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Hydrogen peroxide imposed deviation in glutathione Production with respect to tissue protein weight of goat liver, in vitro

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ABSTRACT

Hydrogen peroxide induce the oxidative stress on the cells. The free oxygen radicle denature the protein by oxidizing the thiole group of protein having cysteine or methionine amino acid that donate proton to free oxygen species thus creating un even di sulphide bonds in protein. Besides H2O2 reduces protein synthesis in various ways with increasing concentration of infusion as well as time. Glutathione which is a natural anti oxidant in cell tries to reduce the oxidative stress in the cell. Glutathione is a tri peptide of glutamate, cysteine and glycine produced by cells. Glutathione prevent oxidation which protect cell and its components from free radicles, reactive oxygen species and peroxides. The ratio of oxidised and reduced glutathione within a cell is indicator of state of oxidative stress on the cell. Thiole group of glutathione donates proton to reduce the oxidative stress on the cell. In an in vitro experiment, liver cell of goat were cultured in M199 media and production of glutathione per milligram of protein with respect to different concentration of hydrogen peroxide as well as different time, glutathione production and protein synthesis was measured by standard method. The production of glutathione per milligram of protein increases with increase in concentration of H2O2 and time.

Introduction

Glutathione is a tri peptide found in many mammalian tissues especially liver. It has a major protective role in the body. I act as scavenger for free radicles, reactive oxygen species and peroxides. Its thiole group or sulfhydryl group of cysteine molecule plays that important role, thus acting as an anti oxidant to remove toxic material and to manage oxidative stress in the cell. Normally the thiole group of glutathione act as a nucleophile that displaces another atom or attack electrophilic site. It may reacts chemically or enzymatically with variety of compounds which are reactive or electrophilic metabolites produced in first phase of reaction. Non enzymatic addition reaction are observed with free radicles and reactive intermediates(Sipes and Gandolfi 1991). The reaction may be catalysed by one of the group of glutathione transferase enzyme. These are widely distributed anand are primarily located in microsomes. The glutathione conjugates are further metabolised that involves glutamyl residue followed by the loss of glycine which in turn is acetylated to produce N Acetylecystine conjugate or mercapturic acid. The enzyme involved in this conjugation is microsomal enzyme found in the liver, kidney and other organs in different forms. The glutathione conjugates or glycine cysteine conjugation is considered as first phase of reaction. These conjugates are excreted directly into the bile and further reaction may be carried out in gastro intestinal tract(Timbrell 1982, 1995). Reduced glutathione plays a major role in protecting cell from toxic chemicals and their metabolites. In additions to production of excretable conjugates it protects the thiole group of certain enzymes and proteins especially oxidative and helps membrane integrity.

These reaction can be studied in cell free, cellular and organ system. The pieces of liver can be used as functional units in culture medium, where the medium is modified to study various influences on live metabolism. It is also true in case of study of free radicles and free radicles modifiers(Borek, 1984). Thus in this experiment in an in vitro system pieces of goat liver were incubated in sterile conditions in tissue culture medium providing varying concentration of hydrogen peroxide (H_2O_2) and at varying intervals of time. In these experimental conditions glutathione was estimated to study the protective tolerance of liver in vitro.

Material and Methods

All he preparations required sterilization. The standard methods of sterilization in glassware preparation and maintenance of culture were strictly observed.

Tissue preparation:

Goat liver was removed immediately on decapitation and was immersed in tissue culture medium at 10 ^{0}C. The pieces of liver were added 100 to 50 mg per ml of culture medium. The liver pieces were equilibrated at 37.5 ^{0}C for 30 minutes and were transferred to the fresh medium 100 to 50 mg per ml of culture medium in Beckmans tubes in closed system.

The protocol of this experiment: In this experiment scheduled concentration of H_2O_2 of 150mM, 300mM, 450mM, 600mM,750mM and 900mM. The formation of radicle is dependent on catalase activity. Its substrate saturation point in vitro is up to 5M H_2O_2 and in the present condition liver slices are used low levels of H_2O_2 are used to mimic in vivo conditions (Hugo 1984) were used. The various alterations were assayed at 30 minutes, 60 minutes and 90 minutes. The weights of the liver were un altered. The parameters considered her include proteins and glutathione. The liver were removed and was used for the estimations of glutathione. Similarly the medium was also used for the estimation of glutathione.

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Bioassay of proteins by (Lowry, 1951)

i) Reagents:

Lowry's reagents:

Lowry's A- 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water.)

Lowry's B- 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartarate solution

Lowry's C- It is prepared at the time of use 50 ml of Lowry's A +1ml of Lowry's B

b)Folin-Ciocalten-Phenol-reagent-

It is made of 100 gm Sodium tungstate, 25gm sodium molydate, 700 ml distilled water, 100 ml concentrated HCl, 50 ml 85% Phosphoric acid(H3PO4). Reflux this mixture for 10 hours in glass apparatus and in reflex mixture add 150gm of Lithium Sulphate, 50ml of double distilled water, 5 drops of bromine water. Boil this mixture to remove excess bromine water. Dilute it to 1N acid and use. Or Folin - Ciocalteau reagent solution (1N) Dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water) (Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J.Biol.Chem 193: 265 (The original method)

Assay: 0.2 ml of extract of + 1.3 ml of Distilled water + 3.0 ml of

Lowry's C : mix well and keep at room temperature for 20 minutes add 5 ml of Folin-phenol reagent mix well and keep at room temperature for 10 minutes. Prepare blank similarly using 1.5 ml distilled water and read Optical Density at 750nm adjusting zero by blank.

Protein content was calculated by preparing standard graph of bovine serum albumen using same method.

Bioassay of glutathione(Lynen 1951):

i)Reagents: 0.067 M Sodium nitroprusside, 0.067M Sodium cynide in 1.5M Na2CO3(Sodium Carboniate)

ii)Assay: 6ml of extract or medium + 1ml of Sodium nitroprusside + 1ml Sodium cynide in 1.5 M Na2CO3(Sodium Carbonate). Similarly blank or control was prepared as sollows 6ml Distilled water+ 1ml of Sodium nitroprusside + 1 ml sodium cynide in 1.5 M Na2CO3

Read OD(Optical Density) at 520 nm adjusting '0' to the blank.

Concentration of Glutathione was calculated using stsndard graph of glutathione prepared by the same method.

Result

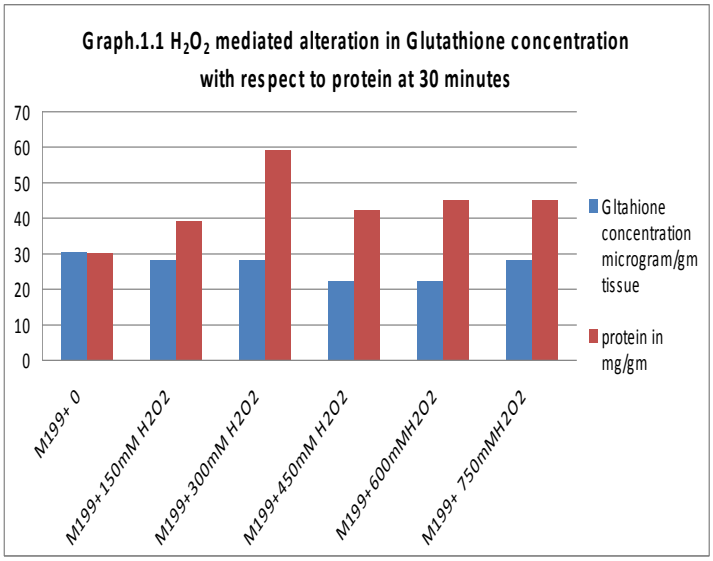
In any of the experiment condition no change in tissue weight was noted Up t 5 hours of duration weight are not altered in vitro under H2O2 influence. In this experiment we tried to study alternation in Glutathione content. Glutathione content was not noted in the medium and hence it is only given in liver expressed in gm/ weight of liver and per mg liver proteins. At 30 minutes of interval the content per gm weight of liver showed 6.60% decrease at 150 mM concentration of H2O2. It remain unaltered at 300nM concentration of H2O2. In 450 mM concentration of H2O2 21.42% decrease was noted. At 600mM concentration of H2O2 no alteration was observed. Glutathione content was not altered. It increased at 700 mM concentration of H2O2 by 1.2 folds

Glutathione content per gram tissue proteins were decreased by 28.90% in 150 mM concentration of H2O2, 33.98% in 300 mM H2O2 concentration and 6.69% in 600 mM concentration of H2O2. Glutathione concentration was increased by 1.103 folds in 450 mM and 1.27 folds in 750 mM Concentration of H2O2

Table.1.1 Hydrogen peroxide mediated alteration in glutathione concentration in µg/gm wet weight of tissue and protein at 30 minutes

Sr.No	Experimental condition	Glutathione in µg/gm wet weight of tissue	Proteins in mg/gm of tissue	Glutathione in µg/gm of tissue protein
1	Blank (M199)	30.30	30.00	1.01
2	M199 + 150 mM H_2O_2	28.00	39.00	0.72
3	M199 + 300 mM H_2O_2	28.00	59.00	0.47
4	M199 + 450 mM H_2O_2	22.00	42.00	0.52
5	M199 + 600 mM H_2O_2	22.00	45.00	0.49
6	M199 + 750 mM H_2O_2	28.00	45.00	0.62

Values are mean of ± SE of 5 sets



Here at 30 minutes with increasing concentration of H2O2 the protein synthesis goes on increasing up to 300 mM concentration of H2O2 after that the protein concentration goes on decreasing while glutathione concentration goes on decreasing upto 600 mM concentration of H2O2 after that it shows slight increase.

At The interval of 60 minutes

At 150 mM concentration of H2O2 glutathione content per gram weight of liver remained un altered which increased by 1.07 folds in 300 mM concentration of H2O2 but it decreased by 13.33% in 450 mM concentration of H2O2. Glutathione concentration remained unchanged at 600 mM concentration of H2O2 and once again increased by 1.23 folds in 750 mM concentration of H2O2

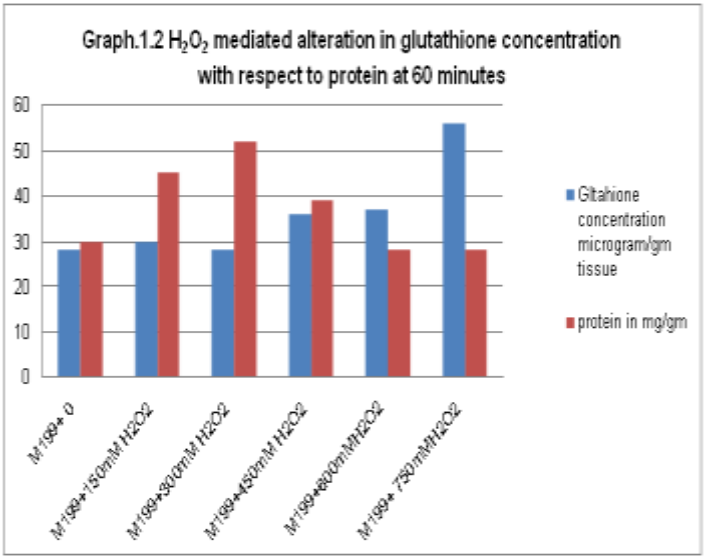
Per mg tissue protein values of glutathione were increasd by 1.55 folds in 300 mM concentration of H2O2, 1.39 folds in 600 mM concentration of H2O2 and 1.23 folds in 750 mM concentration of H2O2. It is found that glutathione concentration decreased by 33.33% in 150 mM concentration of H2O2 and 31.12% decrease in 450 mM concentration of H2O2

At intervals of 90 minutes

Glutathione per gram weight of liver showed increase by 1.67 folds in 150 mM concentration of H2O2, 1.28 folds in 450mM 1.33 folds in 600 mM and 1.51 folds in 750 mM concentration of H2O2. But glutathione concentration was decreased at 300mM concentration of H2O2

Table.1.2 Hydrogen peroxide mediated alteration in glutathione concentration in µg/gm wet weight of tissue and protein at 60 minutes

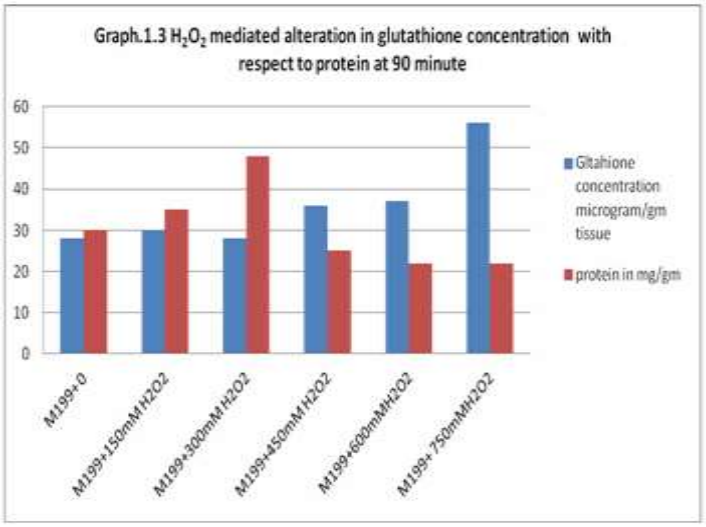
Sr.No	Experimental condition	Glutathione in µg/gm wet weight of tissue	Proteins in mg/gm of tissue	Glutathione in µg/gm of tissue protein
1	Blank (M199)	28.00	30.00	0.93
2	M199 + 150 mM H_2O_2	28.00	45.00	0.62
3	M199 + 300 mM H_2O_2	30.00	52.00	0.97
4	M199 + 450 mM H_2O_2	26.00	39.00	0.67
5	M199 + 600 mM H_2O_2	26.00	28.00	0.93
6	M199 + 750 mM H_2O_2	32.00	28.00	1.14



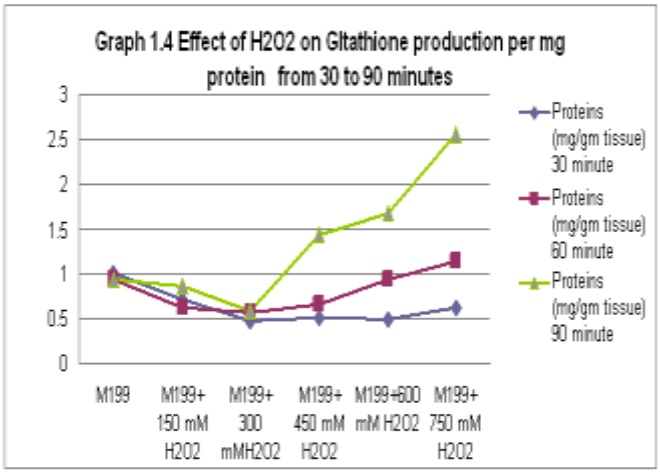
Glutathione concentration per mg tissue protein showed increase by 2.26 folds at 450 concentration of H2O2 , 1.16 folds at 600 mM concentration of H2O2 and 1.51 folds at 750 mM concentration of H2O2 and it is found to be marginally decrease by 8.14% at 150 mM concentration of H2O2

Table.1.3 Hydrogen peroxide mediated alteration in glutathione concentration in µg/gm wet weight of tissue and protein at 90 minutes

Sr.No	Experimental condition	Glutathione in µg/gm wet weight of tissue	Proteins in mg/gm of tissue	Glutathione in µg/gm of tissue protein
1	Blank (M199)	28.00	30.00	0.93
2	M199 + 150 mM H_2O_2	30.00	35.00.	0.86
3	M199 + 300 mM H_2O_2	28.00	48.00	0.58
4	M199 + 450 mM H_2O_2	36.00	25.00	1.44
5	M199 + 600 mM H_2O_2	37.00	22.00	1.68
6	M199 + 750 mM H_2O_2	56.00	22.00	2.55



Discussion



In zero concentration of H2O2 the glutathione concentration milligram of protein remains same at 30, 60 and 90 minute. At 150 mM concentration of H2O2 also there is no much change in glutathione concentration per mg protein only at 60 minute there is slight decline in glutathione concentration per mg protein because high protein synthesis in comparison to low synthesis of glutathione and further at 90 minutes there is 1.3 fold increase in glutathione synthesis per mg protein because of increased glutathione synthesis and decreased protein synthesis. At 300 mM concentration of H2O2 from 30 minutes 90 minutes the glutathione concentration per mg protein is almost same, this is because of decreasing concentration of protein in similar proportion of increasing concentration of glutathione. After that the glutathione per mg proteins increase rapidly with increasing time up to 90 minutes. At 450 mM concentration of H2O2 glutathione concentration increase by 1.3 folds in 60 minutes and rapidly increase by 2.1 folds up to 90 minutes. With increasing time the gap between protein synthesis and glutathione synthesis increase, at 600 mM concentration of H2O2 the glutathione concentration per mg protein increased by 1.8 folds up to 60 minutes and similarly up to 90 minutes, which is because of decreasing protein synthesis and increasing glutathione synthesis. The gap further widens at 750 mM concentration of H2O2 glutathione concentration per mg protein increases by 1.8 folds up to 60 minutes and by 2.23 folds by 90 minutes. Further studies need to study the detailed mechanism.

References

1) Bird.R.P and Draper.H.H(1984) Comparative studies on different methods of Malonaldehyde determination . Oxygen radicles in biological systems(Methods in Entomology -105)

page 299 Edited by L.Packer.Published by Academic press Inc. New York, oLndon.
2)Borek, C. (1984) In vitro cell cultures as Tools in the study of free radical modifier in Carcinogenesis in Oxygen radicles' In biological Systems (Methods in Entomology -105) page 464 Edited by F.Fleischer and L.Packer Published by Academic press New York, London
3)Haliwell , B and Gutteridge, J.M.C (1990) 'Role of free radicles and catalytic metal ions in Human diseases: an over view in Oxygen radicles in Biology Part B / Oxygen radicals and antioxidants (Methods in Entomology -186 Edited by L.Packer and Galzer A.N Published by American press New York and London.
4)Hugo A (1984) ' Catalase in vitro ' Oxygen radicals in Biological system (Methods in Entomology - 105) Edited by L. Packer Published by Academic Press Inc. New York London page 121
5)Alberts, Bruce (2002). *Molecular biology of the cell*. New York: Garland Science. pp. 760. ISBN 0-8153-3218-1
6)Qureshi, SA; Bell, SD; Jackson, SP (1997). "Factor requirements for transcription in the archaeon Sulfolobus shibatae". *EMBO Journal* 16 (10): 2927–2936.doi:10.1093/emboj/16.10.2927. PMC 1169900.PMID 9184236.
7) Dvir, A (Sep 2002). "Promoter escape by RNA polymerase II". *Biochimica et Biophysica Acta* 1577 (2): 208–223.ISSN 0006-3002. PMID 12213653. edit
8)Stryer, Lubert (2002). *Biochemistry; Fifth edition*. W. H. Freeman and Company. p. 826. ISBN 0-7167-4684-0)
9)Alirezaei M, Marin P, Nairn AC, Glowinski J, Prémont J. Source Chaire de Neuropharmacologie J Neurochem. 2001 Feb;76(4):1080-8. Inhibition of protein synthesis in cortical neurons during exposure to hydrogen peroxide., INSERM U114, Collège de France, Paris, France)
10) Patel J, McLeod LE, Vries RG, Flynn A, Wang X, Proud (Eur J Biochem. 2002 Jun;269(12):3076-85.Cellular stresses profoundly inhibit protein synthesis and modulate the states of phosphorylation of multiple translation factors. CG.Source Department of Biosciences, University of Kent at Canterbury, Canterbury, UK.)
11) Sen and Packer FASEB 10: 709-720 1996
12) Sun and Oberley, Free Radic Biol Med 21:335-348, 1996
13) Yang Xu1, Cynthia Bradham2, David A. Brenner[2], and Mark J. Czaja[1] Hydrogen peroxide-induced liver cell necrosis is dependent on AP-1 activation Author Affiliations Accepted in final form30 May 1997.)
14) Bird.R.P and Draper.H.H(1984) Comparative studies on different methods of Malonaldehyde determination . Oxygen radicles in biological systems(Methods in Entomology -105) page 299 Edited by L.Packer. Published by Academic press Inc. New York, London.
15) Borek, C. (1984) In vitro cell cultures as Tools in the study of free radical modifier in Carcinogenesis in Oxygen radicles' In biological Systems (Methods in Entomology -105) page 464 Edited by F.Fleischer and L.Packer Published by Academic press New York, London
16) Haliwell , B and Gutteridge, J.M.C (1990) ' Role of free radicles and catalytic metal ions in Human diseases: an over view in Oxygen radicles in Biology Part B / Oxygen radicals and antioxidants (Methods in Entomology -186 Edited by L.Packer and Galzer A.N Published by American press New York and London.
17) Lowery O H , Rosebrough N.J Farr A.L and Randall R J (1951)
18) Dean P.Jones and Frances G.Kennedy 'Function of Glutathione Peroxidase in Decomposition of Hydrogen peroxide in Isolated liver and heart Cells' edited by Larsson et al Raven Press, New York 1983
19) Sies.H and Chance B (1970): The steady state level of catalase compound I in isolated haemoglobin free perfused rat liver FEBS Lett., 11:172-176
20) Sies,H.,Gerstenecker,C., Menzel,H and Flohe,L(1972): oxidation in NADP system and release of GSSG from haemoglobin – free perfused rat liver during peroxidase oxidation of glutathione by hydrogen peroxides. FEBS Lett., 27:171-175
21) Sies,H and Summer,K-H.(1975): Hydro peroxide metabolizing systems in rat liver.Eur.J.Biochem.57:503-512.
22) Protein measurement with Folin Phenol reagent. Journal of Biochemistry 193, 265- 275
23) Lynen F (1951) Glutathione Ann. Chem (574) 33
24) Mastero R.D (1984): Systemic consequences of O2 radicals in biological system(Methods in Enzymology -106)0Edited by L.Parker Published by Academic Press London Page 386
25) Mehendale M.H (1985) Modern toxicology Volume I, Edited by Gupta. P.K and Salunkhe D.K Published by Metropolitan Book Co.Pvt.Ltd, Netaji Subhash Marg New Delhi 110 002 India Page 226
26) Sipes I.G and Gandolfi A.J (1991) 'Biotransformation of toxicants in Cassarett and Doulls Toxicology, Edited by Amdur M.O , Doull J and Klasen C.D Published by Pragmon Press New York Page 88-126
27) Timbrell J.A (1982) Principles of biochemical toxicology. Taylar and Francis, London.
28) Timbrell J.A (1995) ' Biotransformation of Xenobiotic' in general and applied toxicology . Edited by Ballantyne, T. Marrs, T.Turner .P Published by MacMillon Press Ltd. Brunnel Road Houndmills Basingstoke,Hants RG212xs England
29) Slater T.F (1978) Biochemical studies on liver injury in biochemical mechanism of liver injury(ed) Published by Associated press New York.P.1
30) Moldeus,P., Hogberg,J., and Orrenius,S. (1978): Isolation and use of liver cells. Methods Enzymol., 51:60-71
31) Jones,D.P, Thor,H., Andresson,B., and orrenius,S.(1978): Detoxification reactions in isolated hepatocytes. Role of glutathione peroxidase, catalase and fromaldehyde dehydrogenase in reaction relating to N-demethylation by the cytochrome p-450 system.J.Biol.Chem.253:6031-6037.
32) Jones,D.P., and Manson,H.(1978): Gradients of O_2 concentration in hepatocytes. J.Biol.Chem.253:4874-4880.