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BACKGROUND LEVELS OF DNA DAMAGE

DAVID S. BERGTOLD and MICHAEL G. SIMIC

National Institute of Standards and Technology, Gaithersburg, MD 20899 U.S.A.

Even without external insults from genotoxic agents, DNA *in situ* might be damaged via undesirable side reactions of normal metabolic processes. We have begun to examine this phenomenon to elucidate the fundamental relationship between endogenous DNA damage, mutagenesis, carcinogenesis, and aging. One promising avenue has been to identify oxidatively altered DNA bases generated under normal conditions and to monitor the removal of these compounds from both the cell and the body, an approach which requires consideration of the mechanisms of formation, excision, and excretion of the products. Complementary information has been obtained by exploration of the effects of metabolic rate and free radical generating genotoxic agents on levels of DNA damage and on excretion of corresponding products.

Examples of possible free radical mechanisms by which DNA might be damaged endogenously include the Haber-Weiss-Fenton reaction and other autoxidation processes.[1] The concept can be described in general by equations 1-3, where ·R is any free radical, P and P′ represent products and P(R) represents products which have incorporated ·R.

$$\text{Metabolic Processes or Genotoxic Agents} \longrightarrow \cdot R \tag{1}$$

$$\text{DNA} \xrightarrow{\cdot R} \cdot\text{DNA-R} \longrightarrow \text{DNA-R} \xrightarrow{\text{Repair Enzymes}} \text{DNA} + \text{P(R)} + \Sigma\text{P} \tag{2}$$

$$\text{DNA} \xrightarrow{\cdot R} \cdot\text{DNA} \longrightarrow \text{DNA}' \xrightarrow{\text{Repair Enzymes}} \text{DNA} + \text{P}' + \Sigma\text{P} \tag{3}$$

Some genotoxic agents, such as alkylating agents, do not go through free radical steps but produce DNA adducts,

$$\text{DNA} \xrightarrow{RX} \text{DNA-R} \xrightarrow{\text{Repair Enzymes}} \text{DNA} + \text{P(R)} + \Sigma\text{P} \tag{4}$$

where R = the alkyl group and P(R) is the resulting excised adduct.

One factor that all of the above reactions have in common is that they may lead to the excretion of specific compounds in the urine, e.g.,

$$\text{P(R)} \xrightarrow{\text{Metabolism}} \text{P(R)}'_{\text{metab}} \xrightarrow{\text{Excretion}} \text{P(R)}'_{\text{urine}} \tag{5}$$

Monitoring the urine for specific products of hydroxyl radical addition to nucleic acid bases was suggested originally by Ames and colleagues,[2] and has been confirmed in our laboratory,[3] as a potentially useful way to measure background levels of DNA damage in different species. Studies from both laboratories demonstrate a relationship between metabolic rate and endogenous base damage levels,[2-5] although we find that DNA damage correlates better with life span potential, an indicator of aging

rate.[6] Hypotheses that urinary excretion of DNA base damage products results only from dead cells or cells sloughed from the bladder wall are not sufficient to explain either the species differences or the quantity of products.

We have extended our earlier work to the examination of urinary excretion of thymidine glycol (dR-TG) in mice[7] exposed to whole body doses of ionizing radiation or humans undergoing cancer radiotherapy.[8] The study was based on the premises that (1) since a large proportion of mammalian tissue is water, substantial amounts of OH radicals are generated in the cellular milieu by ionizing radiation and (2) reaction of ·OH with DNA yields numerous specific products, including thymidine glycol, which are excised by repair processes as either the free base (e.g. thymine glycol) or nucleoside (e.g. dR-TG) moieties and then are excreted unaltered in the urine.

The data of Table 1 reveal that mice normally excrete greater levels of dR-TG in the urine than humans. We postulate that these results reflect an elevated background of DNA alteration in the mouse, so that generation of damage by genotoxic agents to levels sufficiently above background for reliable detection would require greater doses in the mouse than in humans. If the rate of metabolic activity is primarily responsible for the endogenous induction of DNA damage, then the production of similar damage by external agents should not be particularly species-dependent and should be additive with existing lesions.

These two predictions are supported by the observations that a given dose of ionizing radiation causes a smaller relative effect in the mouse and that the amount of thymidine glycol excreted per unit of radiation dose is the same in both species (Table 1). We conclude that total damage has an endogenous component due to metabolic processes and an exogenous component due to genotoxic agents and processes,

$$(\text{dR-TG})_{\text{total}} = (\text{dR-TG})_{\text{endo}} + (\text{dR-TG})_{\text{exo}} \quad (6)$$

where the level of $(\text{dR-TG})_{\text{endo}}$ is dependent on metabolic rate, while the level of $(\text{dR-TG})_{\text{exo}}$ is a function of the degree of exposure to external agents. Furthermore, increased excretion of dR-TG in irradiated individuals supports the conclusion that radiation-generated OH radicals can produce thymidine glycol *in vivo* and substantiates experimentally the hypothesis[1] that endogenous dR-TG may result from metabolically generated hydroxyl radicals.

TABLE 1
Yield of thymidine glycol excreted in the urine before and after exposure to ionizing radiation.

Species	Dose (Gy)	Excreted dR-TG (nmol/kg per day)	Yield of dR-TG (nmol/kg/day/Gy)
Man	0	0.3 ± 0.1	
			0.67 ± 0.2
	1.8	1.5 ± 0.5	
Mouse	0	7.2 ± 1.1	
			0.61 ± 0.2
	9.0	12.7 ± 3.1	

Error values are standard deviations of multiple measurements for 8 mice and 2 humans. The entire body of each mouse was exposed to ^{60}Co gamma photons and the data are from samples collected during the time period from 12–24 hours after exposure; the human subjects were exposed only to limited fields as part of a cancer radiotherapy regimen and the data are for samples collected over the time period from about 12–20 hours after the first exposure.

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