

Age- Related Changes In The Lipid Peroxidation

And Antioxidant Systems Of Male Subjects

Saad A. Hussian

Hameed M. Majeed

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College of Pharmacy

College of Education for pure Science

Abstract

The effect of ageing on the lipid peroxidation and antioxidant system was evaluated in this study . for this purpose , we determined MDA as a marker of lipid peroxidation , reduce glutathione , uric acid as well as catalase and glutathione-s-transferase in serum of 84 male healthy individuals (non-smokers).

These subject were divided in to three groups: group 1 (n=28;25-34 years old) ; group2 (n= 28;35-44 years old); group3 (n= 28;45-63years old).

The result showed increased level of MDA and were observed as the stage of age increased (p >0.05). whereas there is a depletion in GSH level related to age increase (p< 0.05), which indicate that the low level of GSH in the circulation may be due to their increased utilization to scavenge lipid peroxide. There was higher levels of catalase and GST in group3 compared with others. Group2 showed lower level in haemoglobin and higher level in albumin in compared with other groups (p<.0.05). no meaningful differences were found in terms of

serum total protein and uric acid levels.

Key words ; Age ,Lipid peroxidation , Antioxidant

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Introduction

Free radicals are highly reactive species characterized by an unpaired electron in their outer orbital. Free radical reactions, including lipid peroxidation, are considered to be important factors in the pathogenesis of a variety of diseases. (MC call MR, Frei B.1999).

Free radicals can damage protein, lipid, carbohydrates, and nucleic acids. Plasma membranes are critical targets of free radical reactions. Oxygen-derived free radicals can easily produce injuries to cell membranes (fig.I) by initiation of polyunsaturated fatty acid, peroxidation, inactivation of membrane enzyme and receptors, and protein cross-linking and fragmentation. (Dasgupta A, et al 1992).

Figure (1) Lipid peroxidation (classic lipid peroxidation)

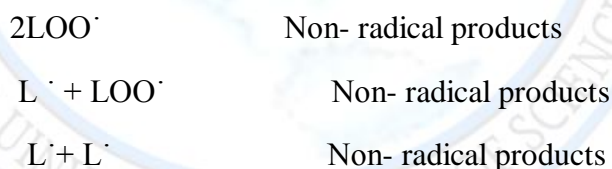
Initiation



Propagation



Termination



Termination may also occur with GSH or other Lipid antioxidants.

(LH) polyunsaturated fatty acid, (x) Free radical atom.

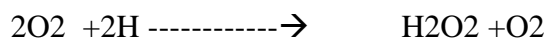
We have evolved antioxidant defenses to protect against free radicals. Superoxide dismutase (SOD) convert superoxide to hydrogen peroxide (H₂O₂) :

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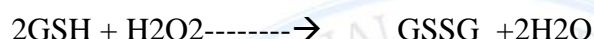
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These enzymes are found in mitochondria and cytosol. (Halli Well B1999). catalase remove hydrogen peroxide ,are found in peroxisomes in most tissues, and probably serve to remove peroxide generated by peroxisomal oxidase enzymes. (Babior BM, Woodman RC.1990).

Glutathione peroxidases are major enzymes that remove hydrogen peroxide generated by SOD in cytosol and mitochondria, by oxidizing the tri peptide glutathione (GSH) into its oxidized form (GSSG) :



GSH also has important roles in xenobiotic metabolism and leukotriene synthesis and it's found at millimolar concentrations in all human cells. (Babior BM, Woodman RC. 1991).

The glutathione peroxidase that removes hydrogen peroxide contains selenium (essential for catalytic function) at its active site, as does a similar enzyme that can remove lipid hydroperoxides from membranes (Maiorino M,etal 1991).

Inborn defects in enzymes of glutathione metabolism can have severe clinical consequences. Since iron and copper ions are powerful promoters of free-radical damage, accelerating lipid peroxidation and causing formation of hydroxyl radical ,(Halli Well B; Gutteridge JMC,1989).

We have evolved a complex system of transport and storage proteins to ensure that these essential metals are rarely allowed to be "free" Ferritin is the usual storage-form of iron. Iron within ferritin will not stimulate free radical reaction (same source above). since antioxidant proteins, remove oxidized fatty acids from membranes, and repair free radical damage to DNA (some of the oxidized bases removed are excreted in urine. All these defences are largely intracellular.

Other antioxidant defences are largely extracellular. They include the plasma iron-transport-protein transferrin and the similar iron-binding-protein Lactoferrin found in many body secretions (eg, tears,nasal lining fluid); iron bound to these proteins can not catalyse free-radical damage. Ceruloplasmin in safe transport form of copper and it assists loading of iron on to transferrin. Haemopexin and haptoglobin bind free heme and heme proteins to minimize their ability to catalyse free radical damage. (Halli Well B, Gutteridge JMC,1990).

Albumin, which has one sulphhydryl group per molecule and is present at about 0.5 mmol/L in plasma can scavenge several radicals and binds copper ions.(Evans p.and HalliWell B,2001).GSH,,Urate,alpha-tocopherol & ascorbate remove free radicals by reacting directly with them catalytically

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Despite all these antioxidants, some free radicals still escape to do damage .thus DNA undergoes constant "oxidative damage" ,and has to be repaired .Free radical-damaged proteins are degraded. End products of lipid peroxidation (eg. The isoprostanes)and of free – radical attack on urate are present in vivo(Kaur h,HalliWell B.,1990).

Akey tenet of the oxidative stress theory of ageing is that levels of accrued oxidative damage increase with age . The aim of our study was first to obtain acomperhensive profile of the serum anti oxidant defense potential & peroxidative damage during ageing . We investigated total protein , albumin, uric acid , glutathione, catalase,& glutathione – s-transferase .

Materials and Methods:

To study the relationship between ageing and the changes in serum lipid peroxidation and antioxidant status, 84 healthy male subjects were used from different working places in baquba city, these subjects were divided into groups: group1 (n=28;25-34 years old); group 2(n=28;35-44 years old); group 3 (n=28;45-63 years old).

- Blood samples were obtained from all subjects and used in the analysis in Baquba hospital laboratory. Venous blood samples werw taken in morning which divided in to two parts part in the EDTA-anti- coagulated samples haematological test and the other part in test tube centrifuged at 3000 rpm for 5min at 4C° to obtain the serum.

Samples were analyzed for malondialdehyde (MDA) by mixed 1ml serum and 2ml thiobarbituric acid(TBA) reagent (o.375gm)thiobarbituric acid, 15gm trichloroacetic acid in 100ml of 0.25N HCL). Light absorbance of supernatant solution is determind at 535nm against blank.

Assay of serum GSH activity:

Glutathione contents in serum (measured as tottral sulfhydryl groups)were measured according to the method of Godin et al (1988), which is based on the reaction of GSH with dithionitro benzene at PH 8 to from yellowish color chromophore which absorbs light at 412 nm. The concentration of GSH was calculated using standard curve prepared for this purpose.

Assay of serum GSH Catalase activity:

Serum catalase activity was assayed according to the method of Aebi(1974), based on spectrophotometric follow-up for the decomposition of 1ml(30mM) h2o2 after rapid mixing with 100UL of serumat PH7.0 after 15 and 30 seconds, relative to control sample containing 1ml of phosphate buffer instead of H2O2.

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Assay of serum GST activity:

Glutathione-transferase activity was determined according to the spectrophotometric method of Itabig et al (1974). The enzyme activity was estimated by monitoring the change in absorbance at 340nm, as a result of conjugating glutathione with 1-color-2,4-dinitrobenzene (CDNB). Specific enzyme activity is defined as the amount of enzyme that catalyzes the formation of one mole of product/minute (which is termed 1 unit)/liter, and can be calculated from the molar absorptivity of the (CDNB) conjugate formed (9.6mole/L/cm).

Selected serum biochemical parameters were also determined in serum (table 1): total protein and uric acid by colorimetric methods: albumin by a photometric method using bromocresol green.

Haemoglobin by MSq instrument.

Statistical Analysis:

All data expressed as the mean value \pm SE. statistical analysis of the results were performed using ANOVA table. Duncan's test was used to compare the result between the groups.

Results:

The table shows elevated levels of serum MDA related to age but not significantly ($p > 0.05$). there were a significant elevated of the catalase and glutathione-transferase in group3 compared with other groups. Whereas there were a significant lowered in GSH between the groups related to aging. There was a significant decrease in a haemoglobin and significant elevated levels in albumin in group2 compared with other groups, whereas total protein and

Table (1) : selected biochemical parameters in the blood of male subjects related to age

Age (year)	25-----34		35-----44		45-----63	
Number	28		28		28	
	M	SE	M	SE	M	SE

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MDA Umol / L	1.318	0.156	1.759	0.16	1.576	0.161
Haemoglobin g/dl	13.6a	0.315	12.325b	0.28	13.971a	0.22
Total protein mg/dl	7.15	0.121	7.008	0.11	7.125	0.14
S.albumin g/dl	3.51 a	0.134	3.88b	0.11	3.47 a	0.09
S.uric acid mg/dl	5.63	0.196	5.78	0.22	5.99	0.25
S.glutathione um/l	0.274 a	0.016	0.22b	0.02	0.178 c	0.015
S. catalase um/l	5.721 a	0.532	4.617 a	0.46	7.2 b	0.508
S.glutathione-s-transferase	29.464a	2.267	29.264a	3.11	38.629b	4.338

Values represent mean + SE ,a,b,c represent significant different ($p < 0.05$)

Discussion:

Under normal conditions, there is a steady state balance between the production of oxygen free radicals and their destruction by the antioxidant systems. The oxygen free radicals, which accumulate via an imbalance generation and scavenging, are believed to induce many disease states. (GonenG a.et al,2002).

Elevated MDA serum levels might be the result of increased lipid peroxidation which an-intermediate product of the oxidation of polyunsaturated fatty acids(Baliga R.et al 1997).

Our data show that there is an age related increase in lipid peroxidation expressed as MDA and low levels of GSH in the circulation which may be due to their increased utilization to scavenge lipid peroxide: GSH acts as a substrate in the detoxification of peroxides like H₂O₂ and lipid peroxides, a reaction involving GPX. This generates oxidized GSH(i.e GSSG), which is subsequently reduced by GR in a reaction mtilising.

There weresignificant increased in catalase NADPH and GST in group3 compared with other groups.

Several enzymatic system can detoxify free radicals cooper/zinc-superoxide dismutase (SOD) catalyzes the conversion of the superoxide anion to hydrogen peroxide and works concomitantly with hydroperoxide, removing enzymes such as catalase and glutathione peroxidase.

The conversion of H₂O₂ to hydroxyl radical may be enhanced by iron, these mechanisms may contribute to the increased serum lipid peroxidation.

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GST provide protection from membrane lipid peroxidation, the increase in GST induction confers resistance to the cells against oxidative stress. (Montserrat M. et al 2001).

Albumin metal-binding proteins is shown to possess free radical scavenging properties, and may be selective antioxidants, in our study serum albumin concentrations showed significant increased in group2 compared with other group while haemoglobin in this group showed significant lowered with other groups.

No significant differences in serum total protein and uric acid with age.

Conclusion

Determination of lipid peroxidation and antioxidant status, may be useful test to evaluate oxidative stress.

The depletion of GSH seems to be a major determination of age effect, whereas increased catalase and GST provide a suitable mechanisms for detoxification.

Future studies are needed to clarify the relations between lipid peroxidation and antioxidative function.

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تأثير العمر على المتغيرات الحاصلة لأكسدة الشحوم ومضادات الاكسدة عند الذكور

حميد محمود مجيد

سعد عبد الرحمن حسين

كلية الصيدلة / جامعة بغداد

كلية التربية للعلوم الصرفة / جامعة ديالى

الخلاصة

ان تأثير فرط الاكسدة يزداد مع تقدم العمر ، مسببة تغيرات شكلية وفسلجية في الجسم مما يزيد من درجة حساسيتها للأمراض ، نتيجة زيادة تولد الجذور الاوكسجينية حيث تستطيع هذه الجذور من احداث الضرر بالغشاء الخلوي ممثلا باكسدة الشحوم ، ضعف الفعالية الانزيمية ، التغير الحاصل في عمليات الاكسدة والاختزال داخل الخلية ، بالإضافة الى الضرر الذي تحدثه في ال DNA .

تم جمع ٨٤ عينة من الدم الوريدي ممثلة لثلاثة مجاميع (٢٨ عينة / مجموعة) تراوحت اعمارهم بين (٢٥ - ٣٤) ، (٣٥ - ٤٤) و (٤٥ - ٦٣) سنة للمجاميع الثلاثة ١، ٣، ٢ على التوالي .

تم اجراء الاختبارات التالية على مصلى الدم :

١- MDA اختبار دال على اكسدة الشحوم مما يعطي فكرة دالة على زيادة الجذور الحرة بزيادته .

٢- تقدير مضادات الاكسدة الانزيمية مثل الكاتليز ، الكلوتاثيون ترانسفيريز لمعرفة الاستنزاف الحاصل بها نتيجة تأثير هذه الجذور . وكذلك بمضادات الاكسدة غير الانزيمية مثل الكلوتاثايون ، البروتين الكلي ، وحامض اليوريك ، بالإضافة الى تقدير متوسط الهيموكلوبين في الدم الكامل . اظهرت النتائج وجود زيادة ملحوظة في انتاج الجذور الحرة وانخفاض في ميكانيكية الدفاع المضاد للتاكسد عند مستوى احتمالية ٥% للكلوتاثايون ، مما يدل على ان الضرر التاكسدي يزداد بتقدم العمر ، حيث ان الكلوتاثايون هو المضاد التاكسدي الاكثر تاثرا بالجذور الحرة ، ووجوده بكميات متدنية في الدوران ربما يعود الى زيادة الاستفاد منه في ازالة البيروكسيدات . كما ان هناك مستويات مرتفعة من الكاتليز والكلوتاثايون ترانسفيريز في المجموعة الثالثة مقارنة ببقية المجاميع . المجموعة الثانية اوضحت مستوى منخفض من الهيموكلوبين ومستوى مرتفع من الالبومين مقارنة مع بقية المجاميع الاخر ($p < 0.05$) . ولا توجد فروقات معنوية في البروتين الكلي لمصلى الدم وحامض اليوريك بين المجاميع .

الكلمات المفتاحية : العمر ، اكسدة الشحوم ، مضادات الاكسدة