I. WYBRAŃSKA, B. MISZCZUK-JAMSKA, E. BACZYŃSKA, P. GOLDSZTAJN, *G. SOWA,
*R. PRZEWŁOCKI, **R. GRYGLEWSKI, A. DEMBIŃSKA-KIEĆ

INFLUENCE OF SIN-1 AND SODIUM NITROPRUSSIDE (NANP) ON OX-LDL METABOLISM IN MACROPHAGES

Department of Clinical Biochemistry, Collegium Medicum Jagiellonian University,
Cracow, Poland
*Institute of Pharmacology Polish Academy of Science, Cracow, Poland
**Department of Pharmacology Collegium Medicum Jagiellonian University, Cracow, Poland

Effects of NO-donors (3-morpholinosydnonimine -SIN-1 and sodium nitroprusside NaNP) on the accumulation and degradation of oxidized LDL (ox-LDL) by macrophages were studied. Ox-LDL, but not native-LDL (n-LDL) suppressed the LPS-stimulated biosynthesis of NO by macrophages. SIN-1 at low concentrations $< 100\,\mu M$ was without any effect while SIN-1 at high concentration ($300\,\mu M$) and NaNP (30—$300\,\mu M$) stimulated the accumulation and degradation of ox-LDL by macrophages. The pretreatment of macrophages with N^G-monomethyl-L-arginine (L-NMMA, $3\,\mu M$) for 24 hours had the same stimulatory effect. The inhibition of endogenous formation of NO, by L-NMMA profoundly changed the pattern of action of NO-donors on ox-LDL catabolism by macrophages; the stimulatory action of SIN-1 was transformed to the inhibitory action on the accumulation and degradation of ox-LDL whereas NaNP lost its stimulatory action entirely. Our interpretation of this unexpected interactions between SIN-1, NaNP and L-NMMA is as follows. Endogenous NO in macrophages inhibits the accumulation of ox-LDL and therefore, the stimulatory effect of L-NMMA has been overcome by exogenous NO from SIN-1. However, NO at high concentrations promotes lipid accumulation in macrophages and thereby, in the absence of L-NMMA, SIN-1 at high concentrations and NaNP produced a paradoxical stimulatory effect in macrophages. NaNP is not a proper NO-donor and its mode of action differed from that of SIN-1. In conclusion, NO at low physiological concentrations keeps scavenger receptors of macrophages downregulated and hence endogenous NO may show anti-atherogenic properties.

K e y w o r d s: *Nitric oxide, NOS, NOS-inhibitors, sodium nitroprusside, SIN-1, LDL-
-accumulation/degradation, scavenger receptor, macrophages*

INTRODUCTION

The role of low density lipoprotein (LDL) in the development of atherosclerosis is well established and the recent evidence suggests that the oxidation of LDL and its accumulation by macrophages in the arterial wall is

a key event in this process (1). Several studies have demonstrated the inhibitory effect of native LDL (n-LDL) and oxidized LDL (ox-LDL) on endothelium dependent vasodilation (2—6). Ox-LDL and n-LDL (to a lesser extend) impair the formation of endothelium derived relaxing factor (EDRF) which has been identified as NO (7, 8). Ox-LDL, but not n-LDL have also been reported to inactivate EDRF/NO after its release from endothelial cells (9, 10). The impairment of the biological activity of EDRF/NO may be important for the blood/arterial wall homeostasis, and may favour the development of inappropriate vasoconstriction, platelet activation (11) and development of atherosclerotic plaque.

Biosynthesis of EDRF/NO is not limited to the endothelial cells and generation of NO has been shown in various mammalian tissues including macrophages, neutrophils, Kupfer cells, adrenal tissue, and cerebellum (for review, see 12). NO is synthetised from L-arginine by NO-synthase (NOS) (13) and this synthesis is inhibited by N^G-monomethyl-L-arginine (L-NMMA) (14). Physiological activities of EDRF/NO are mimicked by nitrovasodilators (NO-donors), such as sodium nitroprusside (NaNP) or 3-morpholinosydnonimine (SIN-1), a metabolite of molsidomine (15—17). Endogenous and exogenous NO leads to intracellular accumulation of c-GMP. The accumulation of lipids by macrophages is inhibited by activation of adenylate cyclase e.g. by PGEs (18). Little is known about the effect of stimulations of guanylate cyclase on this process. Presently we have investigated the influence of SIN-1, NaNP and L-NMMA on metabolism of ox-LDL by rat macrophages.

MATERIALS AND METHODS

Macrophages

Monocyte-derived residual macrophages were obtained from Wistar rats by peritoneal lavage with solution of 0.15 M NaCl and 0.5 I.U. heparin/ml. For every series of experiments, peritoneal lavage fluid obtained from 10—15 rats was pooled. Cells were separated by centrifugation at 700 g for 15 minutes and washed three times with 1640-RPMI medium. After suspension of the cells in 1640-RPMI medium containing 10% foetal calf serum (FCS), glutamine (4mM), penicyllin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml), 1 ml aliquots containing minimum 5 mln cells per dish were dispensed into plastic Petri dishes (diameter = 35 mm) and incubated at 37°C and 5% CO_2. After 16 hours of incubation the dishes were extensively washed with medium without foetal calf serum until no non-adherent cells in medium were found upon microscope.

LDL isolation, modification and labelling

Human LDL (density 1.019—1.063 g/ml) were obtained from the pooled plasma of normolipemic subjects and prepared by differential ultracentrifugation (19). After the extensive dialysis against PBS, LDL was oxidized and thereafter labelled with [125]J by the Iodogen method

(20). Oxydation of LDL was performed by incubation with 5μM $CuSO_4$ at 37°C (21). The LDL protein was determined by Lowry method (22). The final specific varied between 50—100 cpm/ng protein.

Preincubation conditions

The monolayer macrophages were incubated in 1640-RPMI medium containing 10% foetal calf serum (FCS), glutamine (4 mM), penicyllin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml) without (control) and with SIN-1 (30—300 μM) or with sodium nitroprusside (NaNP) (30—300 μM) during 18 hours at 37°C in the 5% CO_2 atmosphere.

The preincubation was performed in the presence or absence of N^G-monomethyl-L-arginine (L-NMMA) (3 μM).

Measurement of ^{125}I-ox-LDL accumulation

The cells after preincubation described above undergo a 6 hour incubation at 37°C in the 5% CO_2 atmosphere with 100 μg/ml of ^{125}I-ox-LDL in 1640-RPMI medium containing 10% foetal calf serum (FCS), glutamine (4 mM), penicyllin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml) and with or without the investigated substances. Thereafter the medium was separated and used for the measurement of ox-LDL degradation products. The cell monolayers were washed three times with 1 ml PBS and dissolved in 500 μl of 1 N NaOH. ^{125}I-ox-LDL radioactivity in the cell lysates was determined in LKB-γ-counter. The cell protein was determined according to Lowry method. The resuts are presented as ng of ^{125}I-ox-LDL protein per μg of the cell protein.

Measurement of ^{125}I-ox-LDL degradation

The degradation of ox-LDL was performed according to Goldstein and Brown (24, 25). The TCA and $AgNO_3$ soluble degradation products of ^{125}I-ox-LDL in culture medium were measured in LKB-γ-counter. The results are presented as ng of ^{125}I-ox-LDL protein degradated per 6 hours per μg of the cell protein.

Generation of NO

The generation of NO by the cells alone or in the presence of LPS (100 μg/ml), native LDL (100—300 μg/ml) or in the presence of investigated NO-donors was performed in the culture media according to Griess (23).

Data analysis

All results are presented as mean ± SD. Statistical significance was calculated by the Student's t-test and presented as $p < 0.001$ — ***; $p < 0.01$ — **; $p < 0.05$ — *.
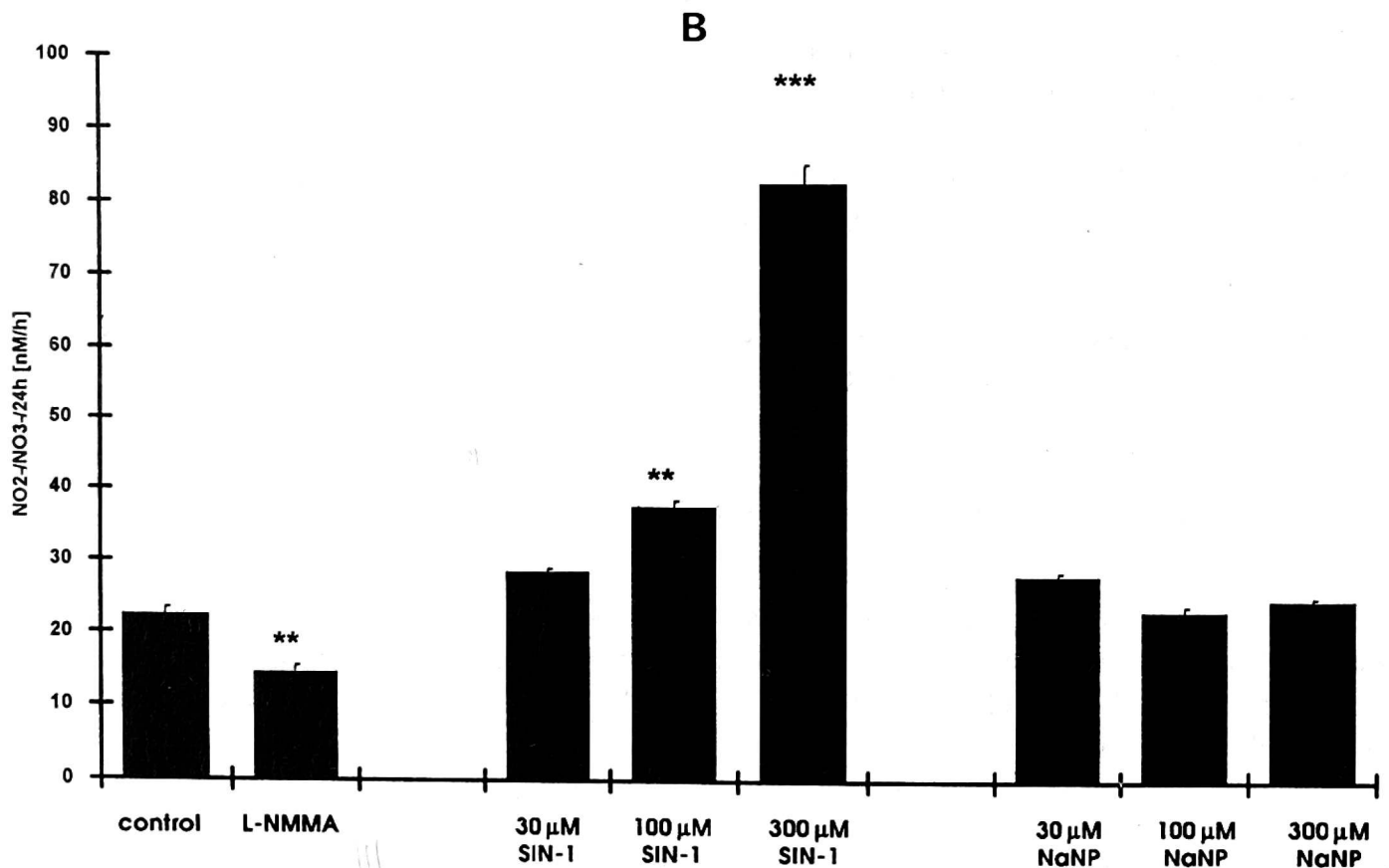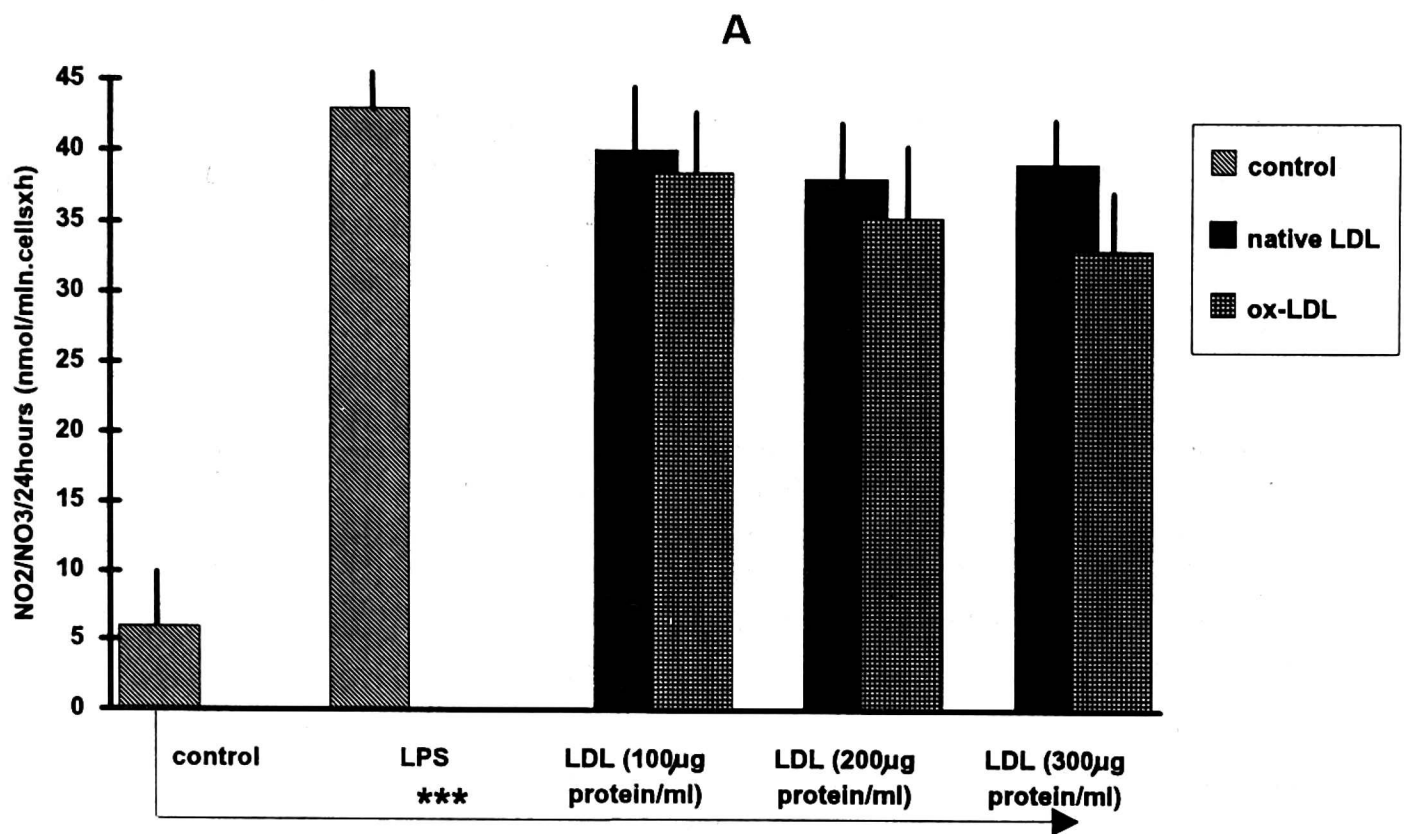
Fig. 1a. Effect of increased concentrations of ox-LDL and n-LDL on NO production by LPS-stimulated rats macrophages. NO (NO2/NO3) was measured according to Griess in medium after 24 hour incubation of cells alone (control), LPS (100 ng/ml) or LPS + n-LDL or LPS + ox-LDL (100—300 μg/ml). All results are presented as mean ± SD $n = 3$—9. Statistical significance: $p < 0.001$ — ***; $p < 0.01$ — **; $p < 0.05$ — * vs. LPS.

Fig. 1b. The amount of NO measured according to Griess in the medium of macrophages co-incubated with NO-donors. Control — 18 hours incubation with 1640-RPMI medium followed by 6 hours with the same medium containing 100 μg/ml [125]l-ox-LDL. L-NMMA (3 μM), SIN-1 (3—300 μM) and NaNP (3—300 μM) were added for 24 hour incubation with cells. All results are presented as mean ± SD $n = 3$—6. Statistical significance: $p < 0.001$ — ***; $p < 0.01$ — **; $p < 0.05$ — * vs. control.

RESULTS

a. Generation of NO as measured by the Griess reaction

In contrast to n-LDL, ox-LDL showed a non-significant tendency to decrease NO generation by the LPS-activated macrophages (*Fig. 1a*).

L-NMMA (3 μM) decreased NO generation in non-stimulated macrophages by 35% (*Fig. 1b*) and then SIN-1 at concentrations of 100 and 300 μM but not NaNP (30—300 μM) increased the release on nitrites to the medium (*Fig. 1b*).

b. Accumulation and degradation of ox-LDL by macrophages

SIN-1 at concentrations of 30 and 100 μM did not influence the accumulation and degradation of ox-LDL (*Fig. 2a, Fig. 3a*). SIN-1 at
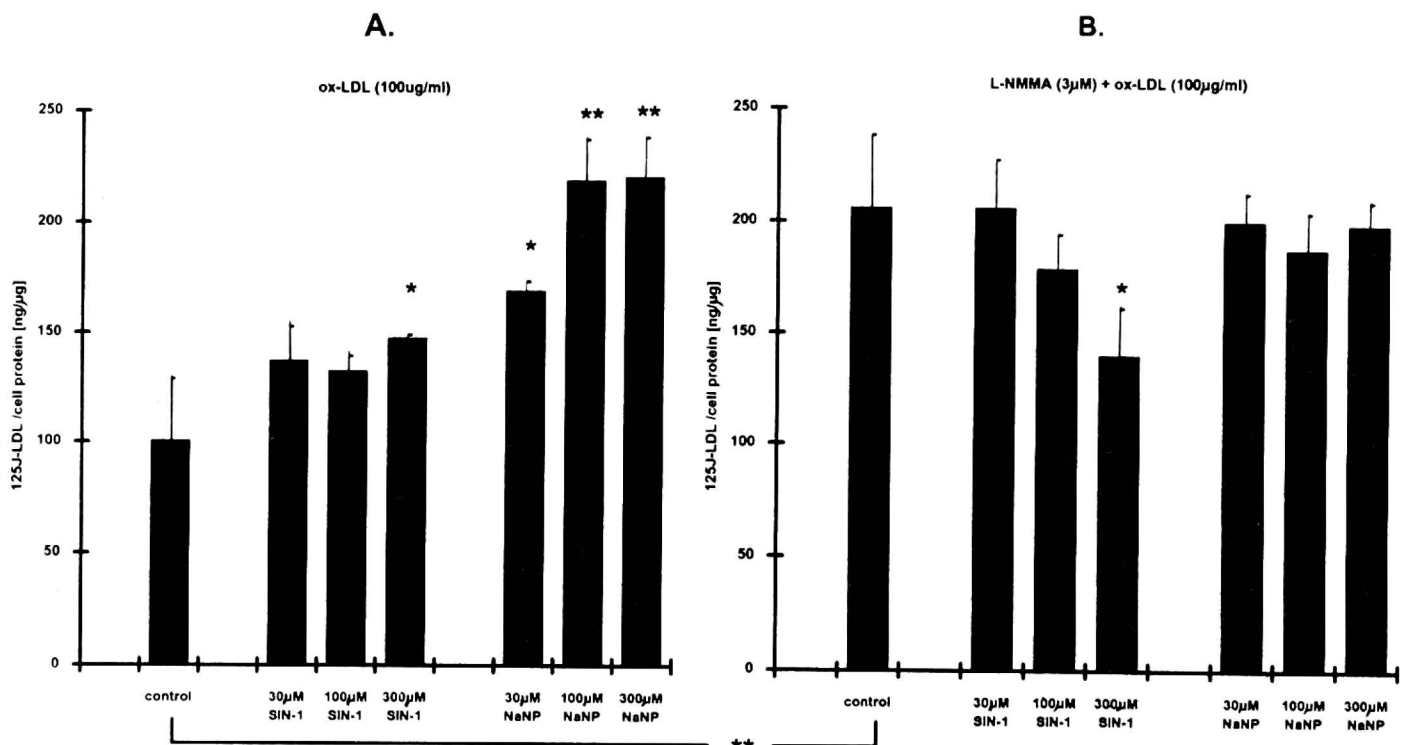

Fig. 2. Effect of SIN-1 and NaNP on ^{125}I-ox-LDL accumulation by macrophages. A. — without, B in the presence of L-NMMA (3 μM). For details, see the legend to *Fig 1b*. Statistical significance: $p < 0.001$ — ***; $p < 0.01$ — **; $p < 0.05$ — * vs. control.

concentrations of 300 μM as well as NaNP at all concentrations used (30—300 μM) increased the accumulation (*Fig. 2a*) and degradation (*Fig. 3a*) of ox-LDL in macrophages. The preincubation of macrophages with L-NMMA (3 μM) significantly increased the accumulation (*Fig. 2b*) and degradation (*Fig. 3b*) of ox-LDL. In the presence of L-NMMA (3 μM), SIN-1 (30—300 μM) dose-dependently inhibited the accumulation (*Fig. 2b*) and degradation (*Fig. 3b*) of ox-LDL, and prevented the NaNP induced potentiation of ox-LDL accumulation by macrophages (*Fig. 2b* and *Fig. 3b*).
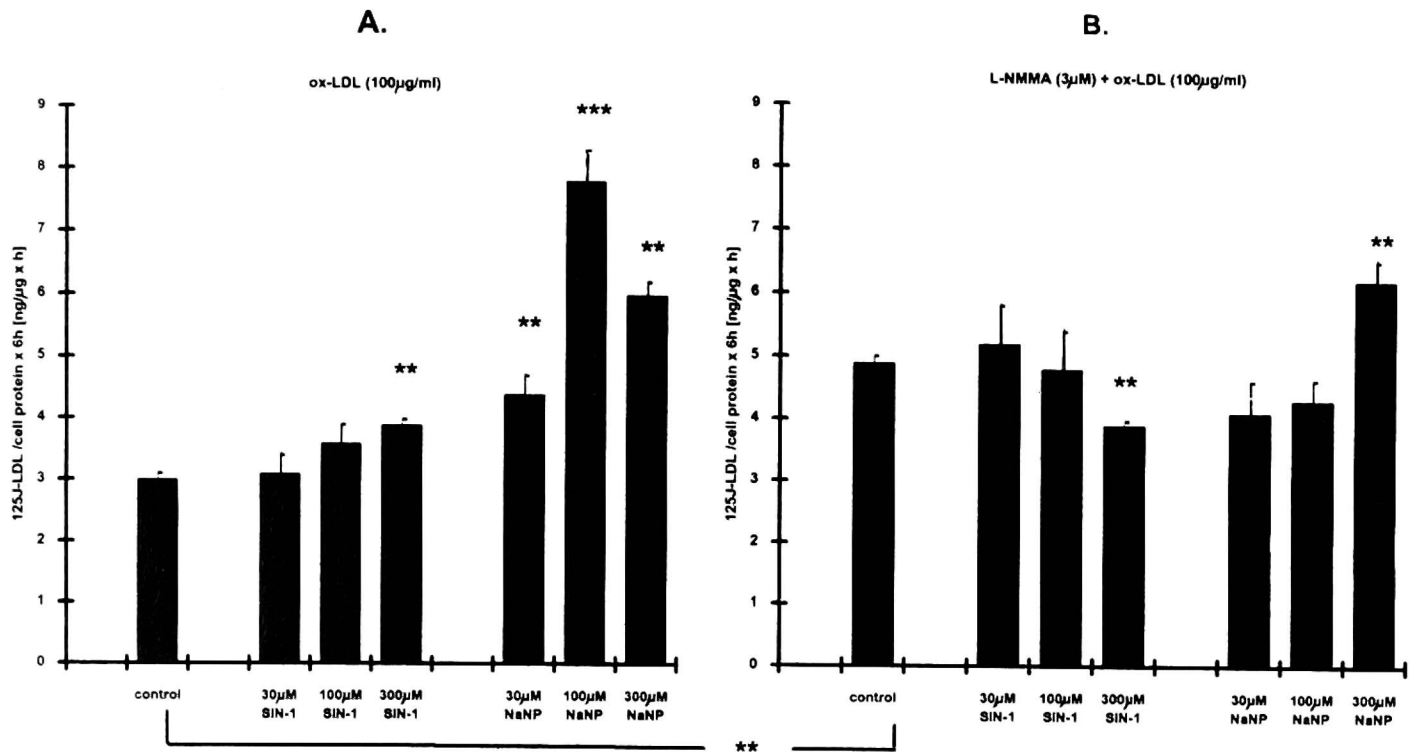
A.

ox-LDL (100µg/ml)

B.

L-NMMA (3µM) + ox-LDL (100µg/ml)

Fig. 3. Effect of SIN-1 and NaNP on ^{125}1-ox-LDL degradation by macrophages. A. — without, B in the presence of L-NMMA (3 µM). For details, see the legend to *Fig 1b*. Statistical significance: $p < 0.001$ — ***; $p < 0.01$ — **; $p < 0.05$ — * vs. control.

DISCUSSION

Our data lead to a conclusion that endogenous NO from macrophages, inhibits the uptake of ox-LDL by these cells. When the biosynthesis of NO is inhibited an exogenous NO from SIN-1 may serve as a replacement for the inhibition of catabolism of ox-LDL by macrophages, however, in intact macrophages which produce their own NO, an additive effect between endogenous NO and exogenous NO from SIN-1 or NaNP may lead to the toxic effects showing up as stimulation of ox-LDL uptake by macrophages.

The rodent macrophages, and macrophage — derived J774 cells (31), as well as human macrophages (32), express the inducible nitric oxide synthase (NOS), which converts L-arginine to NO and citrulline (31, 32). The impaired generation of the constitutive NOS (33, 34), or the increased inactivation of NO (9) by ox-LDL, but not n-LDL in endothelial cells have been recently demonstrated. Also the loading of murine macrophages with ox-LDL resulted in a diminished release of NO, measured by the Griess reaction (35). Here we have also observed a tendency of decreasing of NO generation by ox-LDL, but not by n-LDL in macrophages stimulated with LPS.

We have shown that the decrease of endogenous NO biosynthesis by L-NMMA results in an increase of intracellular accumulation of ox-LDL, suggesting that endogenous NO protects macrophages against loading with ox-LDL.

The mechanism by which NO may participate in the regulation of ox-LDL catabolism remains unknown. Lenten et al (36) demonstrated that LPS, which is a potent activator of NO synthesis by macrophages (31, 32, 35) at concentrations as low as 1 ng/ml selectively prevents the expression of the scavenger receptor activity, and inhibits the ox-LDL binding and accumulation in human monocyte-macrophages. The concentration of LPS as high as 100 ng/ml completely prevented the uptake of ox-LDL but had no effect on n-LDL binding, secretion of apoE, phagocytic activity, or the protein content of monocyte-macrophages (36). In a similar way, Interferon-γ (IFN-γ), a known activator of NO biosynthesis also in macrophages (37), was demonstrated to inhibit the degradation of acetyl-LDL by mouse macrophages (38). The authors (38) observed a slowing movement of internalised acetyl-LDL to the lysosomes and the inhibition of the receptor recycling, which resulted in the decrease in the expression of the scavenger receptors for acetyl-LDL on the cell membrane of macrophages activated with IFN-γ (38). On the other hand, both LPS (39) and IF-γ (37) induce the activation of protein kinase C (PKC), activity of which is necessary for the expression of NOS in macrophages (37).

In other than macrophages cells such as human monocytic leukemic cell line THP-1 and in the human hepatocarcinoma cell line Hep-G2, the accumulation of cAMP as well as the stimulation of PKC resulted in an increase in n-LDL binding and in increase in the cellular n-LDL receptor mRNA (40, 41). The differences in the cell line, and in the receptor character may explain heterogenity of cell behaviour since prostanoids, which accumulate cAMP in macrophages (42, 43) also reduce the number of LDL receptors and sterol synthesis in these cells (44, 45).

In contrast to the abundant information about the influence of adenylate cyclase stimulators on the LDL catabolism in various cell types including macrophages (18), there is hardly any literature available in this respect on guanylate cyclase stimulators, such as endogenous NO or NO-donors (46).

cGMP interacts with three types of intracellular receptor proteins: cGMP-dependent protein kinases, cGMP-regulated ion channels, and cGMP-regulated cyclic nucleotide phosphodiesterases (47). Recently it has been suggested that both cAMP and cGMP are able to cross-activate other kinases in tissues (48). Thus, it well can be that the accumulation of cGMP may promote the cAMP function in macrophages, the effect observed in the synergistic activity of PGI_2 and NO-donors on the inhibition of platelet function (46).

In our experimental model, when the endogenous biosynthesis of NO was suppressed by a NOS inhibitor L-NMMA, SIN-1 decreased the accumulation of ox-LDL by macrophages. However, when the endogenous formation of NO was preserved, SIN-1 and NaNP activated the accumulation of ox-LDL by macrophages. The biphasic effect of NO was described in several experimental models. For example, NO depending on its concentration may inhibit or

394

stimulate the release of noradrenaline from cardiac nerve endings (49), whereas NO-donors may be either cytoprotective or cytotoxic for the granular cells of the cerebellum and retina (50, 51) again depending on concentrations of nitrovasodilators. An increase in the accumulation of n-LDL by human lymphocytes in the presence of SIN-1 and NaNP at high concentrations has also been reported (44) (our results prepared for publication).

Thus, NO or NO-donors at high concentrations seem to induce the adverse effects in macrophages. The NO-mediated apoptosis of macrophages was demonstrated to be prevented by L-NMMA (52), pointing that even in the cells which generate NO, the toxic effect may be observed. In our experimental model the increased accumulation of ox-LDL seems to be related to the stimulation of the cellular cGMP levels, since on the contrary to SIN-1, the presence of NaNP in the medium was not connected with the increase of the NO production measured by the Griess reaction in the medium. NaNP belongs to the unique NO-donors, which due to the presence of NO in their structure, immediately activates the cellular guanylate-cyclase (46). The concentrations used in our experimental model were rather high (comparing with the anti-platelet potency of this compound). Thus the activation of ox-LDL accumulation observed at all concentrations used seems to be related to the accumulation of cGMP. The inhibition of endogenous NO by L-NMMA was sufficient to reverse the effect of this compound on ox-LDL accumulation, that argues for the involvement of the cGMP activation, but not the toxic effect on ox-LDL accumulation by macrophages by NO itself.

We have not observed any additional effects of NO-donors on the ox-LDL degradation by macrophages. The increased accumulation of ox-LDL was followed by the parallel increase in the efflux of metabolised radioactive proteins of ox-LDL from the cells, while the decreased catabolism followed the decreased accumulation. Thus, we conclude that the removal of the metabolised proteins of ox-LDL from macrophages does not seem to be influenced by the investigated compounds.

We conclude that endogenous NO may play a role in preventing of atherosclerosis not only as a platelet suppressor, but also as an inhibitor of the ox-LDL accumulation by macrophages. Thereby, the formation of foam cells may be hindered. In a negative feedback loop ox-LDL show a tendency to impair the generation of endogenous NO by macrophages. NO-donors at low concentrations are substitutes for endogenous NO and thus, they inhibit the accumulation of ox-LDL by macrophages, whereas the overproduction of endogenous NO or overdosage of NO-donors may produce a "paradoxic" effect leading to the accumulation of ox-LDL by macrophages.

REFERENCES

1. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witzum JL. Beyond cholesterol; Modification of low-density lipoprotein that increase its atherogenity. *N Engl J Med* 1989, 320: 915—924.
2. Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M. Low density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. *Nature* 1987; 327: 237—239.
3. Kugiyama K, Kerns SA, Morriset JD, Roberts R, Henry PD. Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low density lipoproteins. *Nature* 1990; 344: 160—162.
4. Vedernikov Y, Lankin V, Tikhaze A, Vikhert A. Lipoproteins as a factors in vessel tone and reactivity modulation. *Basic Res Cardiol* 1988; 83: 590—596.
5. Tomita T, Ezaki M, Miwa M, Nakamura K, Inoue Y. Rapid and reversible inhibition by low density lipoprotein of the endotelium-dependent relaxation to hemostatic substances in porcine coronary arteries: Heat- and acid-labile factors in low density lipoprotein mediate the inhibition. *Circ Res* 1990; 66: 18—27.
6. Simon BC, Cunningham LD, Cohen RA. Oxidized low density lipoproteins cause conraction and inhibit endothelium-dependent relaxation in the pig coronary artery. *J Clin Invest* 1990; 86: 75—79.
7. Furgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylocholine. *Nature* 1980; 288: 373—376.
8. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524—526.
9. Galle J, Mülsch A, Busse R, Bassenge E. Effects of native and oxidized low density lipoproteins on formation and inactivation of endothelium-derived relaxing factor. *Atherosclerosis and trombosis* 1991; 11: 198—203.
10. Chin JH, Azhar S, Hoffman BB. Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. *J Clin Invest* 1992; 89: 10—18.
11. Gryglewski RJ, Botting RM, Vane JR. Mediators produced by the endothelial cell. *Hypertension* 1988; 12: 530—548.
12. Ignarro LJ. Nitric oxide. A novel signal transduction mechanism for transcellular communication. *Hypertension* (Dallas). 1990; 16: 447—483.
13. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthetize nitric oxide from L-arginine *Nature* (London) 1988; 333: 664—666.
14. Palmer RMJ, Moncada S. A novel citrulline forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun* 1989; 158: 348—352.
15. van Overveld FJ, Bult H, Vermiere PA, Herman AG. Nitroprusside, a nitrogen oxide generating drug, inhibits release of histamine and tryptase from human skin mast cells. *Agents Actions* 1993, 38: 337—338.
16. Dembińska-Kieć A. Żmuda A, Marcinkiewicz J, Sinzinger H, Gryglewski RJ. Influence of no-donor (SIN-1) on functions of inflamatory cells. *Agents Actions*, 1991; 1/2 32: 37—40.
17. Pallapies D, Jirman KU, Rademann J et al. Effect of prostaglandin E2 and 3-morpholinosydomine (SIN-1) on arachidonic acid metabolism in fMLP-stimulated rat neutrophils and on thrombin-induced human platelet aggregation. *Agents Actions* 1992, 36: 77—82.
18. Hajjar DP, Pomerantz KB. Signal transduction in atherosclerosis: integration of cytokines and the eicosanoid network. *FASEB J* 1992; 6: 2933—2941.
19. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition by ultracentrifugally separeted lipoproteins in human serum *J Clin Invest* 1958; 34: 1345.

20. Fraker PJ, Speck JC. Protein and cell membrane iodination with a soluble chloramide 1, 3, 5, 6-tetrachloro 3a, 6a-diphenylglycoluril. *Biochem Biophys Res Commun* 1987; 80: 849—857.

21. Steinbrecher UP, Lougheed M, Kwan WC, Dirks M. Recognition of oxidized low density lipoprotein by the scavenger receptor of macrophages results from derivatization of apoprotein B by products of fatty acid peroxidation. *J Biol Chem* 1989; 264: 15216—15223.

22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265.

23. Di Rosa M, Radomski M, Carnuccio R, Moncada S. Glucocortycoids inhibit the induction of nitric oxide synthase in macrophages *Biochem Biophys Res Commun* 1990, 173: 1246.

24. Goldstein J, Brown M. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu Rev Biochem* 1977; 46: 897—930.

25. Goldstein JL, Brown MS, Anderson RGW, Russell DW, Schneider WJ. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu Rev Cell Biol* 1985; 1: 1—39.

26. Ignarro LJ, Endothelium-derived nitric oxide: action and properties. *FASEB J* 1989; 3: 312—336.

27. Schmidt K, Graier WF, Kostner GM, Mayer B, Böhme E, Kukovetz WR. Stimulation of soluble guanylate cyclase by endothelium-derived relaxing factor is antagonised by oxidized low-density lipoprotein. J. Cardiovasc Pharmacol 1991; Suppl. 3: 83—88.

28. Jessup W, Mohr D, Gieseg SP, Dean RT, Stoker R. The participation of nitric oxide in cell free- and its restriction of macrophage-mediated oxidation of low density lipoprotein. *Biochim Biophys Acta* 1992; 1180: 73—82.

29. Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effect of homocysteine are modulated by endothelium-derived relaxing factor and related oxigens nitrogen. *J Clin Invest* 1993; 91: 308—318.

30. Mathur SN, Albright E, Field FJ. Decreased prostaglandin production by cholesterol rich macrophages, *J Lipid Res* 1989; 30: 1385.

31. Di Rosa M, Radomski M, Carnuccio R, Moncada S: Glucocortycoids inhibit the induction of nitric oxide synthase in macrophages *Biochem Biophys Res Commun* 1990; 172: 1246.

32. Sakai N, Milstein S. Aviability of tetrahydrobiopterin is not a factor in the inability to detect nitric oxide production by human macrophages. *Biochem Biophys Res Commun* 1993; 193 (1): 378—383.

33. Jacobs M, Plane F, Bruckdorfer KR. Native and oxidized low-density lipoproteins have different inhibitory effects on endothelium-derived relaxing factor in the rabbit aorta. *Br J Pharmacol* 1990; 100: 21.

34. Tanner FC, Noll G, Boulanger CM, Lüscher TF. Oxidized low density lipoproteins inhibit relaxations of porcine coronary arteries. Role of scavenger receptor and endothelium-derived nitric oxide. *Circulation* 1991; 83: 2012.

35. Jorens PG, Rosseneu M, Devreese AM, Bult H, Marescu B, Herman AG. Diminished capacity to release metabolities of nitric oxide synthase in macrophages loaded with oxidized low-density lipoproteins. *Eur J Pharmac* 1992; 212: 113—115.

36. van Lenten BJ, Fogelman AM, Seager J, Ribi E, Haberland M, Edwards P: Bacterial endotoxin selectively prevents the expression of scavenger — receptor activity on human monocyte-macrophages. *J Immunol* 1985; 6: 3718.

37. Severn A, Wakelam MJ, Liev FY. The role of protein kinase C in the induction of nitric oxide synthesis by murine macrophages. *Biochem Biophys Res Commun* 1992, 188 (3): 997—1002.

38. Frong LG, Frong TAT, Cooper AD. Inhibition of Mouse Macrophage degradation of acetyl-low density lipoprotein by interferon-γ. *J Biol Chem* 1990, 285 (20): 11751—11760.

39. Grove RI, Allegretto NJ, Keiner PA, Warr GA. Liposaccharide (LPS) alters phosphatidylcholine metabolism in elicited peritoneal macrophages; *J Leukocyte Biol* 1990; 48: 38—42.

40. Auverx CH, Chait A, Wolfbauer G, Deeb SS. Involvement of second messengers in regulation of the low-density receptor gene; *Mol Cell Biol* 1989, 9 (6): 2298—2302.

41. Auverx CH, Chait A, Deeb SS. Regulation of the low density lipoprotein receptor and hydroxymethylglutaryl coenzyme A reductase genes by protein kinase C and putative negative regulatory protein. *Natl Acad Sci USA* 1989; 86 (4): 1133—1137.

42. Hijar D, Weksler B, Falcone D, Hefton J, Tack-Goldman K, Minick C. Prostacyclin modulates cholesteryl ester hydrolitic activity by its effect on cyclic adenosine monophosphate in rabbit aortic smooth muscle cells. *J Clin Invest* 1982, 70: 479—488.

43. Koo J, Mahoney E, Steinberg D. Neutral cholesterol esterase activity and its enhancement of c-AMP dependent protein kinase C. *J Biol Chem* 1981; 256: 12659—12661.

44. Krone W, Kaczmarczyk P, Muller-Wieland D, Gretten H. The prostacyclin analog iloprost and prostaglandin E1 supress sterol synthesis in freshly isolated human mononuclear leukocytes. *Biochem Biophys Acta.* 1985; 835: 154—157.

45. Krone W, Klass A, Nagele H, Behnke B, Greten H. Effect of prostaglandins on LDL receptor activity and cholesterol synthesis in freshly isolated human mononuclear leukocytes. *J Lipid Res* 1988; 29: 1663—1669.

46. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology pathophysiology, and pharmacology. *Pharmacol Rev* 43 (2): 109—142.

47. Lincoln TM, Cornwell TL. Intracellular cyclic GMP receptor proteins. *FASEB-J* 1993; 7 (2): 328—338.

48. Jiang H, Shabb JB, Corbin JD. Cross-activation: overriding cAMP/cGMP selectivities of protein kinasesin tissues. *Biochem Cell Biol* 1992; 70 (12): 1283—1289.

49. Feelish M, Bloch W, Addicks K. Control of intraaxonal catecholamine storage in cardiac storage in cardiac symphatetic nerve fibers by endogenous nitric oxide. *J Endothelial Cell Res* 1993; 1 Supp. A 25.

50. Olesen SP, Drejer J. Release of ^{3}H-D-aspartate and ^{3}H-GABA is inhibited by nitric oxide. *J Endothelial Cell Res* 1993; 1: Suppl. A 24.

51. Haberecht MF, Redbum DA, Nakane M, Schmidt HW. Immunocytochemical and histochemical localisation of nitric oxide and soluble guanyl cyclase in rabbit aorta. *J Endothelial Cell Res* 1993; 1: Suppl A 29.

52. Albina JE, Cui S, Matheo RB, Reichner JS. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *J Immunol* 1993; 150 (11): 5080—5085.

Author's address: A. Dembińska-Kieć, Department of Clinical Biochemistry, Collegium Medicum Jagiellonian University, 15A Kopernika str, 31-501 Cracow, Poland