Free Rad. Res. Comms., Vol. 11, Nos. 1–3, pp. 89–97
Reprints available directly from the publisher
Photocopying permitted by license only

ENZYMIC PATHWAYS INVOLVED IN CELL RESPONSE TO H_2O_2

EVA M. LINK

Department of Chemical Pathology, Academic Unit, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, UK

(Received December 15, 1989; in final form, April 2, 1990)

An influence of possible interaction of glutathione peroxidase and cyclooxygenase on the clonogenic survival of epithelial cells exposed *in vitro* to H_2O_2 was investigated. Indomethacin served as the inhibitor of cyclooxygenase, and the use of alkaline (7.5) or acidic (6.5) pH combined with controlled supply of glucose modified glutathione peroxidase activity. Indomethacin affected survival of cells exposed to H_2O_2 in a biphasic manner, enhancing cytotoxicity at lower hydrogen peroxide concentrations, and diminishing it at higher concentrations. The turning point moved gradually to higher concentrations of H_2O_2 corresponding to the augmented decomposition of hydrogen peroxide caused by increased activity of glutathione peroxidase. The data revealed that both enzymic pathways interact in the presence of H_2O_2, resulting in the overall cell survival different from that obtained after inhibition of either.

KEY WORDS: Hydrogen peroxide cytotoxicity, glutathione peroxidase, cyclooxygenase, indomethacin, enzymic pathway interaction.

INTRODUCTION

Detection of hydrogen peroxide generated by common exogenous agents such as ultraviolet and ionizing radiations, as well as its occurence in several pathological states including inflammation, prompted investigations concerning a mechanism(s) involved in cytotoxic effects of H_2O_2. Some of disturbances in enzyme activities[1,2] or even in whole biochemical pathways[2–4] and structural changes including perturbations of the cytoskeleton and plasma membrane[1,5] caused by hydrogen peroxide, undoubtedly contribute to the subsequent mitotic and metabolic cell death. Since most of such alterations precede DNA damage, their appearance is likely to be a direct consequence of H_2O_2 action rather than one mediated by DNA impairment.[1,2,4,6]

Previous observations pointed out to the particular importance of glutathione peroxidase in cell protection against exogenous hydrogen peroxide.[7–9] A significance of the maintenance of this enzymic activity by pentose phosphate pathway and glutathione reductase,[8,9] as well as the pronounced dependence of the survival of cells exposed to H_2O_2 on the environmental conditions influencing the activity of glutathione peroxidase,[9,10] stimulated further investigations that explore a possibility of interactions of glutathione peroxidase with different enzymic pathways which might be involved in the cellular response to H_2O_2. In particular, the possibility of interaction of glutathione peroxidase with prostaglandin endoperoxide synthase (PES) characterised by two enzymic activities: cyclooxygenase and peroxidase, seem worth

Correspondence: Dr. E.M. Link, Department of Chemical Pathology, Academic Unit, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, UK.

exploring. Cyclooxygenase activity of PES catalyses fatty acid oxidation in the reaction occuring slowly initially. Exogenous hydroperoxides eliminate such kinetic lag phase at the concentrations of 10^{-7} to 10^{-8}M which are far below the K_m (= 10^{-5}M) of the peroxidase activity of PES.[11,12] In contrast, glutathione peroxidase and GSH terminate the cyclooxygenase-catalysed substrate oxidation.[11] An interaction of both enzymic pathways could, therefore, modify the efficiency of hydrogen peroxide decomposition by glutathione peroxidase due to a competition between H_2O_2 and PGG_2-hydroperoxide produced by cyclooxygenase. This, however, would inhibit cyclooxygenase unless exogenous H_2O_2 replaces PGG_2, since the hydroperoxide is used by cyclooxygenase in a positive feedback reaction for autoacceleration.[13,14] Consequently, formation of prostanoids might be limited or even terminated. Since an excess of exogenous hydrogen peroxide indeed inhibits the production of prostaglandins,[2,3] the aim of the present investigations was to examine the mechanism responsible for such inhibition with the initial data described here indicating a crucial rôle of glutathione peroxidase and cyclooxygenase interaction in the survival of cells exposed to H_2O_2.

MATERIALS AND METHODS

Chemicals

Indomethacin (1-[p-Chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid) was from Sigma Chemical Co. (Poole, UK). D-glucose was purchased from BDH Chemicals Ltd. (Poole, UK). Minimum essential medium (Eagle's modified) and fetal bovine serum were supplied by Aldrich Chemical Co. (Gillingham, UK). Phosphate-buffered saline (PBS) was prepared by dissolving 8.0 g NaCl, 0.2 g KCl, 0.132 g $CaCl_2 \cdot 2H_2O$, 0.1 g $MgCl_2 \cdot 6H_2O$, 1.15 g $Na_2HPO_4 \cdot 2H_2O$, and 0.2 g KH_2PO_4 in 1 litre of deionized water.

Cell Line

An established epithelial cell line (GPK) grown exponentially in Eagle's minimum essential medium (MEM) supplemented with 200 mM L-glutamine (1 ml/100 ml MEM), 7.5% $NaHCO_3$ (0.4 ml/100 ml MEM), two antibiotics: penicillin (10,000 U/100 ml MEM) and streptomycin (10 mg/100 ml MEM), and 10% fetal bovine serum, was used for the experiments as described previously.[9] Briefly, cells grown in a 25 cm^2 tissue culture flasks (Falcon Scientific Supplies, London, UK) for 7 days at 37°C in a 5% CO_2 atmosphere were trypsinised using 0.25% trypsin and passaged to 75 cm^2 Falcon flasks (5×10^5 cells/flask) 3 days before the experiment. Medium from each flask was replaced with fresh one 48 h later to assure an exponential cell growth.

Cell Exposure to Hydrogen Peroxide

Plain phosphate-buffered saline (PBS) or supplemented with either indomethacin (0.25 mg/100 ml), glucose (4.7 mg/100 ml) or both as indicated, replaced growth medium in falcon flasks 1 h before cell incubation with H_2O_2 . Similarly prepared PBS adjusted to the desired pH by titration with 1 N HCl or 1 N NaOH was filtered through a 0.22 μm Millipore filter and 22 ml aliquots were distributed into 25 ml

conical flasks. Afterwards, 1 ml of the appropriate medium containing 1.6×10^6 cells was added to each flask and mixed with 1 ml of H_2O_2 diluted in PBS to the desired concentration. The cells incubated with H_2O_2 for 1 h at 37°C were continuously stirred with a magnetic stirrer and subsequently recovered by centrifugation and resuspension in the growth medium.

Determination of Cell Survival

The cell survival was determined using a clonogenic assay according to the method described previously.[15] Shortly, after determination of cell number with a Neubauer haemocytometer and dilution to the desired density, cells were seeded into Petri dishes (NUNC Products, Denmark) and left for 9 days at 37°C in 5% CO_2 atmosphere. The formed colonies stained with crystal violet were counted to determine the plating efficiency (P.E.) and a surviving fraction calculated as a ratio of P.E. for treated cells over P.E. of control cells.

RESULTS

An influence of indomethacin as an inhibitor of cyclooxygenase activity on cellular response to hydrogen peroxide exposure was investigated in the presence or absence of glutathione peroxidase activity. A modification of activity of the enzyme was achieved by the use of alkaline (7.5) or acidic (6.5) pH[8,9] combined with controlled supply of glucose which, by supporting pentose phosphate pathway, influences glutathione reductase efficiency.[8,9]

Cell Response to H_2O_2 at pH 6.5

At pH 6.5 when glutathione peroxidase activity is significantly diminished and in the absence of glucose removed from the medium 1 h before cell exposure to H_2O_2, survival of the cells decreased linearly with increasing hydrogen peroxide concentration [Figure 1a]. Indomethacin added to cells 1 h before the incubation with hydrogen peroxide resulted in their sensitisation to H_2O_2. Cell survival decreased by additional 40% as compared with the values obtained in the absence of indomethacin when the concentration of hydrogen peroxide did not exceed 70 μM, and less significantly between 70 and 145 μM H_2O_2 [Figure 1a]. Still higher concentrations of hydrogen peroxide led to a surviving fraction lower than 10%, and indomethacin did not influence the cell response to it; the overlap of both survival curves was observed [Figure 1a].

When glucose was present before cell exposure to H_2O_2 but removed for the period of incubation with H_2O_2, the cell survival was diminished by 20% in comparison with the corresponding curve shown in Fig. 1a [Figure 1a and 1b]. Indomethacin did not affect cell response to H_2O_2 (0–70 μM) as markedly as in the previous experiment, with the survival almost equal to that without indomethacin [Figure 1b]. In contrast, a sharp peak of protection by indomethacin was observed within the narrow range of 100–125 μM H_2O_2 used for cell incubation; the surviving fraction increased from 12% in the absence of indomethacin to 33% with the compound [Figure 1b].

The presence of glucose during the incubation with H_2O_2 resulted in a sigmoidal shape of the survival curve of cells exposed to hydrogen peroxide without any

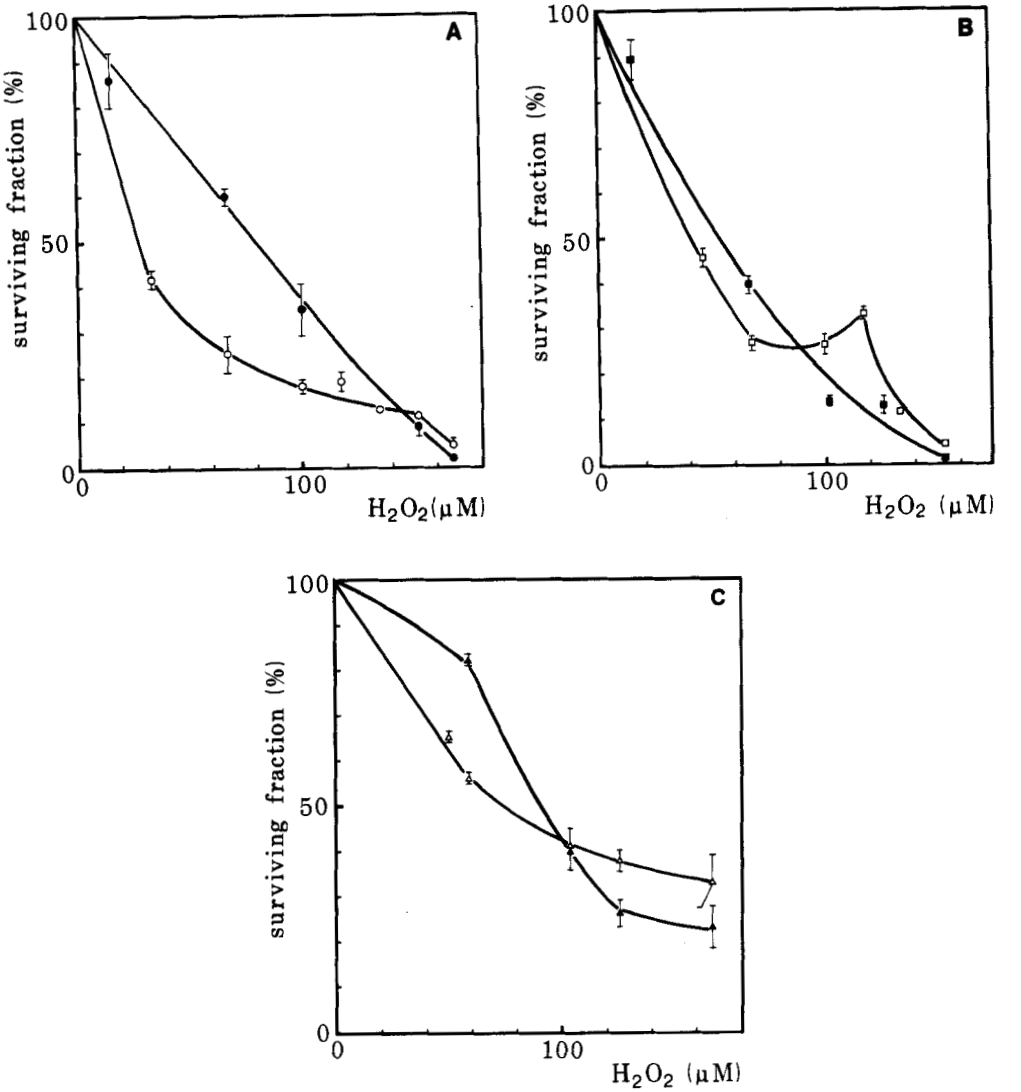

FIGURE 1 Dose-survival curves of cells exposed to H_2O_2 at pH 6.5 after pre-incubation with (open symbols) or without (closed symbols) indomethacin. 1 ml of H_2O_2 diluted in PBS to the required concentration was added to the suspension of 1.6×10^6 cells: A. deprived of glucose 1 h prior to and during exposure to H_2O_2; B. deprived of glucose only during exposure to H_2O_2; C. with glucose present both prior to and during exposure to H_2O_2. The H_2O_2 concentrations shown are recalculated per 1×10^5 cells. The points represent means of at least four independent determinations; the error bars, ±S.D.

additional compound [Figure 1c]. Indomethacin affected this cell response in double manner: at H_2O_2 concentration up to 100 μM the cells were sensitised by indomethacin, whereas above 100 μM H_2O_2 a protective effect of the compound was observed [Figure 1c].

Cell Response to H_2O_2 at pH 7.5

Glutathione peroxidase activity is much higher at pH 7.5 than at 6.5.[8,9] Since a maintenance of the maximum enzyme turnover depends significantly on the pentose phosphate pathway and glutathione reductase activity, the availability of glucose

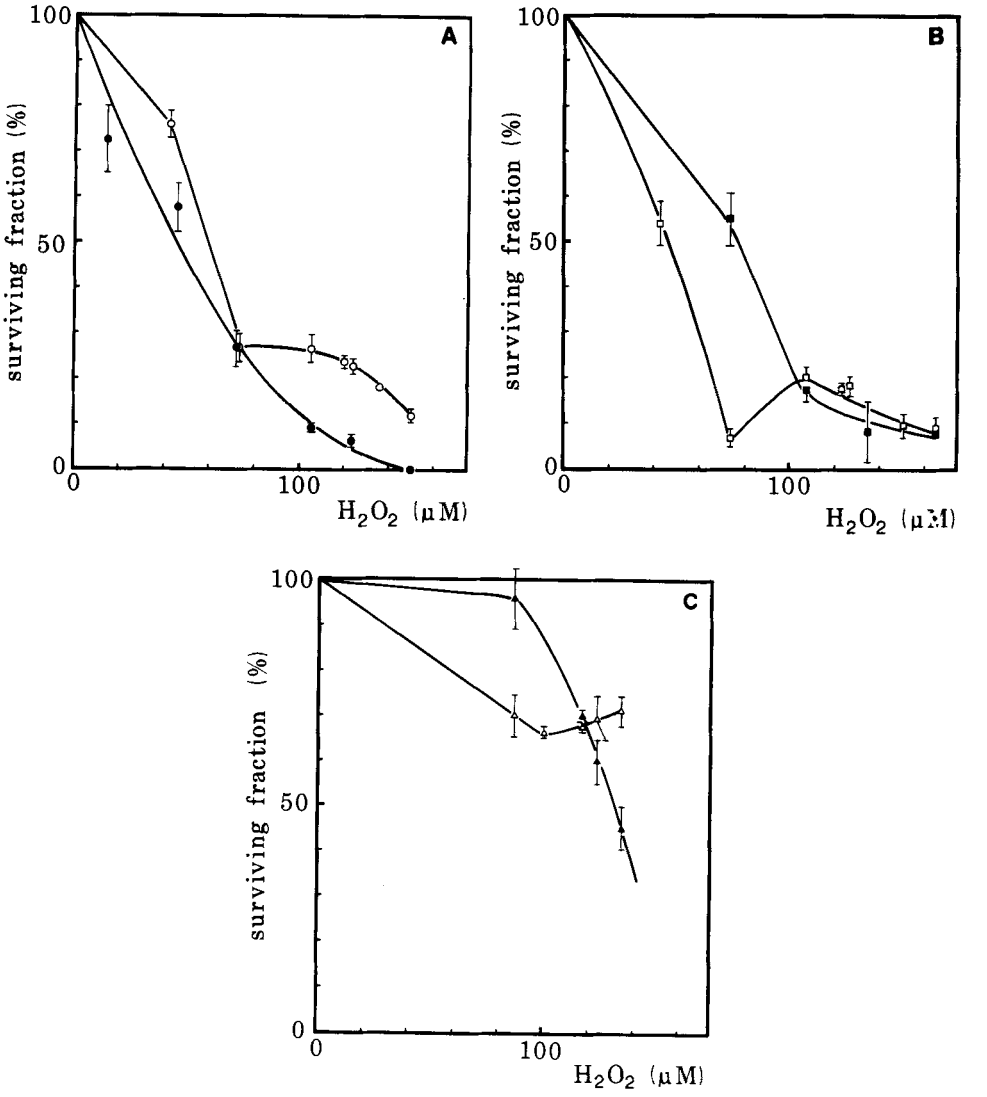

FIGURE 2 Dose-survival curves of cells exposed to H_2O_2 at pH 7.5 after pre-incubation with (open symbols) or without (closed symbols) indomethacin. 1 ml H_2O_2 diluted in PBS to the required concentration was added to the suspension of 1.6×10^6 cells: A. deprived of glucose 1 h prior to and during exposure to H_2O_2; B. deprived of glucose only during exposure to H_2O_2; C. with glucose present both prior to and during exposure to H_2O_2. The H_2O_2 concentrations shown are recalculated per 1×10^5 cells. The points represent means of at least four independent determinations; the error bars, ± S.D.

indispensable for pentose phosphate pathway is crucial for a continuity of the overall enzymic reactions.[8,9] 1 h deprivation of glucose prior to and during cell exposure to H_2O_2 led to a sharp decrease of cellular survival in a manner observed at pH 6.5 when cells were also deprived of glucose before the incubation with H_2O_2 [compare Figure 1b and 2a]. Similarly to those conditions, indomethacin did not affect significantly cellular response to lower doses of H_2O_2, whereas it protected cells against H_2O_2 concentrations higher than 80 μM by approximatley 20% [Figure 2a, compare with Figure 1b].

Removal of glucose only for the period of incubation with hydrogen peroxide resulted in an increased cell survival illustrated by a sigmoidal-like curve with the initial slope equal to that observed at pH 7.5 in the absence of glucose but with indomethacin [Figure 2a and 2b]. In contrast, pre-treatment with indomethacin diminished the initial surviving fraction in a manner characteristic for cells incubated without glucose or indomethacin prior to their exposure to H_2O_2 [compare Figure 2a and 2b]. Further decrease of the survival was, however, significantly enhanced, with the lowest surviving fraction of 7% at 74 μM H_2O_2. This corresponds to 55% found for cells exposed to hydrogen peroxide in the same conditions in the absence of indomethacin [Figure 2b]. The following cell response to increased H_2O_2 concentrations became less dependent on the indomethacin action attaining values even higher (by approximately 5%) than those obtained without indomethacin [Figure 2b].

The presence of glucose during cell incubation with H_2O_2 resulted in a pronounced long shoulder in the survival curve of the cells [Figure 2c]. Up to H_2O_2 concentration of 87 μM, the surviving fraction decreased only by a negligible 3%. However, afterwards the cell survival diminished almost linearly with the increased concentration of hydrogen peroxide [Figure 2c]. Pre-treatment with indomethacin resulted in a biphasic cellular response to H_2O_2 action. The first component represented an augmented cell sensitivity to H_2O_2 as compared with the survival of cells exposed to it without initial treatment with indomethacin [Figure 2c]. The survival curve was characterised by the similar slope as found for the same conditions at pH 6.5 without indomethacin [compare Figure 1c and 2c]. The minimum of 66% in the surviving fraction appeared when 100 μM of hydrogen peroxide was used and for its higher concentrations a linear increase in the cell survival was observed, suggesting a progressive protection by indomethacin against H_2O_2 action [Figure 2c]. Similar, but less pronounced, effects were found at pH 6.5 [compare Figure 1c and 2c].

DISCUSSION

The data presented in this paper revealed that the cytotoxic action of exogenous hydrogen peroxide is significantly affected by preincubation of cells with indomethacin and further modified by pH and availability of glucose before and during cell exposure to H_2O_2. Two factors, i.e., pH and glucose, allowed a manipulation of glutathione peroxidase activity at physiological conditions without using any inhibitors of the enzyme which could also affect different cellular pathways in uncontrolled way. As described previously,[8,9] the glutathione peroxidase activity is strongly dependent on pH with no enzymic activity at pH 6.0 and its maximum at pH 8.5.[16-18] The optimum efficiency in the enzyme turnover is maintained by the pentose phosphate pathway and glutathione reductase products, i.e., NADPH and GSH, respectively. Therefore, the availability of glucose crucial for the pentose phosphate pathway

activity is of a high importance. Consequently, by manipulating both pH and the supply of glucose, glutathione peroxidase activity was either included or excluded from the cellular response to hydrogen peroxide toxicity as described previously.[8,9]

Indomethacin is a commonly recognized inhibitor of cyclooxygenase activity of prostaglandin endoperoxide synthase.[19] Therefore, the use of the compound in the conditions in which glutathione peroxidase activity was limited almost to its minimum (pH 6.5 and removal of glucose from the medium 1 h before exposure to H_2O_2) allowed an observation of the effect of cyclooxygenase almost alone on the survival of cells incubated with H_2O_2. A gradual supply of glucose and, subsequently, an increase of pH to 7.5 with a varying availability of glucose from its initial absence to the excess at the final stage, not only enabled a detection of the progressive involvement of glutathione peroxidase in the cellular response to H_2O_2 but led also to the conclusion concerning a significant influence of the interference with both enzymic pathways on the cell survival after exposure to H_2O_2.

It was evident that the surviving fraction of cells with glutathione peroxidase activity reduced to its minimum decreased linearly with increasing H_2O_2 concentration. Furthermore, the results showed that in such conditions the cyclooxygenase activity (determined by the effect of indomethacin on the survival of cells incubated with H_2O_2) was crucial in the protection of cells against H_2O_2 toxicity when the concentration of hydrogen peroxide did not exceed 3.5×10^{-5} M (approximately 3.5×10^{-10} M H_2O_2 per cell). Above these H_2O_2 concentrations, cyclooxygenase was progressively less effective in cell protection. Increased activity of glutathione peroxidase emphasised a double function of cyclooxygenase: protective–first, sensitizing–afterwards, with the turning point between them gradually moved to higher concentrations of H_2O_2. This regularity corresponds to augmented decomposition of H_2O_2 by glutathione peroxidase, as illustrated by the increasing angle between the ordinate and the linear part of the survival curves obtained in the presence of indomethacin. Such double action of cyclooxygenase was also reflected in the survival curves generated in the absence of indomethacin changing their slopes from less to more steep at the H_2O_2 concentration matching the turning point of corresponding curves obtained with indomethacin (see Figure 1c and 2c).

The most direct illustration of glutathione peroxidase interaction with cyclooxygenase activity in the presence of exogenous H_2O_2 is shown in Figures 1b and 2a. At pH 6.5 and in the absence of glucose during the H_2O_2 exposure only, GSH remained at its physiological level at the beginning of the incubation with H_2O_2. Since the low pH limits the rate of glutathione peroxidase turnover and, consequently, the use of GSH by the enzyme per unit of time, more GSH was initially available for cyclooxygenase leading to its significant acceleration and, subsequently, enlarged production of PGG_2. Such sudden increase in the intracellular hydroperoxide level also turns glutathione peroxidase activity towards PGG_2 decomposition. This, in turn, slows down or even inhibits the cyclooxygenase activity, since PGG_2 decomposed by glutathione peroxidase could not be replaced by H_2O_2 in its accelerating action as effectively as initially due to a fast decline in GSH level which does not recover by a reduction of GSSG in the absence of glucose.

Indomethacin, when added, inhibited cyclooxygenase and, consequently, interrupted the formation of hydroperoxide enabling glutathione peroxidase to decompose proportionally more H_2O_2. However, since at the same time inactivated cyclooxygenase did not use H_2O_2 for its acceleration, the overall decomposition of H_2O_2 in the presence or absence of indomethacin remained comparable and, consequently,

the cell survival was similar under both sets of conditions (Figure 1b). In contrast, at pH 7.5 H_2O_2 decomposition by glutathione peroxidase dominated the use of hydrogen peroxide by cyclooxygenase. Therefore, in the conditions in which glutathione peroxidase turnover was significantly limited by the insufficient supply of GSH, any additional source of hydroperoxide such as cyclooxygenase activity, resulted in diminishing the efficiency of glutathione proxidase decomposition of exogenous H_2O_2. Thus, the cyclooxygenase inhibition by indomethacin restored a full capacity of glutathione peroxidase restricted only by a low level of GSH and, consequently, the increased cell survival was observed (Figure 2a). Further increase in the GSH level by a prolonged supply of glucose until cell incubation with H_2O_2 and then even during the exposure to H_2O_2, gradually balanced a diminished capacity of glutathione peroxidase to decompose H_2O_2 (caused by a competitive action of PGG_2) with the use of H_2O_2 by cyclooxygenase for its acceleration (Figure 2b) and, finally, led to the conditions under which a full availability of GSH, unlimited by insufficient GSSG reduction, enabled maximal decomposition of H_2O_2 by both enzymes almost regardless of their concomitant interactive actions (Figure 2c).

Biochemical data concerning an inhibition of cyclooxygenase by glutathione peroxidase[11] and those indicating the activation of an impaired cyclooxygenase reaction system by exogenous H_2O_2[20] together with investigations *in vitro* demonstrating gradual acceleration of prostaglandin production in the presence of glutathione and H_2O_2 within the concentration range of 10^{-8} to 10^{-5} M[3] and a subsequent progressive inhibition of the prostaglandin formation above these H_2O_2 concentrations,[2,3] as well as a protective action of A23187[21]- a calcium ionophore which is known to stimulate prostaglandin production from endogenous arachidonic acid by an elevation of intracellular level of Ca^{2+} - support the proposed mechanism and its involvement in the observed cellular responses to H_2O_2. Furthermore, the conversion from a stimulation to the inhibition of prostaglandin production with the increased doses of H_2O_2 significantly correlates with the observed double function of cyclooxygenase; H_2O_2 concentrations at the turning point of cyclooxygenase action correspond precisely with those of prostaglandin formation. This allows a further suggestion concerning previously observed inhibition of prostaglandin production:[2,3] it is due to a disproportion between the efficiency of cyclooxygenase and peroxidase activities of prostaglandin synthase in the presence of exogenous H_2O_2 rather than a direct inhibition of cyclooxygenase itself, since indomethacin significantly influences the cell survival within the entire range of H_2O_2 doses used. Such disproportion in both enzyme activities would lead to the excess of the intermediate product - PGG_2 which, when not converted to PGH_2, could be highly cytotoxic.

Certainly, more detailed studies are required to confirm the proposed mechanism with its considerable consequences for survival of cells exposed to H_2O_2. However, the conclusions drawn initially[22] are further supported here: whatever enzymic reactions are involved in the observed double action of indomethacin in the presence of hydrogen peroxide, the demonstrated modifications in the cellular response to H_2O_2 caused by the preincubation with indomethacin cannot be accounted for by the free radical involvement.

References

1. M.J. Welsh, D.M. Shashby and R.M. Husted (1985) Oxidants increase paracellular permeability in a cultured epithelial cell line. *Journal of Clinical Investigation*, **76**, 1155–1166.

2. A. Barchowsky, R.S. Kent and A.R. Whorton (1987) Recovery of porcine aortic endothelial cell prostaglandin synthesis following inhibition by sublethal concentrations of hydrogen peroxide. *Biochimica et Biophysica Acta*, **927**, 372–381.
3. C. Deby and G. Deby-Dupoint (1980) Oxygen species in prostaglandin biosynthesis *in vitro* and *in vivo*. In: *Biological and clinical aspects od superoxide and superoxide dismutase* (ed. Bannister, W.H. and Bannister, J.V.), Elsevier, Amsterdam, 84–97.
4. P.A. Hyslop, D.B. Hinshaw, W.A. Hanslye, I.U. Schraufstätter, R.D. Sauerheber, R.G. Spragg, J.H. Jackson and C.G. Cochrane (1988) Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. *Journal of Biological Chemistry*, **263**, 1665–1675.
5. D.B. Hinshaw, L.A. Sklar, B. Bohl, I.U. Schraufstätter, P.A. Hyslop, M.W. Rossi, R.G. Spragg and C.G. Cochrane (1986) Cytoskeletal and morphologic impact of cellular oxidant injury. *American Journal of Pathology*, **123**, 454–464.
6. H.R. Massie, H.V. Samis and M.B. Baird (1972) The kinetics of degradation of DNA and RNA by H_2O_2. *Biochimica et Biophysica Acta*, **272**, 539–548.
7. P.E. Starke and J.L. Farber (1985) Endogenous defences against the cytotoxicity of hydrogen peroxide in cultured rat hepatocytes. *Journal of Biological Chemistry*, **260**, 86–92.
8. E.M. Link (1988) Why is H_2O_2 cytotoxicity pH-dependent? In: *Free Radicals, Methodology and Concepts* (ed. Rice-Evans, C., Halliwell, B.), Richelieu Press, London, 539–548.
9. E.M. Link (1988) The mechanism of pH-dependent hydrogen peroxide cytotoxicity *in vitro*. *Archives Biochemistry and Biophysics*, **265**, 362–372.
10. E.M. Link (1989) Histidine contribution to pH-dependent mechanism of hydrogen peroxide cytotoxicity. In: *Medical, Biochemical* and *Chemical Aspects of Free Radicals* (ed. Hayaishi, O., Niki, E., Kondo, M., Yoshikawa, T.), Elsevier, Amsterdam, 157–160.
11. W. Lands, R. Lee and W. Smith (1971) Factors regulating the biosynthesis of various prostaglandins. *Annals of the New York Academy of Sciences*, **180**, 107–122.
12. P. Needleman, J. Turk, B.A. Jakschik, A.R. Morrison and J.B. Lefkowitch (1986) Arachidonic acid metabolism. *Annual Review of Biochemistry*, **55**, 69–102.
13. M.E. Hemler, G. Graff and W.E.M. Lands (1978) Accelerative autoactivation of prostaglandin biosynthesis by PGG_2. *Biochemical and Biophysical Research Coummunications*, **85**, 1325–1331.
14. W.E.M. Lands (1988) Enzymatic assay of hydroperoxides. In: *Free Radicals, Methodology and Concepts* (ed. Rice-Evans, C., Halliwell, B.), Richelieu Press, London, 213–224.
15. E.M. Link and P.A. Riley (1988) Role of hydrogen peroxide in the cytotoxicity of the xanthine/xanthine oxidase system. *Biochemical Journal*, **249**, 391–399.
16. K.G. Paul (1963) Peroxidases. In: *The Enzymes* (ed. Boyer, P.D., Lardy, H., Myrbäck, K.), 2nd ed., Academic Press, New York, 227–274.
17. J. Chaudiere, E.C. Wilhelmsen and A.L. Tappel (1984) Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans. *Journal of Biological Chemistry*, **259**, 1043–1050.
18. K.R. Maddipati, C. Gasparski and L.T. Marnett (1987) Characterization of the hydroperoxide-reducing activity of human plasma. *Archives of Biochemistry and Biophysics*, **254**, 9–17.
19. W.L. Smith and W.E.M. Lands (1971) Stimulation and blockage of prostaglandin biosynthesis. *Journal of Biological Chemistry*, **246**, 6700–6702.
20. P.J. Marshall, M.A. Warso and W.E.M. Lands (1979) Prostaglandin biosynthesis can be triggered by lipid peroxides. *Archives Biochemistry and Biophysics*, **193**, 340–345.
21. H.C. Birnboim and M. Kanabus-Kaminska (1985) The production of DNA strand breaks in human leukocytes by superoxide anion may involve a metabolic process. *Proceeding of the National Academy of Sciences USA*, **82**, 6820–6824.
22. E.M. Link (1989) Hydrogen peroxide cytotoxicity in a system *in vitro*. *Biochemical Society Transactions*, **17**, 704.

Accepted by Prof. H. Sies