Role of Lead-binding Proteins in Renal Cancer

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High-dose lead exposure in rodents has been shown to produce pathognomonic lead intranuclear inclusion bodies and to result in an increased incidence of renal adenocarcinomas. Studies from this laboratory and others have demonstrated the presence of high-affinity renal lead-binding proteins in rat kidneys which act as tissue sinks for lead at low dose levels. Cell-free nuclear translocation studies have shown that these molecules are capable of facilitating the intranuclear movement of lead and that they are associated with chromatin. These data suggest that renal lead-binding proteins may play a role in mediating known alterations in renal gene expression associated with formation of intranuclear inclusion bodies. More recent studies from this laboratory have demonstrated the presence of chemically similar lead-binding proteins in kidneys of both monkeys and humans. Such observations suggest that a similar mechanism may be operating in primates since lead intranuclear inclusion bodies are also observed in these species. These data provide a testable mechanistic approach for assessing the possible role(s) of lead-binding proteins in mediating the intranuclear movement of lead and lead-induced renal cancer in primate species. — Environ Health Perspect 102(Suppl 3):115–116 (1994).

Key words: lead, lead-binding proteins, DNA binding, nuclear translocation, altered gene expression, oncogenes, renal cancer

Introduction

Chronic exposure to high dosages of lead has been known for many years to produce a marked increase in the incidence of renal adenocarcinoma in rodents (*1,2*). However, the lower end of the dose-response curve or the underlying mechanism(s) for this effect have not been delineated. The possible relevance of these data to renal adenocarcinomas in humans is not clear. Studies have shown that chronic exposure to lead acetate or acute intravenous injection of lead in rats and mice produces a marked stimulation of mitosis in renal proximal tubule cells. Such exposures are also associated with increases in total DNA, RNA, and protein synthesis in the kidneys of these animals (*3–5*). More recent intravenous injection studies (*6*) have shown that there is both up- and down-regulation of a number of gene products in rat kidneys which is temporally linked with the reversible formation of lead-induced intranuclear inclusion bodies. In other words, there is a clear relationship between the presence of lead within the nucleus and a number of specific alterations in renal gene expression.

At present, the exact molecular mechanisms underlying the above processes are unclear. It has been hypothesized (*7,8*) that kidney-specific lead-binding proteins (*8,9*), which are capable of facilitating the intranuclear movement of lead and its binding to DNA (*6,10*), may act as tissue-specific "receptors" for lead; this may explain why only certain cells in certain tissues are preferentially affected by this metal. The renal lead-binding protein in rats was found to be an N-terminal cleavage product of α-2μ-globulin (*8*), which underwent aggregation in the presence of lead (*11*). This suggests that the protein plays an early role in the formation of lead inclusion bodies in both the cytoplasm and the nucleus.

More recently, studies in this laboratory have demonstrated the presence of chemically similar but immunologically distinct renal lead-binding proteins in monkeys (*12*) and humans (*13,14*). The presence of these lead-binding molecules in primate species suggests that they may play a similar role with regard to mediating the low-dose effects of lead in the kidney. Research is in progress to identify these proteins in primates via N-terminal sequence analyses so that a clearer picture of how the proteins relate to those in rodents may be established. This information may provide a useful "bridge" between rats, monkeys, and humans for risk assessment (*12*), and may provide a mechanistic basis for determining possible linkages between lead-induced renal adenocarcinomas in rodents and in workers exposed to lead (*15–17*).

The role of lead-binding proteins in mediating renal cancer in rodents (and possibly in primates) may be explored through the following working hypothesis: the renal carcinogenic effects of lead are mediated through a group of processed kidney-specific proteins which act as receptors for lead and facilitate its intranuclear movement and interactions with DNA in renal proximal tubule cells. This in turn leads to cancer via chronic altered renal gene expression, including expression of some oncogenes, in those cells.

The probability of such an event occurring in a given individual is thus directly proportional to the tissue concentrations of the lead-binding proteins in the target cell population (renal proximal tubule cells) and not only the administered dosage or total tissue concentration of lead. A diagrammatic representation of this hypothesis is presented in Figure 1. This hypothesis is based upon studies in rats, and it is not clear whether the proteins identified in monkeys and humans behave in a similar manner.

Two lines of evidence that support this working hypothesis, aside from that listed in the introduction, are the following:

(a) Lead-binding proteins are found in highest concentrations in target cell populations. Immunohistochemical studies of renal proximal tubule cells using a polyclonal antibody showed that only certain clusters of cells stained positive for α-2μ-globulin (*8*), indicating that not all cells contained this protein. This is consistent with the idea that cancerous cells arise from discrete loci within proximal tubule cell populations. Intercellular differences in the presence of α-2μ-globulin may have a physiologic basis, since only certain nephrons are operational under normal conditions. Further, the observed presence of α-2μ-globulin in nuclear and cytoplasmic areas, in addition to lysosomes, is consistent with

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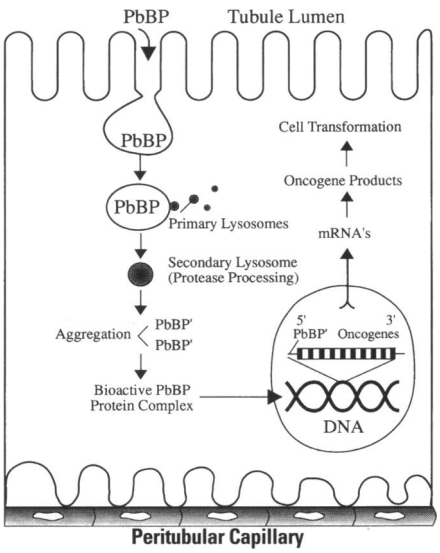

Figure 1. Diagram of renal proximal tubule cell showing hypothesized mechanism by which reabsorbed lead-binding proteins could alter renal gene expression, activate oncogenes, and ultimately lead to cellular transformation.

the idea that certain populations of proximal tubule cells preferentially take up this protein because they possess the necessary receptors on their apical surfaces.

(b) The renal lead-binding protein facilitates the intranuclear transport of lead and binding to DNA. Cell-free nuclear translocation studies have shown that the renal lead-binding protein facilitated the intranuclear transport of lead into renal nuclei, and they provided evidence of chromatin binding of the lead-binding protein complex (*6,10*). Binding of lead to the lead-binding protein and the nuclear uptake of this complex was also effected by *in vitro* levels of cadmium and zinc. Cadmium greatly inhibited the nuclear uptake of lead-binding protein, while zinc actually increased nuclear uptake. This effect of cadmium is consistent with other *in vivo* studies in which intranuclear inclusions did not form in renal proximal tubule cells from rats concurrently exposed to both lead and cadmium, while they did form in animals receiving only lead (*18*).

These observations, combined with the data of DuVal et al. (*11*) indicate that the renal lead-binding protein may be the mechanism by which lead enters renal proximal cell nuclei, and that the lead-binding protein aggregation phenomenon observed *in vitro* is an initial step in the formation of intranuclear inclusion bodies. The observation that zinc is a highly effective competitor to lead and facilitates the nuclear uptake of lead-binding protein suggests that it is the normal metal cofactor for this protein. This is consistent with some studies of hormone receptors, in which zinc enhanced the nuclear uptake of specific target molecules.

Conclusions

The above-mentioned data suggest that the renal lead-binding protein shares a number of properties with receptors for other biologically active molecules such as hormones. It may be that the lead-binding protein (in rodents) acts in a similar manner as those other biologically active molecules, and that lead substitutes for zinc, thereby altering its normal biological activity. Implicit in this idea is the concept that this metal-binding protein(s) is normally acting as an interorgan system messenger capable of regulating renal gene expression. This brief overview is intended to provide a testable hypothesis for explaining how lead could produce renal cancer in rodents, and perhaps in primates as well, by leading to the expression of elevated levels of these metal-binding proteins.

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