



Title	Radiation-induced DNA damage : Analysis of the "quasi-direct" effect by evaluating the generation of 8-hydroxyguanine, alkali-labile sites and DNA strand breaks in $\gamma$ -irradiated frozen aqueous solutions of DNA at 77 K
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some autophagic vacuoles or free in the cytoplasm. In addition, virus-containing electron-dense bodies were found in the cytoplasm, which contained varying numbers of viral particles within fine electron-dense granular matrix and were surrounded by a single limiting membrane. Viral particles were released by exocytosis through cytoplasmic vesicles or appeared to be discharged through disrupted cell membranes.

It is concluded that epithelial cells of the lower nephron and ducts are the primary target cells in IBV-infected kidneys.

Lastly, in order to study the effect of infectious bursal disease virus (IBDV) on the pathogenesis of renal lesions caused by IBV infection, the MA-87 strain of IBV was inoculated intra-tracheally into 14-day-old SPF chicks or ones previously inoculated with highly virulent IBDV at 7 days of age. The renal lesions were examined histopathologically and immunohistochemically at intervals up to 30 days PI.

At the early stage of infection, the histo-

pathological changes in the kidneys were similar in both groups, but the ducto-tubular changes were more severe in the dually infected chicks. At the late stage of infection, the renal lesions were characterized by chronic interstitial nephritis with formation of lympho-plasmacytic follicles in IBV-inoculated chicks and by chronic active nephritis which consisted of tubular degeneration, lymphoid cell reaction and interstitial fibrosis in IBDV+IBV-inoculated ones.

The IBV antigen-positive cells were first detected in the trachea. Subsequently, these were seen in the kidneys, particularly in the CD, CT and DCT. The IBV antigen were of greater numbers and persisted longer in the kidneys of dually infected chicks than in those of IBV-inoculated ones. The antigen was also detected in the renal epithelial cells in chronic active renal lesions of IBDV+IBV-inoculated chicks.

These findings suggest that the pathogenicity of nephropathic strains of IBV is increased in chicks previously infected with IBDV.

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Radiation-induced DNA damage: Analysis of the "quasi-direct" effect by evaluating the generation of 8-hydroxyguanine, alkali-labile sites and DNA strand breaks in  $\gamma$ -irradiated frozen aqueous solutions of DNA at 77 K.

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Ionizing radiation induces DNA damage generally through two types of pathways; one is called the "direct" and the other the "indirect" effect. In the direct effect, ionizing radiation induces both electron-loss centers on DNA through the ejection of electrons at purine bases

and electron-gain centers through the capture of the electrons ejected by pyrimidine bases. The indirect effect is caused by the reactions of DNA with hydroxyl radicals ( $\cdot\text{OH}$ ), hydrated electrons ( $e^-_{\text{aq}}$ ) and, to a small extent, atomic hydrogens ( $\cdot\text{H}$ ) formed by the radiolysis of water surround-

ing DNA. However, recent studies indicated that two different types of water exist in aqueous DNA solution, "free (or bulk) water" constituting a large proportion of water surrounding DNA and "bound water" in the hydration layer of DNA. The mechanism for damaging DNA originating from the radiolysis of bound water is called a "quasi-direct" effect because its radiolysis is thought to cause DNA damage in different way from that of free water. Therefore, it is important to fully clarify the mechanism of the quasi-direct effect to induce DNA damage. Since the free water is frozen to form ice crystals and the bound water in the hydration layer of DNA forms a glassy structure at 77 K,  $\gamma$ -irradiation at this temperature is expected to induce the quasi-direct effect on DNA in addition to the direct effect on DNA. For this reason, aqueous DNA solutions were exposed to  $\gamma$ -rays in the frozen state at 77 K and subsequently analyzed in the thawed solutions by measuring 8-hydroxyguanine, alkali-labile sites and DNA strand breaks.

As preliminary experiments, X-irradiation was carried out with deoxyguanosine (dGuo) as a DNA model at room temperature. The spin-trapping method using  $\alpha$ -phenyl-N-tert-butyl nitron (PBN) as a spin trap, was employed to obtain fundamental information about the mechanisms of radiation-induced degradation of water as well as alteration of dGuo. For this purpose, aqueous solutions of dGuo were X-irradiated in the presence or absence of PBN. The HPLC-ECD method was also employed to measure 8-hydroxydeoxyguanosine (8-OHdG) to observe the effects of PBN on the radiation-induced alteration of dGuo. The ESR observation provided evidence of production of both  $\cdot\text{OH}$  and  $\cdot\text{H}$  in the aqueous solution of dGuo, but not for that of dGuo radicals. The amount of  $\cdot\text{OH}$  trapped by PBN was reduced by dGuo, whereas the amount of  $\cdot\text{H}$  trapped by PBN was increased by dGuo. The measurements of 8-OHdG with

HPLC-ECD showed that the alteration of dGuo to 8-OHdG was enhanced by PBN. From the quantitative comparison of both increased  $\cdot\text{H}$  and 8-OHdG, the following reactions were inferred as the mechanisms of the alteration of dGuo to 8-OHdG: (1) the attack of  $\cdot\text{OH}$  on dGuo to induce the dGuo base radical, and (2) electron and subsequent proton transfer reactions from the dGuo radical to PBN to form 8-OHdG.

Next, experiments were carried out at 77 K to evaluate the quasi-direct effect. Frozen aqueous DNA solutions with or without PBN were exposed to  $\gamma$ -rays at 77 K. After thawing the solutions, ESR, HPLC-ECD and agarose gel electrophoresis experiments were carried out to examine what kinds of water radiolysis products were induced in the frozen aqueous solutions of DNA and whether the resulting products acted as an inducers of DNA strand breaks, base alterations and alkali-labile sites. The ESR signals from PBN-OH adducts and PBN-H( $e^-$ ) adducts were observed after irradiating the solution containing PBN and DNA, whereas few signals were present in the solution containing PBN alone, suggesting that reactive  $\cdot\text{OH}$  and  $e^-$  (or  $\cdot\text{H}$ ) were produced in the glassy layer (the hydration layer of DNA) but not in ice crystals (free water surrounding DNA). Examination of the solutions with HPLC-ECD showed that PBN suppressed the formation of 8-OHdG by scavenging  $\cdot\text{OH}$  formed in the glassy layer. Agarose gel electrophoresis of irradiated DNA showed that PBN had no effect on the formation of strand breaks. These results indicated that  $\cdot\text{OH}$  generated in the hydration (glassy) layer of DNA induced 8-OHdG but not strand breaks of DNA. As for the  $e^-$  (or  $\cdot\text{H}$ ) generated in the hydration layer of DNA, since the amount of radiation-induced alkali-labile sites was found to be enhanced by PBN, the high electron affinity of this compound was inferred to bring about the increase of alkali-labile sites by scavenging  $e^-$  (or  $\cdot\text{H}$ ) and thereby increasing the amount of DNA

base cation radicals.

The present study demonstrated that  $\cdot\text{OH}$  and  $e^-$  (or  $\cdot\text{H}$ ) were produced not only in bulk water but also in the hydration layer of DNA (DNA-bound water). However, though  $\cdot\text{OH}$  generated in the hydration layer could produce base damage such as 8-OHdG, they could not produce DNA strand breaks. Since scavenging of  $e^-$  (or  $\cdot\text{H}$ ) generated in the hydration layer

resulted in an increase of alkali-labile sites, these reactive species might negatively participate in the formation of alkali-labile sites. These results concerning the reactivities of  $\cdot\text{OH}$  as well as  $e^-$  (or  $\cdot\text{H}$ ) in the hydration layer will be helpful for understanding the mechanisms of radiation-induced damage in DNA in an intact environment with a small content of free water.

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### Mechanisms of catecholamine secretion and elevation of intracellular $\text{Ca}^{2+}$ concentration induced by muscarine and purine receptor in adrenal chromaffin cells of guinea pig

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Effects on catecholamine secretion and intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) of muscarine and purine receptor activation were examined using perfused adrenal glands and dispersed chromaffin cells of the guinea pig.

1. Muscarine (1–300  $\mu\text{M}$ ) and pilocarpine (10–300  $\mu\text{M}$ ) caused a dose-dependent increase in catecholamine secretion from perfused adrenal glands. Muscarine was much more effective than pilocarpine. In dispersed adrenal chromaffin cells, all muscarinic agonists tested caused increases in catecholamine secretion in a dose-dependent manner. Muscarine and methacholine were more effective than bethanechol, oxotremorine and pilocarpine.

2. Muscarine caused a small increase in catecholamine secretion even in the absence of extracellular  $\text{Ca}^{2+}$  in both perfused adrenal glands and dispersed chromaffin cells.

3. Both 4-DAMP (0.1  $\mu\text{M}$ ) and pirenzepine

(0.1  $\mu\text{M}$ ), but not methoctramine (0.1  $\mu\text{M}$ ), shifted the dose-response curve for muscarine-induced catecholamine secretion to the right and inhibited increase in  $[\text{Ca}^{2+}]_i$ .

4. ATP (2–10 mM) caused a dose-dependent increase in catecholamine secretion from perfused adrenal glands. ADP, but neither AMP nor adenosine, was also effective in increasing catecholamine secretion, though its potency was much less than that of ATP.

5. ATP-induced secretory response was also observed under  $\text{Na}^+$  deficient conditions, but was reversibly abolished by removal of extracellular  $\text{Ca}^{2+}$ . In dispersed chromaffin cells, ATP (500  $\mu\text{M}$ ) caused increases in catecholamine secretion and  $[\text{Ca}^{2+}]_i$ , both of which were abolished after the removal of extracellular  $\text{Ca}^{2+}$ .

6. In fura-2 loaded-single chromaffin cells, KCl (40 mM), nicotine (100  $\mu\text{M}$ ) and muscarine (100  $\mu\text{M}$ ), all caused a rapid increase in  $[\text{Ca}^{2+}]_i$