCully and Carvalho 1987). McGarrigle et al. (2006) showed that Hcy and HTL promote activation of platelet integrin α\textsubscript{IIb}β\textsubscript{3}, that is involved in platelet adhesion or aggregation. On the other hand, Luo et al. (2006) observed that Hcy potentiates collagen type I induced-platelet activation through signaling components of glycoprotein VI and integrin α\textsubscript{2}β\textsubscript{1} pathway. Prontera et al. (2007) indicate that a potential molecular target of Hcy is the CD40/CD40L system in platelets; and upregulation of CD40/CD40L signaling may represent a link between hyperhomocysteinemia and an increased risk of cardiovascular diseases.

Blood platelets, in analogy to other circulating blood cells, can generate different reactive oxygen/nitrogen species (ROS/RNS) that may behave as second messengers and may regulate platelet functions. In blood platelets are postulated several sources of ROS (Pignatelli et al. 1998, Wachowicz et al. 2002, Krotz et al. 2004). Intracellular sources of reactive oxygen species in platelets are arachidonic acid pathway (via cyclooxygenase or 12-lipoxygenase) stimulated by different agonists, the glutathione cycle (Jahn and Hansch 1990, Pignatelli et al. 1998,) and metabolism of phosphoinositides (Gopalakrishna et al. 2000, Wachowicz et al. 2002). ROS are generated in platelets mostly by activation of NAD(P)H oxidase (Forde and Fitzgerald 1997, Krotz et al. 2004, Begonja et al. 2005) and xantine oxidase (Miller et al. 1993). Our recent data, where we used in our experiments the method of cytochrome c reduction for the estimation of the level of O\textsubscript{2}• showed that not only Hcy, but
also HTL induces the production of $O_2^\cdot$ in resting and thrombin-activated platelets (Olas et al. (in press)).

In blood platelets peroxynitrite may be formed (Lufrano and Balazy 2003). It has been demonstrated that reaction of protein with ONOO$^-$ resulted in the nitration and oxidation of some amino acid residues (Hernandez-Hernandez et al. 1999, Olas and Wachowicz 2007). Free and protein-bound 3-nitrotyrosine, a stable product of tyrosine nitration may be measured as a biomarker of protein damage induced by peroxynitrite and other reactive nitrogen species (Forde and Fitzgerald 1997, Begonja et al. 2005), since peroxynitrite nitrates tyrosine residues in different proteins in vivo and in vitro (Miller et al. 1993). During platelet activation spontaneous nitration of proteins may also take place, without the addition of exogenous peroxynitrite (Muruganandam and Mutus 1994). When proteins from activated and resting platelets were separated on gels and immunoblotted with antinitrotyrosine antibodies, a number of proteins in the low molecular weight region were nitrated. Moreover, in our study, we observed that reduced form of Hcy and HTL distinctly reduced nitration of platelet proteins (Olas et al. (in press)). It is suggested that the nitrosation of homocysteine or its metabolite – HTL in blood platelets may exist. Nitrosation of Hcy may be responsible for a decrease in the level of $^\cdot$NO, because Hcy reacts with $^\cdot$NO to form S-nitroso-homocysteine (Ignarro and Gruetter 1980). Signorello et al. (2007) showed that in type 2 diabetic patients high plasma Hcy is associated with increased platelet ROS level and reduced $^\cdot$NO formation in blood platelets. On the other hand, Hcy induces cell death in H9C2 cardiomyocytes through the generation of peroxynitrite and can activate key signaling cascades in the myocardium (Levrand et al. 2007). Moreover, Erol et al. (2007) have showed that Hcy increases $^\cdot$NO release from stimulated coronary microvascular endothelial cells without affecting basal $^\cdot$NO production, which is probably accompanied by increased production of reactive oxygen species. It can be postulated that endothelial cells generate $^\cdot$NO in order to
minimize the damage caused by Hcy. There is possibility that nitration of tyrosine may directly inhibit the tyrosine phosphorylation of proteins, which is well – recognized mechanism of signal transduction in blood platelets (Ischiropoulos 2003). It was showed that the addition of peroxynitrite to platelets, at relatively high concentrations, brought about the nitration of proteins and a rapid increase in the phosphorylation of tyrosine residues (Mondoro et al. 1997). However, if platelets were activated by the low doses of thrombin, the amount of phosphorylation was decreased; if high doses of thrombin were used, peroxynitrite caused the increase of the phosphorylation. There is no direct evidence that phosphorylation and nitration on the same proteins occurs. Probably relationship between nitration and tyrosine phosphorylation is a competitive process (Low et al. 2002). Marcondes et al. (2006) suggested that nitration of α–actinin interferes with its phosphorylation and contributes consequently to the inhibitory role of *NO on platelet adhesion. Some results showed that homocysteine stimulates the tyrosine phosphorylation and activation of platelet phospholipase Cγ2 (Leoncini et al. 2003) and Src kinase (Luo et al. 2006). The stimulation of this pathway by Hcy requires signals through oxygen free radicals and thromboxane A₂. It is possible that the same mechanism may exist when blood platelets are treated with thiolactone homocysteine.

**Homocysteine, coagulation and fibrinolysis**

Fibrinogen is the main substrate for coagulation cascade and form a polymerized fibrin clot. Fibrinogen is a 340 kDa glycoprotein, consisting of three pairs of nonidentical polypeptide chains, Aα, Bβ and γ, interconnected by disulfide bonds. In the course of blood coagulation, fibrinogen, after the thrombin-induced cleavage of short fibrinopeptides A and B from the amino termini of α and β chains, is converted to fibrin monomers. The monomers
thereafter interact spontaneously and form half–staggered protofibrils flowed by the laterally associated fibers. The initial clot is stabilized by the formation of covalent cross-links in a reaction catalyzed by activated coagulation factor XIII. Fibrinogen and other plasma proteins can be covalently modified by Hcy or its thiolactone. Lysine homocysteinylation is a plausible mechanism for protein modification \textit{in vivo}; and is likely an important pathogenic mechanism. Mass spectrometric analysis of fibrinogen treated with Hcy revealed twelve lysines that were homocysteinylated. Several of these are close to tissue plasminogen activator (tPA) and plasminogen binding sites. Moreover, lysines are major binding sites for fibrinolytic enzymes and are also sites of plasmin cleavage (Sauls et al. 2003, 2005, 2006 and 2007). Sauls et al. (2007) suggest that homocysteinylation of lysine residues in fibrinogen may be linked to three important functional consequences. First, modification in the $\alpha$C domain could alter the lateral association of fibers and thereby alter clot structure. Second, the alteration of the protein conformation may interfere with calcium binding, which could contribute to alterations in fibrin clot structure. Third, modification of lysine sites that are directly involved in fibrinolytic enzyme binding and activity could lead to increased resistance to fibrinolysis – the process by which a fibrin clot is dissolved. Recent reports showed that compounds with thiol groups enhance plasma factor XIII-mediated fibrinogen cross linking. Since Hcy-SH is involved in oxide-reduction reactions or disulfide exchange reactions, it is possible that some components of fibrin formation such as fibrinogen and coagulation factor XIII can be altered. Lauricella et al. (2002 and 2006) observed that clots formed from human plasma incubated \textit{in vitro} with Hcy have been more compact structure, with shorter and more frequently branched fibers, than those formed in the absence of Hcy. Harpel et al. (1992) showed that Hcy enhances the binding of lipoprotein(a) to fibrin, and this results may suggest a biochemical relationship between thiol compound metabolism, thrombosis and atherogenesis.
In mildly hyperhomocysteinemic human subjects dysfibrinogemia (which is characterized by formation of clots composed of abnormally thin, tightly packed fibers with an increased resistance to fibrinolysis) could play a role in the increased risk of atherothrombotic disease (Undas et al. 2006, Souls et al. 2007). On the other hand, Lijfering et al. (2007) showed that the increased risk of cardiovascular diseases in hyperhomocysteinemia is mainly related to elevated coagulation factor VIII levels. Moreover, Ebbesen and Ingerslev (2005) found reduced functional activities of coagulation factors XII, X and II in hyperhomocysteinemia-induced by folate deficiency in rat, whereas the functional factor VII activity was unchanged. Contrary, Al-Obaidi et al. (2000) observed that levels of coagulation factor VIIa and Hcy correlate in plasma of acute coronary syndrome patients. This changes may enhanced risk of thrombotic events in hyperhomocysteinemic patients. Recent reports showed that activity of antithrombin, which is one of the most important inhibitors of blood coagulation, is inhibited by HTL (Gugliucci 2008). Proposed targets of Hcy and HTL action on coagulation and fibrinolysis process are presented in Figure 2.

**Conclusion**

The mechanism of homocysteine and its thiolactone action on haemostasis process is complex and still unclear. Hcy or HTL may modulate the signal transduction in different cells and sometimes act in opposite ways. Homocysteine and its metabolite cause the changes in the level of reactive oxygen species and reactive nitrogen species (special *NO) and may be responsible for the modification of haemostasis induced by these compounds (Fig. 3). The biological significance of haemostatic protein modification (fibrinogen and other coagulation factors) induced by Hcy or HTL is not well known, although especially N-homocysteinylation
induced by HTL may play an important role in different pathophysiological consequences leading to cardiovascular diseases.


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Legend to Figures

**Fig. 1A and B.** The chemical structure of homocysteine (Hcy) and its thiolactone (HTL). The mechanism of N- and S-homocysteinylation of proteins

**Fig. 2.** Proposed targets of homocysteine (Hcy) and its thiolactone (HTL) action on coagulation and fibrinolysis

**Fig. 3.** Proposed model for the regulatory role of Hcy and HTL in vascular wall (smooth muscle, endothelial cells) and in blood platelets. Abbreviations: Hcy – homocysteine, HTL – homocysteine thiolactone, LDL – low density lipoproteins, Hcy-LDL – homocysteinylated LDL, ox-LDL – oxidized LDL, ROS – reactive oxygen species, *NO – nitric oxide, ONOO\(^{-}\) – peroxynitrite, NO-LDL– nitrated LDL, TXA\(_2\) – thromboxane A\(_2\)
Fig. 3

endothelial cells

Hcy/LDL protein nitration ONOO-••• NO

LDL LDL ROS LDL NO-LDL ox-LDL

blood platelets

platelet activation:
↑ aggregation
↑ production of ROS
↑ protein phosphorylation
↑ TXA2 release
↓ generation of •••NO

endothelial cells

Hcy/LDL (+)

(+) (+) (+)

apoptosis of endothelium G1 arrest of endothelium

smooth muscle cells proliferation

TXA2

damage

endothelial cells
Fig. 1(B)

Protein-Lys-ε-NH₂ + Homocysteine thiolactone (HTL)

\[ \text{Protein-Lys-ε-NH₂} + \text{Homocysteine thiolactone (HTL)} \rightarrow \text{N-homocysteinylation} \]

\[ \text{Protein-Lys-ε-NH₂} + \text{Lys-ε-NH-C-CH-CH₂-CH₂-SH} \rightarrow \text{Protein-Lys-ε-N-Hcy} \]
Fig. 1(A)

\[
\text{Cys-SH} + \text{HS-CH}_2\text{-CH}_2\text{-CH-COOH} \rightarrow \text{Protein-SH} \rightarrow \text{S-homocysteinylation} \rightarrow \text{Protein-S-S-Hcy}
\]

- **Cys-SH**
- **Protein-SH**
- **Homocysteine (Hcy)**
- **S-homocysteinylation**
- **Protein-S-S-Hcy**