DAMAGE AND REPAIR OF HUMAN LYMPHOCYTE DNA UNDER ACTIVATION OF MOLECULAR OXYGEN

B. Binkova, G. Topinka,
R. Shram, R. R. Lideman,
and A. N. Erin

UDC 612.112.94.015.3:547.963.32/.06:612.262

KEY WORDS: DNA; repair; activated oxygen.

Activation of molecular oxygen is one of the principal mechanisms of modification of cellular structures. The phenomenon of modification of cell membranes as a result of peroxidation of membrane lipids has been studied the most [1]. However, molecular oxygen is not always activated directly in biomembranes (as when under the influence of cytochrome P-450 or monoamine oxidase) [2, 3]. It can be activated also in the cytoplasm and, in particular, during function of aldehyde and xanthine oxidases [8], during auto-oxidation of some low-molecular-weight compounds such as adrenalin [10], during interaction of oxygen with metals of transitional valency [1], and so on. In these cases activated oxygen can interact with components of the cytoplasm. Such interaction has been demonstrated for cytosol enzymes involved in oxygen activation [8]. In this connection, and considering the key role of the genome in the maintenance of cellular homeostasis, the solution to the problem of the possibility of interaction of activated oxygen with the genetic apparatus of the cell is of undoubted interest.

We have studied the intensity of DNA repair in cells of a short-lived culture of human lymphocytes during activation of oxygen.

EXPERIMENTAL METHOD

Lymphocytes were obtained from the blood of healthy donors (men aged 20-22 years) by differential centrifugation by the method in [4]. The lymphocytes were washed with medium of the following composition: 0.01% glucose, 50 μM $CaCl_2$, 0.98 mM $MgCl_2$, 5.4 mM KCl, 126 NaCl, 145 mM Tris, and kept in short-term culture in medium PM-1640. The protein concentration of the lymphocytes was determined by Bradford's method [5]. The level of reparative DNA synthesis of the lymphocytes was estimated by measuring uptake of ^{3}H-thymidine by the method in [9] with some modifications: hydroxyurea (10 mM) and 5-fluorodeoxyuridine (2 μM) were used as inhibitors of replication synthesis. The concentration of ^{3}H-thymidine was 10 μCi/ml. Reparative synthesis was expressed as radioactivity (cpm) per microgram of DNA. Radioactivity was measured on a Beckman Sl-5801 liquid scintillation counter (Austria). The DNA concentration was measured by a colorimetric method using a diphenylamine reagent [9]. The control level of reparative DNA synthesis in short-term lymphocyte fultures varied depending on the donor from 30 to 100 cpm/μg DNA. When studying the effects of different factors on reparative DNA synthesis, its changes were therefore expressed as M ± m, in percent of the control level. The intensity of lipid peroxidation (LPO) was estimated as accumulation of malonic dialdehyde (MDA). The MDA concentration was measured colorimetrically by the reaction with 2-thiobarbituric acid [11]. The control level of MDA also varied in lymphocytes from different donors within limits of 1.1 ± 0.4 nmoles/mg protein. Changes in the MDA level were therefore also expressed as percentages of the control level. The spectroscopic measurements were made on a Specord M40 spectrophotometer (East Germany). The following reagents were used: hydroxyurea, 5-fluorodeoxyuridine, methyl-N-nitro-N'-nitrosoguanidine, Tris, D,L-α-tocopherol, and Na ascorbate were obtained from Serva, West Germany; PColl 400 from Pharmacia, Sweden; 2-thiobarbituric acid from Reakhim, USSR; medium RPMI-1640 from Usol, Czechoslovakia, 6-^{3}H-thymidine from UVVVR, Czechoslovakia), and SLD scintillation fluid from Spovena, Czechoslovakia. The remaining reagents were of the analytical grade and of Czechoslovak origin.

Central Institute of Psychiatry, Ministry of Health of Czechoslovakia, Prague. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 675-678, December, 1988. Original article submitted October 15, 1987.

0007-4888/88/0012-1705$12.50

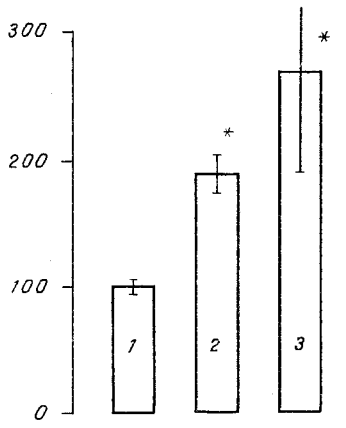

Fig. 1. Effect of oxygen activation and action of a mutagen on incorporation of ^{3}H-thymidine into DNA fraction of human lymphocytes. 1) Control, 2) Fe^{++} (2 μM) and ascorbate (30 μM), 3) methyl-N-nitro-N'-nitrosoguanidine (10^{-4} M). Variations in repeats of lymphocytes from the same donor taken into account in the control. *) Significance of difference from control, $p < 0.001$. Ordinate, incorporation of ^{3}H-thymidine (in %).

EXPERIMENTAL RESULTS

Reparative synthesis in short-term lymphocyte culture reflects the level of injuries in DNA that are repaired by the cell. In the experiments of series I, to assess the action of activated oxygen on DNA we therefore studied changes in the rate of repair under the influence of Fe^{++} and ascorbate as an oxygen activation system. As the results in Fig. 1 show, during incubation for 30 min at 37°C with Fe^{++} and ascorbate the level of repair of the lymphocyte DNA rose to twice the control level, reflecting damage to DNA associated with oxygen activation. How severe this damage is can be judged from the fact that the rise of the repair level under the influence of Fe^{++} and ascorbate is comparable with the effect of near-maximal doses of the chemical mutagen methyl-N-nitro-N'-nitrosoguanidine, at which death of the cells does not yet occur [9] (see Fig. 1). It is important to note that the effect of DNA damage under the influence of Fe^{++} and ascorbate is achieved under conditions of not very effective oxygen activation: The level of LPO products in the lymphocyte membranes was increased only twofold compared with the steady-state level. Damage to membrane structures is observed in the presence of much higher intensities of LPO.

Damage to DNA can be linked both with the direct action of activated oxygen on DNA and with its indirect effect — through the formation of lipid radicals. To solve this problem the following technique was used. We know that ascorbate in high concentrations is an antioxidant, protecting lipids of biomembranes [6]. It must therefore be expected that with a fall in the concentrations of Fe^{++} and ascorbate, LPO may be intensified, although the effectiveness of oxygen activation will be reduced. In fact, as Table 1 shows, if the concentrations of Fe^{++} and ascorbate were reduced by half, increased accumulation of MDA was observed, whereas the amount of DNA repair, reflecting the degree of its damage was reduced by 50%. These results are evidence that activated oxygen interacts directly with DNA.

This conclusion was confirmed by the results of the next series of experiments. As Table 1 shows, addition of the lipophilic antioxidant α-tocopherol to a culture of human lymphocytes leads to virtually complete inhibition of LPO, whereas the repair level fell by only 20% through the action of Fe^{++} and ascorbate. This proves that the action of activated oxygen on DNA is not mediated through the generation of lipid radicals. The effect of partial protection of DNA by α-tocopherol in the presence of oxygen activation is in good agreement with views that vitamin E can perform a chromatin stabilizing function in the cell [11].

Oxygen activation is thus a powerful factor causing damage (modification) to DNA. We know that the effectiveness of repair processes can never be absolute. It can be tentatively suggested that this factor plays an important role for tissues with low mitotic activity, in

TABLE 1. Effect of Fe^{++} and ^3H-Thymidine Incorporation into DNA Fraction and on MDA Accumulation under the Influence of Fe^{++} and Ascorbate

Experimental conditions	^3H-thymidine uptake, % of control	MDA concentration, % of control
Control	100±5,5	100
Fe^{2+} + ascorbate	191±14	211
Fe^{2+} + ascorbate + tocopherol	172±8	104
α-Tocopherol	106±10	100

Legend. Concentrations: Fe^{++} 2 μM, ascorbate 30 μM, α-tocopherol 10^{-4} M.

which the intensity of repair processes is depressed [7]. In our view, therefore, the study of processes of this kind in brain neurons in the postmitotic phase, which is characterized by low activity of reparative enzymes, and the partial oxygen consumption is very high, may therefore be particularly interesting.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. V. Z. Gorkin, Mol. Biol., 10, No. 4, 717 (1976).
3. C. Auclair, M. Jones, and J. Hakim, FEBS Lett., 89, 26 (1978).
4. A. Böyum, Scand, J. Clin. Lab. Invest., 21, Suppl., 97 (1968).
5. M. M. Bradford, Anal. Biochem., 72, 248 (1975).
6. G. W. Burton and K. U. Ingold, Acc. Chem. Res., 19, 194 (1986).
7. J. E. Cleaver, Biochim. Biophys. Acta, 516, 489 (1978).
8. R. E. Lynch and I. Fridovich, Biochim. Biophys. Acta, 571, 195 (1979).
9. C. N. Martin, A. C. McDermid, and R. C. Garner, Cancer Res., 38, 2621 (1978).
10. H. P. Misra and I. Fridovich, J. Biol. Chem., 247, 3170 (1972).
11. R. N. Patnaik and P. P. Nair, Arch. Biochem., 178, 333 (1977).

^{31}P-NMR SPECTROSCOPY OF HUMAN LIVER AND BILE

D. S. Khristianovich, V. I. Reshetnyak
A. S. Loginov, V. E. Yushmanov,
M. A. Tumanyan, and L. A. Sibel'dina

UDC 612.351+612.357.1/.088.1:543.422.23

KEY WORDS: ^{31}P-NMR spectroscopy; liver; bile; orthophosphate; lecithin.

The study of the composition and physicochemical properties of bile, a product of the external secretory activity of the liver, can be used to evaluate both the synthetic and the excretory function of the liver. In particular, changes in the concentration of one component of native bile — phosphatidylcholine (PCh) — reflect changes in its acid-dependent (dependent on secretion of bile acids) secretion by the hepatocyte [3, 7, 8]. Meanwhile, secretion of inorganic phosphorus (P_i, orthophosphate) and of other inorganic ions is acid-independent (does not depend on secretion of bile acids), and is effected by hepatocytes along the concentration gradient [1]. As a result of this, the P_i concentration in bile is directly linked with its concentration in the hepatocytes and, consequently, it depends on the level of metabolic processes taking place in the liver cells with the participation of orthophosphate.

Institute of Chemical Physics, Academy of Sciences of the USSR. Central Research Institute of Gastroenterology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. S. Loginov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 678-681, December, 1988. Original article submitted June 15, 1987.

0007-4888/88/0012-1707$12.50