PREVENTION OF IFOSFAMIDE NEPHROTOXICITY BY N-ACETYLCYSTEINE: CLINICAL PHARMACOKINETIC CONSIDERATIONS

Nancy Chen[1], Katarina Aleksa[5], Cindy Woodland[5,6], Michael Rieder[1,3,4] ,Gideon Koren[1,2,3,5,6]

[1]Department of Physiology and Pharmacology, University of Western Ontario, London, Canada; [2]Ivey Chair in Molecular Toxicology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada; [3]Department of Paediatrics, University of Western Ontario, London, Canada; [4]CIHR-GSK Chair in Paediatric Clinical Pharmacology, Children's Hospital of Western Ontario, London, Canada; [5]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, Canada; [6]Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Canada.

Corresponding Author: gkoren@sickkids.ca or gkoren@uwo.ca

ABSTRACT

Background
Ifosfamide, which is routinely given to treat a variety of solid tumours in children, causes serious nephrotoxicity in treated children. Previous *in vitro* studies have shown that depletion of intracellular glutathione can enhance ifosfamide nephrotoxicity. Presently, there is no therapeutic agent that can prevent ifosfamide nephrotoxicity. We have recently shown that N-acetylcysteine (NAC) at 0.4mM prevents ifosfamide-induced nephrotoxicity *in vitro.* However, this *in vitro* concentration of NAC needed to be compared to those used in human pharmacokinetic studies since the *in vitro* pharmacological effect of a compound is achieved at concentrations exceeding those used in clinical.

Objective
The aim of the present study was to verify whether the *in vitro* concentration of NAC, which was found to protect renal cells from ifosfamide-induced damages, is comparable to the currently used clinical concentrations.

Methods
A systematic literature review of all published papers reporting on the pharmacokinetics of NAC in humans was conducted.

Results
The steady state concentrations of NAC administered intravenously to humans ranged from 0.04mM to 0.9mM and the urine concentration of NAC was 2mM.

Conclusion
This suggests that the concentration chosen for *in vitro* studies is well within the range of clinical levels.

Keywords: *Ifosfamide, N-acetylcysteine, nephrotoxicity, steady state concentration, pharmacokinetics, total body clearance, clinical relevance*

Ifosfamide (IF), an alkylating antineoplastic drug, is widely used to treat a variety of tumors in both adults and children.[1,2] Initially the clinical use of IF was restricted by severe hemorrhagic cystitis and nephrotoxicity. The former problem has been resolved by the concurrent administration of MESNA (sodium 2-mercaptoethanesulfonate), a synthetic thiol compound, which binds to toxic species of IF in the bladder to form stable nontoxic thioether compounds.[3,4] Despite the decrease in

urotoxicity with MESNA, nephrotoxicity still remains a serious adverse effect, particularly in children.[5,6] Approximately 30% of pediatric patients experience renal dysfunction, which is typically presented as Fanconi syndrome, with a generalized renal loss of amino acids, glucose, phosphate, and bicarbonate[5] as well as of small molecular-weight proteins. In addition, there is a decrease in the glomerular filtration rate (GFR).[7-9] As a result, children may survive their cancer but have to endure chronic renal failure.

Our recent *in vitro* studies have documented that N-acetylcysteine (NAC) prevents ifosfamide-induced nephrotoxicity[10] at a concentration of 0.4mM. A porcine renal tubular cell line, LLCPK-1, was treated with a combined treatment of 50mM BSO and 1mM IF with 0.4mM NAC for 96 hours. The cellular cytotoxicity caused by the combined treatment of BSO and IF was significantly diminished by NAC treatment. With these promising results, it is important to determine their clinical relevance. One of the major issues with *in vitro* models is that pharmacological effects are usually achieved at concentrations exceeding those achieved clinically.

Presently there is no proven modality to mitigate IF nephrotoxicity. However, N-acetylcysteine (NAC) is perceived as an attractive option since it has been used clinically as an antioxidant in conditions characterized by GSH depletion and it is the drug of choice as an antidote for acetaminophen poisoning both in adults and children.[11] Specifically, NAC enhances the biosynthesis of GSH by donating the cysteine which is a precursor.[12] In addition, NAC can prevent the formation of carcinogen-DNA adducts and suppress or delay the development of tumors through its antioxidant activity towards reactive oxygen species (ROS) and the conjugation of electrophilic metabolites, including endogenous sources or those produced by chemotherapeutic drugs.[12-14] This suggests that not only does NAC prevent the adverse effects caused by pharmacological agents; it also benefits the chemoprevention of mutations and cancer. The objective of the present study was to investigate whether the concentrations of NAC rendering the protective effect in our recent *in vitro* studies are achievable in the context of current clinical use of NAC. Our aim was to estimate the therapeutic concentration of NAC found in the plasma of children who receive the drug for treatment of acetaminophen overdose from published pharmacokinetic values, as well as comparing the estimated pediatric steady state concentration (Css) of NAC with those reported in the literature.

METHODS

Search Strategy

We systematically reviewed all published papers in Pubmed, Medline, and EMBASE, including all relevant articles about the pharmacokinetics of NAC in humans with search terms including "pharmacokinetics of NAC in humans", "steady state serum levels of NAC in humans", and "clearance rate of NAC in humans". Search of human studies included adults, children, and neonates. We included studies of pharmacokinetics of NAC after both oral and intravenous administration.

Calculation of *in vivo* Plasma NAC Concentrations

During the literature search, if steady state NAC concentrations were not reported directly, they were estimated by the following formula:

$$Cl_{NAC} = \frac{\text{Rate NAC of Infusion}}{[Css]}$$

with Cl being the clearance rate and Css being steady state concentration.

Calculation of *in vivo* NAC Concentrations in the Urine

The renal tubular cell is surrounded by blood on the basolateral membrane side and by urine on the brush border side. The urine concentration of NAC was calculated based on the reported amount of NAC excreted in urine over a 12-hour time course.[15] The concentration of NAC in the urine was determined using the following formula:

$$\text{Concentration}_{NAC(urine)} = \frac{\text{Amount of NAC in urine}}{\text{Volume of the urine}}$$

The volume of the urine in healthy adults was obtained from Addis and Watanabe[13].

RESULTS

Our systematic review identified 8 studies describing the pharmacokinetics of NAC (Css) in healthy adults or in neonates (Table 1). We estimated the pediatric steady state concentration of NAC, based on the NAC dose schedule given intravenously for acetaminophen overdose in children and the published total clearance rate of NAC in healthy volunteers.

TABLE 1 Steady State Concentration [Css] and Total Clearance Rate of NAC in Adults, Neonates and Children

Reference	Patient Group	Route of Administration	[Css]	Clearance Rate (Total)	Urine Concentration
Jones et al. (1997)	Healthy adults	Intravenous	0.21mM	0.0929 L/kg/h	NA
Brown et al. (2004)	Healthy adults	Intravenous	0.93mM	0.164 L/kg/h	NA
Prescott et al. (1989)	Healthy adults	Intravenous	0.23mM	0.1866 L/kg/h	NA
Olsson et al. (1988)	Healthy adults	Intravenous	0.037mM	0.11 L/kg/h	NA
Borgstrom et al. (1986)	Healthy adults	Intravenous	0.2mM	0.207 L/kg/h	2mM
Ahola et al. (1999)	Pre-term newborns	Intravenous	0.51mM	0.037 L/kg/h	NA
Holdiness M. (1991)	Healthy adults	Oral	0.065mM	0.286 L/kg/h	NA
De Caro et al. (1989)	Healthy adults	Oral: a) 600mg single dose; b) 200mg t.i.d.	a) 0.033mM b) 0.0023mM	NA	NA
Borgstrom et al. (1986) & Marzullo (2005)	Estimated pediatrics	Intravenous	0.4mM	NA	NA

Ahola et al.[17] studied newborn infants weighing between 500g and 1500g at gestational ages between 24 weeks and 32 weeks who required assisted ventilation. They found that at a constant 24 hour infusion of 4.2 mg/kg/h, the steady state concentration of NAC was 0.51mM and the total body clearance rate was 0.037 L/kg/h.

The steady state concentration of NAC in healthy adults ranged from 0.037 to 0.93mM. Prescott et al.[18], who studied the adult disposition and kinetics of intravenous NAC in both healthy subjects and patients with acetaminophen overdose, found a steady state concentration of NAC of 0.2mM and total clearance rate of 0.1866 L/kg/h in healthy subjects. Similarly, Jones et al.[19], who studied the pharmacokinetics of NAC in both healthy subjects and patients with chronic liver disease, found that the Css of NAC was also about 0.2mM in healthy subjects.

However, the total body clearance rate in Prescott's study was about twice the rate reported by the Jones study (0.1866 L/kg/h vs. 0.0929 L/kg/h). The Css of NAC found in healthy subjects by Olsson et al.[20] was 0.037mM and the value in Brown et al.[21] was 0.9mM, both of which were different from the values found by Prescott and Jones. Two pharmacokinetic studies of orally administered NAC in healthy adults found the Css of NAC were 0.06mM[22] and 0.03mM.[23] These concentrations are several folds lower than the ones from intravenous NAC.

The amount of NAC excreted in 12h urine was estimated at 29% of the given dose.[15] Hence the estimated concentration of NAC in urine was:

$$[C_{NAC(urine)}] = \frac{\text{Dose}}{\text{Vol of the urine (in 12 h)}}$$

$$= \frac{600\text{mg} * 0.29}{0.5\text{ L}}$$

$$= 348\text{ mg/L}$$

Based on the molecular weight of NAC, the urine concentration of NAC would be 2mM.

DISCUSSION

The clearance rate of intravenous NAC determines its therapeutic steady state concentration. In our *in vitro* study, the protective effect of NAC against ifosfamide-induced nephrotoxicity was achieved at 0.4mM in LLCPK-1 cells. Of importance, very similar values of steady

state plasma concentrations of NAC have been reported in the only pediatric use of the drug (0.51mM).[17] Although the pharmacokinetics of NAC have not been studied in toddlers and children, studies on the ontogeny of other drugs suggest that beyond the neonatal period, serum concentration in children are not higher than those in adults.[24] The wide range of the Css of NAC found in healthy adults may be due to the use of different pharmacokinetic analysis programs. Prescott and Jones used the SIPHAR pharmacokinetic curve fitting and modeling program to analyze the plasma concentration-time data, whereas Olsson used the MAXFIT program, and Brown used the NONMEM program. Despite these discrepancies, the Css of NAC found in the Prescott and Jones studies were quite close. Our estimated NAC concentration (0.4mM) for children lies in between the values found in pre-term infants and in healthy adults. On the other hand, the levels of NAC achieved by oral dosage are expected to be lower since NAC, given orally, undergoes extensive first pass metabolism in the gut wall and liver, resulting in bioavailability of only 10%.

The results of the existing studies of NAC pharmacokinetics in healthy volunteers may have varied due to different analytical methods, dose, formulation, and route of administration. Borgstrom et al.[15] presented the first study after intravenous administration of NAC in a group of healthy volunteers. In this study, the analytical method used only permitted determination of total NAC concentration in deproteinized plasma, which includes the reduced form of NAC and NAC in mixed disulphides. Under such conditions, more than 50% of the drug may be lost due to the fact that it is precipitated together with the proteins.[15,20] Although other studies[18-20] estimated the total NAC concentration, this probably does not reflect its true pharmacokinetics *in vivo*. This is because total NAC consists of free reduced and oxidized forms, disulphides, and mixed disulphides with other low molecular weight thiols such as cysteine and glutathione.[20] As NAC acts primarily as a precursor of cysteine[12,25],only the free reduced form can diffuse through the cell and be deacetylated to cysteine.[13] Hence, the plasma concentration of total NAC at any time may not be a reliable indication of its biological activity. It would be advantageous to look at the active form that is available with respect to questions of biological activity.

It has been recognized that 20 to 30% of an intravenous dose of NAC is excreted unchanged in the urine.[15,18] This implies that at least 70% of a given dose stays in the body whether it is compartmentized in the plasma, being absorbed, or being metabolized. The calculated concentration of NAC in the urine is much higher than that in the plasma. Hence, the renal tubular cell is clinically surrounded by a range concentration of NAC between 0.04mM in the plasma and 2mM in the lumen of tubular cell, well within the range of levels used by us *in vitro*. These data suggest that the protective effect of NAC on *in vitro* nephrotoxicity is clinically relevant, which further provides validation for our *in vitro* studies and merits future human studies.

Furthermore, the risk of NAC decreasing the chemotherapeutic effect of ifosfamide is minimal as studies have shown that NAC did not interfere with the antitumour activity of ifosfamide in patients with lung carcinoma.[26] This has further strengthened the potential of NAC as an option to prevent ifosfamide-induced nephrotoxicity. Following investigation of the protective effect of NAC in an animal model of ifosfamide nephrotoxicity, it will be reasonable to study its prevention of renal damage in children treated for cancer. In Canada, the protocol for acetaminophen overdose uses NAC intravenous administration (unlike the oral administration in the US); therefore, it will be reasonable to benefit from the intravenous dose, rendering more optimal protective levels of NAC.

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