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Letter to the Editor

Correspondence re: R. H. Earhart *et al.* Improvement in the Therapeutic Index of Cisplatin (NSC 119875) by Pharmacologically Induced Chloruresis in the Rat. Cancer Res., *43:* 1187–1194, 1983[1]

In the March 1983 issue of *Cancer Research*, Earhart *et al.* report that the therapeutic index of cisplatin is improved after rats drink 0.9% sodium chloride solution, receive injections of ammonium chloride, and are given thiomerin (2). The authors conclude that it is the high urine chloride produced by such maneuvers which, by virtue of its inhibition of the production of toxic aquated metabolites of cisplatin, is responsible for reduced nephrotoxicity. However, several defects in these studies need to be addressed before we accept this view too hastily.

The first serious problem in the design of the study is that it did not provide adequate controls for potentially important effects of the diets and drugs used on cisplatin nephrotoxicity. For example, thiomerin given to the chloruretic group may protect rats. Furosemide, a diuretic with a similar site of action as thiomerin (16), is known to partially protect rats from cisplatin nephrotoxicity (11). The mechanism for its protective effect is unknown, but it is generally believed that the diuresis it produces is important for this effect (8). Although the authors conclude, on the basis of 24-hr urine collections (data not presented), that chloruretic animals do not have larger urine volumes, the data in Table 1 clearly show that this is not so when urine is collected over a shorter time period. Rats given thiomerin have 3 times higher urine volume. As cisplatin is given with thiomerin, this diuresis encompasses the time in which the rate of cisplatin excretion and renal accumulation is maximal (9, 14). It is well known that the nephrotoxicity of cisplatin (11), and many other chemically dissimilar nephrotoxins (8), can be ameliorated by the production of high rates of urine and solute excretion. The chloride-deprived group, who do not receive chloride supplementation, ammonium chloride, or thiomerin and become more azotemic after cisplatin, may have several changes in cellular and extracellular fluid composition that could potentiate cisplatin nephrotoxicity independent of any effect of urinary chloride. For example, rats fed a low-chloride diet and given sodium phosphate (1.2%, no pH indicated) may be intravascularly volume depleted, hypereninemic, hypokalemic, hypocalcemic, and alkalotic (3, 10); have significant changes in intracellular ion composition (3); have reduced whole kidney and single nephron glomerular filtration rate; and have enhanced tubuloglomerular feedback responses (10). None of these changes was adequately assessed in these studies. Furthermore, gentamicin, a nephrotoxin chemically unrelated to cisplatin and excreted unchanged in the urine (17), produces more renal failure in the background of chloride depletion as shown in a recent report by Kahn (4). To conclude that, of all the possible changes produced by chloride deprivation, it is only the change in urine chloride concentration that is responsible for the increased nephrotoxicity of cisplatin is extremely hazardous.

A second serious problem in the study is the authors' interpretation of the morphological studies. The authors state that the tissue section shown in Fig. 4, p. 1194, shows predominantly collecting duct and distal tubule necrosis. This figure is a section through the inner cortex and outer stripe of the outer medulla. The most numerous tubular structure at this level of the kidney is the proximal straight tubule (5). This is true for several reasons. (*a*) The proximal straight tubules of superficial nephrons descend together in the outer stripe among the proximal tubules of juxtamedullary nephrons. (*b*) Straight proximal tubules have taller cells than the straight distal tubules. (*c*) At least 6 distal tubules empty into a single collecting duct above this region, so there are fewer collecting ducts compared to proximal segments (6). Although the degree of necrosis in this section obscures characteristic features which might help to identify the segment damaged, it is not possible that collecting ducts and distal tubules alone could account for the number of tubules damaged. On the other hand, necrosis of the proximal straight tubule would account for the changes seen, as we (15) and others (1) have shown.

Additional theoretical and experimental objections can be raised against the proposal that prior aquation of cisplatin in the tubule fluid is an important determinant of the nephrotoxicity of cisplatin. Using the published value of the forward rate constant for the initial loss of chloride from cisplatin in distilled water [7.6×10^{-5}/sec, at 35° (12)], and using a generous estimate for the intratubular transit time of fluid passing from the glomerulus to the ureter [180 sec (18, 19)], no more than 1.4% of excreted cisplatin could be converted to other species if the formation of such products depended on cisplatin aquation. This is a maximum estimate of cisplatin conversion by aquation, as even 10 mM chloride would slow the rate of aquation markedly (12), and the transit time through the distal nephron and collecting duct, the site where significant chloride gradients are generated, is no more than 60 sec. Imposing such physiological constraints on the calculation, it is unlikely that even 0.5% of cisplatin would undergo aquation. Yet Table 1 shows that 8.8% of the administered platinum is in a noncisplatin, nonaquated form. We (13) and others (7) have found even larger percentages of biotransformed cisplatin in urine of rats and humans who were not chloride deprived. Thus, whatever the mechanism of its formation, it is unlikely that such a compound is formed by prior aquation.

Consider, too, that hydration with hypertonic mannitol, which should also reduce urinary chloride concentration (20), has been shown by others to ameliorate cisplatin nephrotoxicity (11) and that the rate of aquation at the proximal straight tubule, the site of greatest cell damage, would be exceedingly low due to the high chloride concentration of fluid at this site, yet cisplatin produces extensive damage at this site. These observations make a causal link between cisplatin nephrotoxicity and tubular fluid chloride concentration very unlikely.

The proposal of Earhart *et al.*, that urinary chloride may be an important determinant of cisplatin nephrotoxicity, is an attractive

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one. However, the data offered in support of this hypothesis do not provide compelling reason to accept it as true.

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