Urinary biomarkers as humane endpoints in toxicological research

R. Poon & I. Chu

Environmental and Occupational Toxicology Division, Environmental Health Center, Health Canada, Ottawa K1A 0L2, Canada [e-mail: raymond_poon@hc-sc.gc.ca]

Summary

Urinary biomarkers can provide early and sensitive indicators of organ perturbation, and the collection of urine can be non-invasive. Examples of their use after the administration of model toxic compounds (xylene and PCB126) are given and the advantages and their limitations are discussed.

From a toxicological research perspective, the characteristics of humane endpoints should include the following: (1) the provision of test results with a minimal amount of pain and distress to the animals; (2) the detection of treatment effects at dose levels that do not produce excessive pain and distress to the animals; and (3) the detection and monitoring of early signs of pain and suffering in treated animals. Ideally, these endpoints should be obtained by non-invasive means such as growth and food consumption, clinical examination, neurobehavioural observation, and physiological measurements. The following is an overview of the current status and the future outlook of urinary biomarkers as humane endpoints.

Urinary biomarkers in toxicological research

There are at least eight categories of toxic effects that can be readily monitored by urinary biomarkers (Table 1). Microscopic examination for urinary formed elements (such as cell casts, microorganisms, crystals) is probably the earliest non-invasive means for assessing kidney damage. At the present time, there are more than 20 biochemical tests that are used for the detection of functional and tubular damage (Mueller *et al.* 1997). Tests for some hepatic effects and metabolic effects are also widely used in chemical toxicity studies. Recent reports indicate that urinary prostanoids (prostaglandins, thromboxanes) and tubular antigens may be sensitive biomarkers of nephrotoxicity in humans (Cárdenas *et al.* 1993a,b, Roels *et al.* 1993).

Advantages of urinary biomarkers

An immediate advantage of urine testing is its non-invasiveness. Specimens can be obtained easily either passively from metabolic cages, or by suprapubic manipulation (see e.g. Hayashi & Sakaguchi 1975). The advantages of urine testing include some of the following. The ready availability and ease of collection of urine specimens enable time course measurements; and if each animal acts as its own control, urinary testing substantially reduces the number of animals used. Depending on the frequency of measurement, changes in urinary analytes represent close to real time or time-averaged *in vivo* events. As an example, the time courses of urinary ascorbic acid excretion in response to treatment with PCB 126 (a persistent organochlorine) and to xylene (which is rapidly metabolized) are described (Fig 1). It is known that increased ascorbic acid excre-

Table 1 Urinary biomarkers in toxicological research

Biomarkers of	Tests in use	New tests
Kidney effects		
Functional	• Proteins (high & low MW) • Osmolality • Blood, haemoglobins	• Prostaglandins, thromboxanes
Tubular damage	• Enzymes • Cell casts and sediments	• Tubular antigens
Hepatic effects		
Modulation of drug metabolic activities	• Caffeine metabolites • 6-β hydroxycortisol • Ascorbic, glucuronic acids	
Hepatocellular damage	• Bilirubin	
Haematological effects		
Interference with haem bio-synthesis	• Porphyrin profile • δ-aminolevulinic acid	
Metabolic effects		
Diabetes, starvation	• Ketone bodies, glucose	
Stress		
Neuroendocrine	• Corticosterone	
Oxidative stress		
Lipid peroxidation	• Malondialdehydes, conjugated dienes	• Isoprostanes
Bone effects		
Bone turnover	• Calcium	• Crosslinked pyridiniums • Hydroxyproline
Lung effects		
Elastin turnover		• Desmosine, isodesmosine

tion is related to the stimulation of the hepatic glucuronic acid pathway activity following exposure to certain organic compounds (Burns *et al.* 1960, Poon *et al.* 1994). As shown in Fig 1, each curve represents the results from a single rat, normalized to day 0. The markedly different magnitude and time course of changes demonstrate the responsiveness and usefulness of urinary ascorbic acid as a biochemical indicator of a hepatic response. It should be noted that changes in ascorbic acid were detected in the absence of clinical signs of toxicity or distress in the animals. Such a high sensitivity to early biochemical changes is a common characteristic of many urinary biomarkers.

While most of the cage-side observations generate binary (yes/no) or subjectively graded results, urinary tests provide objective, quantitative data which are amenable to statistical treatment. In addition, due to the unique physiological function of the kidneys, the magnitude of changes in urinary analytes can often be quite large. For example, glucose levels in urine can change from a trace amount to over 300 mM depending on the extent to which blood glucose levels exceed the kidney threshold. The urinary β_2-micro-

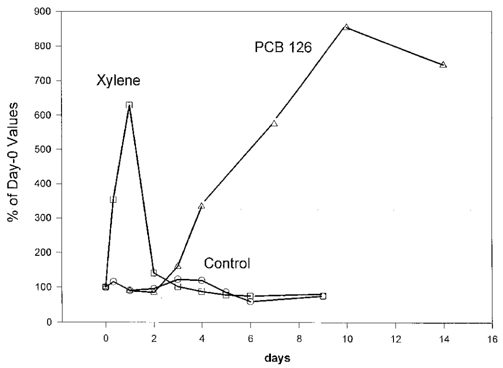

Fig 1 Urinary ascorbic acid as a non-invasive biomarker of hepatic effect induced by xylene and PCB 126 in rats

globulin level is another good example: this protein is present in the glomerular filtrate but is almost completely reabsorbed in the proximal tubules. When the tubular function is compromised, even a minor decrease in the reabsorption ability of the tubules will result in many-fold increases in urinary β_2-microglobulin.

The underlying mechanisms of urinary biomarkers for kidney damage have been thoroughly studied, and validated biomarkers specific for functional, glomerular, and tubular damage are available. Progress has been made in identifying the location of various enzymes along the segments of the nephron tubules (see WHO 1991 for a review). For some biomarkers the biochemical mechanisms are known: e.g. increased urinary ketone bodies reflect biochemical changes occurring during starvation and diabetic conditions; alteration in porphyrin profiles is correlated with inhibition of haem biosynthesis (Franklin *et al.* 1997).

Most urinary biomarkers reflect biochemical changes and, therefore, allow the detection of early subclinical effects. As a result, treatment-related effects can be obtained at lower dose levels. Our laboratories have routinely incorporated urinary biomarkers in range-finding and sub-chronic studies. In the tris(4-chlorophenyl)methanol study, it was observed that urinary ascorbic acid (hepatic biomarker) was elevated at 10 mg/kg/day while N-acetyl-glucosaminidase activity (kidney biomarker) was increased at 100 mg/kg/day (Poon *et al.* 1997). These results guided the design of a longer-term study in which the dose levels were rationally set at a range just high enough to elicit treatment effects but not too high to cause unnecessary suffering in the animals.

As biomarkers of stress on the hypothalamic-pituitary-adrenocortical axis, corticosteroids (corticosterone in rodents and cortisol in primates) serve as an important humane endpoint. Blood glucocorticoid levels are traditionally used as an indicator of stress in animals due to environmental, experimental, or treatment-related factors. Recently, non-invasive tests for glucocorticoids have been reported using urine (Kanda *et al.* 1993) and saliva (Fuchs *et al.* 1997) specimens.

Limitations of urinary biomarkers

A limitation of urinary biomarkers is that they might indicate changes that are adaptive or reversible. For example, changes in caffeine metabolite ratios are related to the specific induction of hepatic cytochrome P450 IA2 enzyme activity (Aldridge *et al.* 1977), which is indicative of an adaptive biochemical change and not irreversible tissue damage. The usefulness of some urinary biomarkers is limited by their uncertain diagnostic specificity. For example, urinary calcium is routinely included amongst tests that assess bone resorption, but other factors such as dietary changes, hormonal and vitamin variations, metabolic acidosis, and kidney diseases can have a significant influence on the results. The underlying mechanism for the modulation of some biomarkers can also be complex. Elevated urinary malondialdehyde levels could be due to a number of causes which include increased lipid peroxidation, compromised antioxidant status (Langley 1997), or changes in dietary lipid contents. Specimen integrity (stability, presence of food particles and faeces, and microbial contaminants) is a primary concern. The marked effect of urinary pH on β_2-microglobulin (Viau *et al.* 1986), and the instability of ascorbic acid in the absence of antioxidants (Poon *et al.* 1994) illustrate the importance of maintaining specimen stability. In animal studies, biomarker concentrations are routinely expressed in terms of volume, creatinine, or 24 h total values. The volume-based units are influenced by conditions that affect urinary output such as diuresis, while the creatinine-based units are invalidated when urinary creatinine levels were altered due to renal dysfunction or changes in muscle mass.

Future outlook

At present, the array of tests covers only a limited spectrum of adverse effects (Table 1). There is a need to develop and validate new tests that can serve as humane endpoints of concern. It would be desirable to develop urinary biomarkers of neurotoxicity that can produce objective and quantitative data at lower doses. The potential of urinary biogenic amines as indicators of neurotoxicity should not be overlooked and, indeed, these analytes were traditionally used in humans to diagnose phaeochromocytoma and neuroblastoma. The effect of polychlorinated biphenyls (PCBs) on rat urinary homovanillic acid has been reported by Seegal *et al*. (1985). In carcinogenicity studies where the endpoint is the detection of tumours at necropsy, non-invasive tests that can detect pre-neoplastic and early neoplastic lesions would be highly desirable. It is recognized that the relationship between neoplastic growth and tumours marker expression is highly complex (Beer & Pitot 1987). However, for specific tumours such as bladder cancer, which is frequently observed in chronic rodent studies, there are established (urine cytology) and investigative tests (tumour markers) that could be incorporated into the inventory of carcinogenicity endpoints.

While urinary corticosteroids can be used as a sensitive indicator of stress due to, for instance, environmental factors, their variations in response to intense or chronic stress and pain have not been fully characterized. There is a need to explore new tests in this area. Other areas of exploration where urinary biomarkers may be appropriate include biochemical tests for irreversible damage to tissues and organs other than the liver and kidneys (e.g. heart, muscle, pancreas, thyroid), and tests for cytokines that are involved in inflammatory and immune responses.

References

Aldridge A, Parsons WD, Neims AH (1977) Stimulation of caffeine metabolism in the rat by 3-methylcholanthrene. *Life Sciences* **2**, 967–74

Burns JJ, Conney AH, Dayton PG, Evans C, Martin GR, Taller D (1960) Observations on the drug induced synthesis of D-glucuronic acid, L-gulonic acid and L-ascorbic acid in rats. *Journal of Pharmacology and Experimental Therapeutics* **129**, 132–8

Beer DC, Pitot HC (1987) Biological markers characterizing the development of preneoplastic and neoplastic lesions in rodent liver. *Archives of Toxicology* **10**(Suppl.), 68–80

Cárdenas A, Roels H, Bernard AM, *et al*. (1993a) Markers of early renal changes induced by industrial pollutants. I. Application to workers exposed to mercury vapour. *British Journal of Industrial Medicine* **50**, 17–27

Cárdenas A, Roels H, Bernard AM, *et al*. (1993b) Markers of early renal changes induced by industrial pollutants. II. Application to workers exposed to lead. *British Journal of Industrial Medicine* **50**, 28–36

Franklin MR, Phillips JD, Kushner JP (1997) Cytochrome P450 induction, uroporphyrinogen decarboxylase depression, porphyrin accumulation and excretion, and gender influence in a 3-week rat model of porphyria cutanea tarda. *Toxicology and Applied Pharmacology* **147**, 289–99

Fuchs E, Kirschbaum C, Benisch D, Bieser A (1997) Salivary cortisol: a non-invasive measure of hypothalamo-pituitary-adrenocorical activity in the squirrel monkey, *Saimiri sciureus. Laboratory Animals* **31**, 306–11

Hayashi S, Sakaguchi T (1975) Capillary tube urinalysis for small animals. *Laboratory Animal Science* **25**, 781–2

Kanda K, Omori S, Yamamoto C, *et al*. (1993) Urinary excretion of stress hormones of rats in tail-suspension. *Environmental Medicine* **37**, 39–41

Langle UW, Wolf A, Cordier A (1997) Enhancement of SDZ ICT 322-induced cataracts and skin changes in rats following vitamin E- and selenium-deficient diet. *Archives of Toxicology* **7**, 283–9

Mueller PW, Lash LH, Price RG, *et al*. (1997) Urinary biomarkers to detect significant effects of environmental and occupational exposures to nephrotoxins. I. Categories of tests for detecting effects of nephrotoxins. *Renal Failure* **19**, 505–21

Poon R, Chu I, Lecavalier P, Bergman A, Villeneuve DC (1994) Urinary ascorbic acid–HPLC determination and application as a non-invasive biomarker of hepatic response. *Journal of Biochemical Toxicology* **9**, 297–304

Poon R, Lecavalier P, Bergman Å, Yagminas A, Chu I, Valli V (1997) Effects of tris(4-chlorophenyl)-methanol on the rat following short-term oral exposure. *Chemosphere* **4**, 1–12

Roels H, Bernard AM, Cárdenas A, *et al*. (1993) Markers of early renal changes induced by industrial pollutants. III Application to workers exposed to cadmium. *British Journal of Industrial Medicine* **50**, 37–48

Seegal R, Brosch KO, Bush B (1985) Oral dosing of rats with polychlorinated biphenyls increases urinary homovanillic acid production. *Journal of Toxicology and Environmental Health* **15**, 575–86

Viau C, Bernard A, Lauwerys R (1986) Determination of rat β_2-microglobulin in urine and in serum. I. Development of an immunoassay based on latex particles agglutination. *Journal of Applied Toxicology* **6**, 185–9

WHO (1991) Principles and methods for the assessment of nephrotoxicity associated with exposure to chemicals. In: *Environmental Health Criteria* 119, Geneva: World Health Organization