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VASCULAR CELLS UNDER PEROXIDE INDUCED OXIDATIVE STRESS: A BALANCE STUDY ON *IN VITRO* PEROXIDE HANDLING BY VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Enzymes such as glutathione peroxidase and catalase play an important role in the cellular defence against (per)oxidative stress. Balance- and inhibitor-studies were undertaken with *in vitro* cultured human vascular endothelial cells (EC) and smooth muscle cells (SMC) to assay the relative importance of these enzymes in the handling of cumene hydroperoxide (Chp) and hydrogen peroxide (H_2O_2). Low concentrations of Chp (up to 80 μM) could be removed to near completion within the first hour of incubation by stimulation of the hexose monophosphate shunt (HMS) of both cell types. The HMS activity reached a plateau upon incubation with higher concentrations of Chp ($>80\,\mu$M). The non-converted Chp in the higher concentrations could be detected quantitatively in the incubation solution. After inhibition of the glutathione reductase by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), incubation with Chp (40 μM) did not result in a stimulation of the HMS activity. Moreover the added Chp could be recovered from the medium. So Chp is exclusively handled by the GSH-redox cycle. When low concentrations of H_2O_2 (up to 80 μM) were added to EC or SMC approximately 50% of the peroxide loss cound not be accounted for. Inhibitor studies with aminotriazole proved that catalase was responsible for the handling of this unaccounted H_2O_2. In both ECs and SMCs at lower concentrations of H_2O_2 the GSH-redox cycle was as effective as catalase and at higher H_2O_2 concentrations the catalase pathway plays the major role.

KEY WORDS: Cumene hydroperoxide, hydrogen peroxide, endothelial cell, smooth muscle cell, hexose monophosphate shunt, catalase, glutathione.

INTRODUCTION

The vascular endothelium is constantly exposed to reactive oxygen species generated from blood cells such as neutrophils (PMNs), platelets and monocytes. The reaction of reactive oxygen with membrane lipids of the endothelial cells (ECs) may have a damaging effect on this celltype which lines the vessel wall.[1] In the subendothelial space the production of reactive oxygen compounds by activated cell types can also have an effect on the smooth muscle cells (SMCs) of the inner media. ECs and SMCs however are not passive in this process of peroxidation. They contain endogenous antioxidant defence systems such as the glutathione peroxidase-reductase system (GSH-redox cycle) and catalase. Many studies have been done using specific enzyme inhibitors of these pathways to study cell-lysis under exogenous peroxidative stress.[2,3,4,5,6,7] These studies indicated a major role of the GSH-redox cycle in the

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detoxification of exogenous peroxides in human endothelium. One study pointed out that ECs from different species and different sites may utilize diversified antioxidant protective mechanisms.[6]

In an earlier study using low concentrations of cumene hydroperoxide (Chp) we could confirm the activation of the GSH-redox cycle to inactivate the organic peroxide in ECs.[8] In the present balance study we investigated quantitatively the handling of various concentrations of Chp and H_2O_2 by the defence systems of the ECs and SMCs.

ABBREVIATION LIST

AT	3-Amino-1,2,4-triazole
Chp	Cumene hydroperoxide
DTNB	5,5'-Dithiobis-(2-nitrobenzoic acid)
EC	Endothelial cells
FCS	Fetal calf serum
GSH	Glutathione
HMS	Hexose monophosphate shunt
LDL	Low density lipoprotein
NADPH	Nicotinamide-adenine-dinucleotide phosphate, reduced
PMN	Polymorphonuclear leucocyte
SMC	Smooth muscle cells
BCNU	1,3-bis(2-chloroethyl)-1-nitrosurea

MATERIALS AND METHODS

Cells, culture and peroxide incubation

Endothelial cells were isolated from human umbilical veins by collagenase digestion according to the method of Jaffe.[9] The cells were plated in culture flasks precoated with fibronectin (10 $\mu g/cm^2$) and grown in culture medium.[8] The endothelial cell strains were subcultivated by 1:5 splits when the culture reached confluency.

Smooth muscle cells were isolated from human umbilical artery. Pieces of arterial wall (3–4 mm) were prepared free and were cultured in a fibronectin coated 24 well plate (Costar) in medium M199 supplemented with 10% FCS, 10% human serum, 100 I.U./ml penicillin, 0.10 mg/ml streptomycin. The muscle cells were positively identified with a monoclonal antibody against smooth muscle α-actin (Sigma clone no 1 A4) and subcultivated by 1:3 splits when the culture reached confluency.

Endothelial and smooth muscle cells were used before passage 4. The confluent cell cultures were incubated with the peroxide-dilutions made in M199 medium without serum and phenol red to avoid interference with the iodometric peroxide determination.

Inhibitor studies

The catalase-inhibitor 3-amino-1,2,4-triazole (AT; Sigma) was added to the cells at 50 mM concentration 16 h before addition of peroxides.[6] In separate experiments the efficiency of AT in inhibiting cellular catalase was tested. AT treated cells were

homogenized and incubated with H_2O_2 and the remaining peroxide was measured after one hour incubation using an iodometric peroxide assay.[11]

Glutathione reductase was inhibited by preincubation with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; Bristol-Myers Co.; 375 μg/ml) for 20 minutes in medium without serum at 37°C.[7]

Assay for hexose monophosphate shunt activity

The flux of glucose through the hexose monophosphate shunt (HMS) during treatment with Cumene hydroperoxide (Chp) or H_2O_2 was monitored by assaying $^{14}CO_2$ formation from 1-^{14}C- or 6-^{14}C-glucose. Cells were grown to confluency in 16 mm wells (approx. 10^5 cells) of a 24 cluster dish (Costar 3524). The medium was then replaced by 0.5 ml M199 supplemented with Chp or H_2O_2, but without serum and phenol red. To each well 0.5 μCi/ml of radioactive D-1-^{14}C-glucose or D-6-^{14}C-glucose (spec. act/58.5 mCi/mmol: Amersham, England) was added. The $^{14}CO_2$ released was collected and counted in a scintillation counter.[8] The formation of NADPH through the HMS was calculated by subtracting the $^{14}CO_2$ liberated from the 6-^{14}C-glucose from the carbon dioxide obtained from the 1-^{14}C-glucose according to Reitzer[10] and multiplying this value by 2.

Peroxide determinations

The peroxides remaining in the solution after incubation with ECs and SMCs were determined using a iodometric peroxide assay.[11]

The peroxide content of the H_2O_2 stock solution (Merck; 30%) was checked by titration with $KMnO_4$.[12]

The Chp content of the stock solution (80%; Sigma) was determined using glutathione peroxidase (10 U/ml; Calbiochem) which catalyses the reduction of hydroperoxides with glutathione as the reductant. To 600 μl of a phosphate buffered saline solution, containing 60 nmol GSH and up to 20 nmol Chp, 10 μl of glutathione peroxidase is added. The mixture is allowed to stand at room temperature for 45 min. After incubation 300 μl of a DTNB reagent is added (150 mM phosphate buffer pH 7.2; 0.04% bovine serum albumin; 15 mM EDTA; 0.3 mM DTNB (Sigma) to measure the GSH remaining in the solution using the molar extinction coefficient of reduced DTNB ($\varepsilon = 13600$) at 412 nm to calculate the remaining GSH.[13] The commercial Chp solution was calculated to be 75.6% w/w.

Protein determination

Protein was determined with a micromethod using the fluorescamine reagent.[14]

Tests on cellular integrity

The permeability of cells to propidium iodide (5 μg/ml) after exposure to peroxides was compared with that of control cells without peroxide treatment. The percentage of propidium iodide negative cells was determined flowcytophotometrically.[15]

TABLE I
Cumene hydroperoxide (Chp) handling by intact endothelial cells (EC) and smooth muscle cells (SMC)

	nmoles Chp added					
	10	20	40	80	160	320
EC						
NADPH[a]	10.8 ± 0.71	18.2 ± 0.49	32.9 ± 1.61	36.3 ± 2.26	40.5 ± 4.42	38.4 ± 4.49
Rest[b]	0.2 ± 0.26	1.6 ± 0.61	7.0 ± 0.38	43.3 ± 6.00	117.1 ± 5.33	267.2 ± 13.79
% Recovery	110 ± 6.9	99 ± 3.9	99 ± 4.5	99 ± 6.1	99 ± 5.7	95 ± 5.1
SMC						
NADPH	7.9 ± 1.45	15.3 ± 2.03	32.5 ± 3.03	39.8 ± 2.23	34.2 ± 3.14	29.5 ± 4.53
Rest	0.9 ± 0.66	2.4 ± 0.88	4.9 ± 2.16	37.1 ± 1.99	119.6 ± 5.37	287.0 ± 4.22
% Recovery	89 ± 14.1	89 ± 6.9	93 ± 5.7	96 ± 1.4	96 ± 1.4	99 ± 0.4

[a]Stimulation of NADPH production (nmoles/well/hr) of HMS by Chp addition.
[b]Remaining peroxides (nmoles/well) in culture fluid after 1 hr incubation.
[c]Mean ± sem; n = 4.

RESULTS

Balance studies with Chp

Addition of 20 nmol of Chp (40 μM final concentration) to a culture of endothelial cells (mean protein: 53.9 μg/well; mean ($\pm$SD) HMS activity; 61.9 $\pm$ 20.3 nmoles NADPH·mg protein^{-1}·hour^{-1}) resulted in an increase in HMS activity during 1 hour incubation of 9.1 nmol of CO_2. This amount of carbon dioxide generated by the HMS is equivalent to 18.2 nmol NADPH produced by the HMS. The amount of peroxide remaining in the solution was 1.6 nmol, so the balance was complete (Table I; recovery 99%). Similar experiments were performed after pre-incubation with the glutathione reductase inhibitor BCNU. This resulted in a near complete inhibition of the HMS stimulation by Chp (Table II). These experiments show that, within one hour, low concentrations of Chp (up to 80 μM) are exclusively handled by the GSH-redox cycle. Higher amounts of Chp (up to 320 nmol/0.5 ml) resulted in a maximum stimulation in HMS activity of 40.5 nmol NADPH per well during 1 hour of incubation. The unmetabolized peroxides could be recovered quantitatively in the incubation solution (Table I). So in endothelial cells also at higher concentrations of Chp, the GSH-redox cycle is the only pathway for the cell to reduce the Chp added.

Vascular smooth muscle cells (mean protein: 78.8 μg/well ($\pm$SD) HMS activity: 45.6 $\pm$ 10.24 nmoles NADPH·mg protein^{-1}·hour^{-1}) showed a similar behaviour to endothelial cells (Table I). At low concentrations of Chp the HMS could reduce almost all the peroxides added within one hour. At higher concentrations the balance was still complete. A maximum stimulation of the HMS activity was reached at 39.8 nmol NADPH per well during one hour of incubation.

The maximum specific stimulation of the HMS amounted to 751 nmoles NADPH·protein^{-1}·hour^{-1} in ECs and 509 nmoles NADPH·protein^{-1}·hour^{-1} in SMCs, which was 68% of the maximum stimulation of endothelial cells (Fig. 1).

After incubation of ECs with 640 μM Chp, ECs were washed, homogenized and intracellular peroxides determined with the iodometric assay. There was only a neglectable amount of peroxide present (< 0.17 nmol peroxide/well; n = 4).

TABLE II
Cumene hydroperoxide[a] (Chp) handling by endothelial cells after BCNU treatment

	− BCNU	+ BCNU[b]
NADPH[c]	16.4 $\pm$ 0.41[e]	0.5 $\pm$ 0.13
Rest[d]	2.6 $\pm$ 0.79	18.7 $\pm$ 0.84
% Recovery	92 $\pm$ 3.3	96 $\pm$ 4.2

[a] 20 nmol Chp/well (0.5 ml).
[b] 375 μg/ml BCNU for 20 minutes at 37°C.
[c] Stimulation of NADPH production (nmoles/well.hr) of HMS by Chp addition.
[d] Remaining peroxides (nmoles/well) in culture fluid after 1 hr incubation.
[e] Mean $\pm$ sem; n = 4.

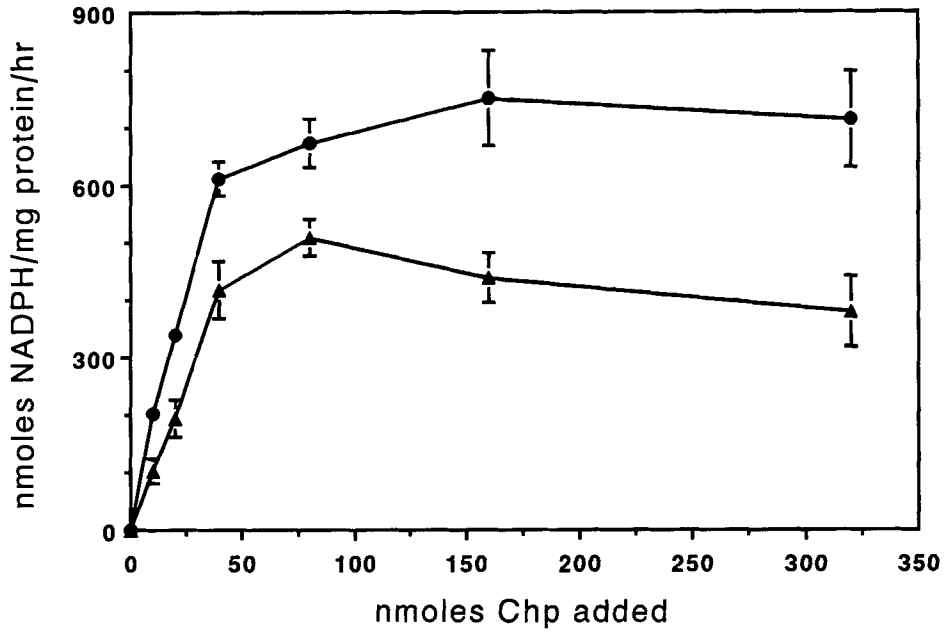

FIGURE 1 Stimulation of the glucose flux through the hexose monophosphate shunt in intact cells subjected to increasing amounts of Chp: (●) endothelial cells; (▲) smooth muscle cells. Values are the mean ± sem of four experiments using four different cell strains.

Balance studies with H_2O_2

During one hour incubation with 40 μM H_2O_2 (20 nmol/0.5 ml) there was an extra CO_2 generation of 5.7 nmol by the endothelial HMS, which is equivalent to 11.4 nmol NADPH. The H_2O_2 remaining in the solution amounted to 0.1 nmol, so there was an unaccounted deficit of 8.6 nmol (Table III: −AT; deficit).

Increasing concentrations of H_2O_2 resulted in an initial increase followed by a decrease in HMS activity at higher H_2O_2 concentrations (Table III: −AT, NADPH). The peroxide remaining in the solution steadily increased at higher concentration together with the unaccounted deficit. In order to account for the missing peroxide in the balance (Table III: deficit) we incubated the cells with the catalase inhibitor aminotriazole (Table III: +AT). Overnight incubation of intact cells with 50 mM AT resulted in a more than 90% inhibition of catalase activity in cellular homogenates (EC: 93 ± 2.1%; SMC: 96 ± 1.4%; n = 3). A comparison between AT treated and non-treated cells indicates that this inhibition of intracellular catalase caused an increase of peroxide remaining in the incubation solution which resulted in an increase in recovery. So the deficit in the balance (Table III: −AT; deficit) could be attributed to enzymatic peroxide removal by catalase.

Table III indicates that at low concentrations of H_2O_2 (up to 80 μM), the HMS is as effective as catalase in the removal of H_2O_2. At high concentrations of H_2O_2 (80–640 μM) the removal by catalase is the most important process.

The balance study of the reaction of SMC to H_2O_2 was comparable to that using EC (Table III). In the low H_2O_2 concentrations the HMS pathway was as effective as catalase, while at higher concentration the catalase removed the bulk of the H_2O_2.

TABLE III
H_2O_2 Handling by intact endothelial cells (EC) and smooth muscle cells (SMC)

			nmoles H_2O_2 added					
			10	20	40	80	160	320
EC	−AT	NADPH[a]	5.5 ± 0.76[c]	11.4 ± 0.89	17.3 ± 1.90	13.3 ± 4.18	7.2 ± 2.19	4.8 ± 1.69
		Rest[b]	0.4 ± 0.35	0.1 ± 0.06	3.1 ± 3.12	8.9 ± 3.1	45.5 ± 8.52	101.8 ± 16.73
		Deficit[d]	4.1 ± 0.64	8.6 ± 0.93	19.5 ± 4.25	57.8 ± 2.67	107.3 ± 7.26	213.3 ± 15.36
		% Recovery	58 ± 6.3	57 ± 4.7	51 ± 10.6	28 ± 3.3	33 ± 4.5	33 ± 4.8
	+AT[e]	NADPH	6.3 ± 0.47	12.1 ± 0.76	24.5 ± 1.87	13.9 ± 0.64	7.8 ± 0.43	4.3 ± 1.43
		Rest	3.1 ± 1.28	6.8 ± 2.67	17.9 ± 6.81	30.3 ± 12.06	125.7 ± 11.43	282.3 ± 15.29
		Deficit	0.9 ± 0.62	2.3 ± 2.23	2.6 ± 2.47	30.3 ± 12.07	27.6 ± 10.76	33.9 ± 15.69
		% Recovery	94 ± 8.2	95 ± 13.6	106 ± 12.6	63 ± 15.4	84 ± 7.4	90 ± 5.0
SMC	−AT	NADPH	7.5 ± 1.57	11.1 ± 1.89	19.1 ± 0.95	26.6 ± 4.17	25.1 ± 3.05	16.2 ± 1.58
		Rest	1.3 ± 0.49	2.7 ± 0.39	4.4 ± 1.82	21.9 ± 7.06	46.4 ± 11.3	101.9 ± 21.52
		Deficit	2.0 ± 1.31	6.2 ± 2.28	16.5 ± 2.69	31.6 ± 2.97	88.5 ± 8.32	201.9 ± 20.09
		% Recovery	88 ± 17.4	69 ± 11.3	59 ± 6.8	60 ± 3.5	45 ± 5.4	37 ± 6.2
	+AT	NADPH	7.7 ± 1.24	11.6 ± 1.56	22.1 ± 1.11	27.5 ± 4.82	9.9 ± 1.19	2.4 ± 1.26
		Rest	1.9 ± 0.58	4.3 ± 0.89	8.2 ± 2.51	41.5 ± 10.89	123.7 ± 8.15	264.6 ± 12.84
		Deficit	1.5 ± 0.89	4.2 ± 2.37	10.1 ± 4.45	11.0 ± 6.10	26.3 ± 8.94	53.1 ± 12.43
		% Recovery	97 ± 16.6	79 ± 12.3	86 ± 7.6	86 ± 7.6	84 ± 5.6	84 ± 3.8

[a]Stimulation of NADPH production (nmoles/well/hr) of HMS by H_2O_2 addition.
[b]Remaining peroxides (nmoles/well) in culture medium after 1 hr incubation.
[c]Mean ± sem; n = 4.
[d]Unaccounted deficit; nmoles/well/hr.
[e]AT: 3-Amino-1,2,4-triazole; 50 mM for 16 hr.

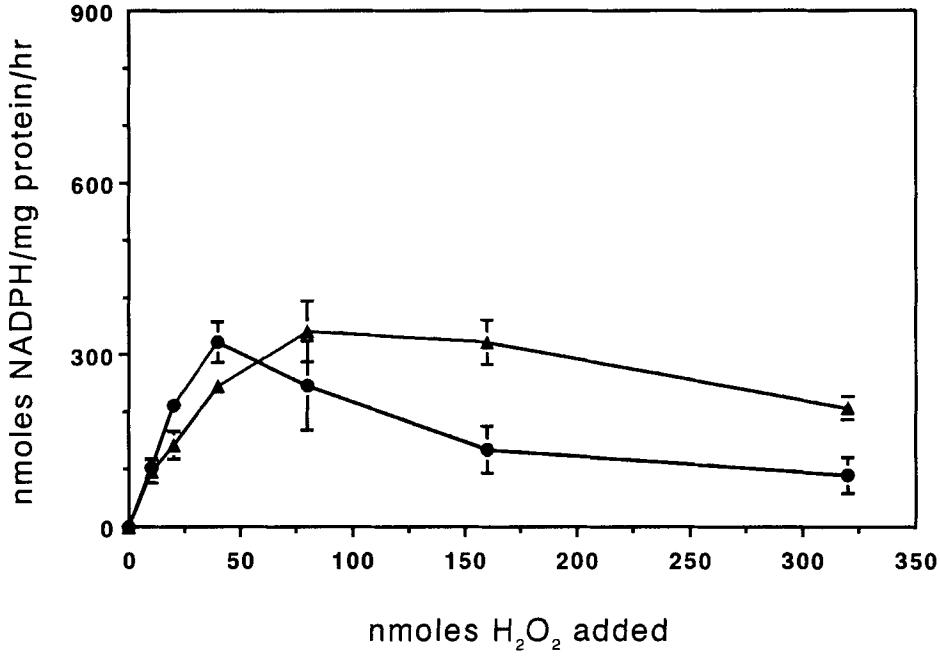

FIGURE 2 Stimulation of the glucose flux through the hexose monophosphate shunt in intact cells subjected to increasing amounts of H_2O_2: (●) endothelial cells; (▲) smooth muscle cells. Values are the mean ± sem of four experiments using four different cell strains.

The maximum specific stimulation of the HMS using H_2O_2 amounted to 322 nmoles NADPH·protein^{-1}·hour^{-1} in ECs and 341 nmoles NADPH·protein^{-1}·hour^{-1} in SMCs (Fig. 2). This stimulation of the HMS activity was much lower than when using Chp (Figs. 1, 2).

Cellular peroxides were determined after incubation with 640 μM H_2O_2 in the presence and absence of AT. After washing and homogenation the intracellular peroxides amounted to 0.06 nmol in the absence of AT and 0.17 nmol/well in the presence of AT ($n = 4$).

Since the balance in experiments with the inhibitor AT is nearly complete (Table III: +AT), we can equalize the deficit in experiments without AT to catalase activity in intact cells. The plot of specific removal of H_2O_2 by catalase against the added H_2O_2 (Fig. 3) indicates, that the stimulation of catalase handling by ECs is higher compared to SMCs.

Cell integrity after peroxide treatment

The permeability of the plasma membrane after one hour treatment in different concentrations of peroxides was tested with propidium iodide (Table IV). After incubation with Chp, the percentage of ECs that were impermeable to propidium iodide decreased slightly from 97 ± 1.47% (control) to 89 ± 2.9% (640 μM). In all other conditions the integrity of the plasma membrane did not change upon incubation with peroxides (Table IV).

TABLE IV
Cell integrity after peroxide treatment[a]

Peroxide	Endothelial cells		Smooth muscle cells	
	Cumene hydroperoxide	H_2O_2	Cumene hydroperoxide	H_2O_2
0 μM	97 ± 1.4	96 ± 1.3	90 ± 0.9	94 ± 1.4
40	95 ± 1.7	95 ± 2.0	87 ± 0.3	92 ± 1.7
80	92 ± 1.9	97 ± 1.3	87 ± 0.9	91 ± 1.0
160	89 ± 2.5[b]	95 ± 1.0	85 ± 1.7[b]	90 ± 1.2
320	87 ± 2.9[b]	96 ± 1.3	85 ± 2.3	90 ± 1.3
640	89 ± 2.9[b]	94 ± 1.4	87 ± 2.3	90 ± 1.4

[a] Percentage of propidium iodide negative cells after 1 hour peroxide incubation (mean ± sem; n = 4).
[b] $p < 0.05$ compared to control (0 μM); student t test.

DISCUSSION

In this study we quantified the major pathways through which hydroperoxides are metabolized in *intact* vascular endothelial and smooth muscle cells. Balance studies were performed with increasing concentrations of Chp and H_2O_2.

Upon incubation with Chp we found up to a 12.1 fold stimulation of the specific HMS activity in ECs and a 11.1 fold stimulation in SMCs. The NADPH produced by the HMS together with the amount of the remaining Chp in the medium, was equal to the amount of Chp added to the cells (Table I). Inhibition of the glutathione reductase by BCNU in ECs resulted in a near complete inhibition of the HMS stimulation in the presence of Chp. Again a complete balance was found (Table II). These experiments show that the extra amount of NADPH formed, by stimulation of the HMS after addition of Chp, is exclusively consumed by the GSH-redox cycle. Since cell integrity (Table IV) is only slightly diminished in ECs and not in SMCs, the balance sheet is not affected by cell viability. The balance is not influenced by the formation of cellular peroxides since they did not show up in the assay even at high concentrations of Chp or H_2O_2.

Handling of H_2O_2 by EC and SMC resulted also in an increase in specific HMS activity (maximum for EC: 5.2 fold; SMC: 7.5 fold). The contribution of intracellular catalase to the removal of H_2O_2 was studied using AT as an inhibitor for this enzyme. After addition of the catalase inhibitor a nearly complete balance was obtained (Table III). The small deficit in the balance with AT can be attributed to the incomplete inhibition of catalase by AT in our experiments. So the unaccounted deficit in the experiments in the absence of AT could completely be attributed to removal by catalase.

The activation of the HMS by H_2O_2 is not as high as the activation by Chp (Figs. 1, 2). This difference in activation could be caused by the fact that H_2O_2 is metabolized primarily by catalase, so a low concentration is left over for the GSH-redox cycle to handle. Addition of the catalase inhibitor AT however did not lead to an increase in HMS activity, so this explanation is not valid. Since the cellular integrity does not change upon incubation with either peroxide, the difference in maximum rate of the GSH-redox cycle probable could be attributed to a different inactivation.

Consistent with this explanation is the fact that only at increasing test concentrations of H_2O_2 is the activity of the GSH-redox cycle decreasing (Table III).

The result of our study about H_2O_2 handling in *intact* vascular ECs and SMCs agree with the work of Cohen and Hochstein[21] in *intact* erythrocytes. They found that glutathione peroxidase is primarily responsible for the elimination of low concentrations and that catalase is important in handling higher levels of H_2O_2.

Little is known about the actual peroxide load of the vascular wall. From the luminal site the H_2O_2 delivered by activated polymorphonuclear leucocytes (PMN) can be of importance.[1] Quantitative data indicate that approximately 1 nmol H_2O_2 is produced by 10^6 activated PMNs/min during the initial linear phase of activation.[3] So local accumulation of 10^6 activated PMNs on endothelial cells will produce maximally 60 nmol per hour. In our experiments, this is the amount of H_2O_2 which could easily be removed by approx. 10^5 endothelial cells, partially by the HMS and partially by catalase (Fig. 3a). When a local production of H_2O_2 should occur in the subendothelium, both endothelial cells and smooth muscle cells easily could manage to reduce the H_2O_2.

The damage done by lipid peroxides on the vessel wall is more difficult to assess. The hydroperoxyeisosatetraenoic acids (HPETEs) from platelets, PMNs, basophils and T-lymphocytes probably never will be excreted, but will be reduced intracellularly by glutathione peroxidase to the hydroxy derivatives as in the case of 12 HPETE in platelets.[16] Another candidate for external but also subendothelial lipid peroxide damage are the peroxidized lipids of the oxidized LDL particle (ox-LDL). The

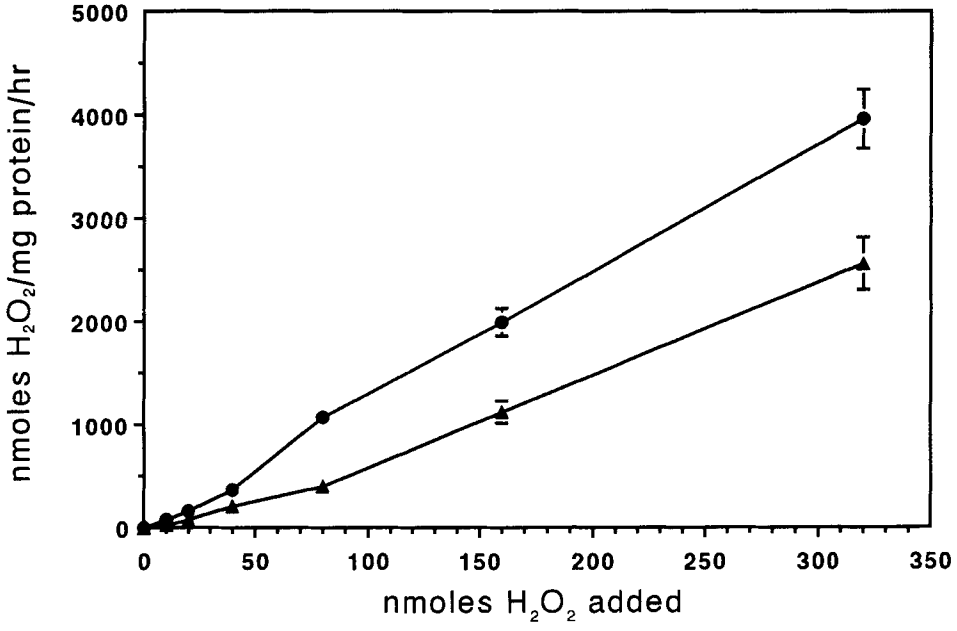

FIGURE 3 Stimulation of H_2O_2 removal by catalase in intact endothelial cells (●) and smooth muscle cells (▲) with increasing amounts of H_2O_2. Values are the mean ± sem of four experiments using four different cell strains. See results section for details of calculation.

toxicity of Ox-LDL is above question (for a review see 17) and recent experiments indicate that LDL isolated from atherosclerotic lesions resembles ox-LDL in its physical, chemical and its biological properties.[18] Immunological studies indicate that at least a portion of the LDL found in atherosclerotic lesions has undergone oxidative modification.[19] Recent experiments indicate that also β-VLDL, with its peroxidized lipids, can be a candidate for oxidative damage of endothelial cells.[20] Whether or not endothelial cells can cope with this challenge by means of glutathione peroxidase and/or catalase is under study.

In an earlier study, the peroxide load due to serum factors was estimated in patients with hyperlipoproteinaemia, by measuring the HMS stimulation in endothelial cells.[8] There was a significant increase in HMS activity by the plasma of these patients, corresponding to 5 nmol Chp/0.5 ml. This amount can easily be handled by the GSH-HMS system in a static system. It is however possible that in the actual bloodvessel, with a flowing content, this peroxide load is permanent and results in a constant low concentration peroxide stress of the endothelial layer.

Although the cellular defence mechanisms against peroxide induced oxidative stress are able to cope with fair amounts of peroxides within one hour, there is evidence that exposure to low concentrations of hydroperoxides can cause both functional changes and DNA damage in ECs. For example incubation with 40 μM Chp evoked an increase in monocyte adherence to human umbilical vein endothelial cells,[22] probably caused by an increased exposure of P-Selectin.[23] Also DNA strand breakage was already detected after 15 minutes of exposure to 50 μM of hydrogen peroxide in bovine pulmonary and aortic endothelial cells.[24]

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