

## Renal transport and disposition of Na-2-mercaptoethane sulfonate disulfide (Dimesna) in the rat

K. Ormstad and N. Uehara

*Department of Forensic Medicine, Karolinska Institutet, PO Box 60400, S-104 01 Stockholm, Sweden*

Received 2 November 1982

The transport and reduction of dimesna (Na-2-mercaptoethane sulfonate disulfide) was studied in vitro using isolated, perfused rat kidney, and isolated renal epithelial cells. Cellular uptake of dimesna was found to be dependent on an active transport mechanism working across the luminal brush border, with an app.  $K_m$  of  $\sim 22 \mu\text{M}$  and  $V_{\max} \sim 1.4 \text{ nmol} \cdot 10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}$ . Among other low molecular thiols or disulfides reduced glutathione was the only one to exert competitive inhibition.  $\gamma$ -GT-activity or cellular GSH status had no influence on renal uptake of dimesna, but the intracellular reduction rate was dependent on access to reduced glutathione as a cofactor.

*Kidney      Mercaptoethane sulfonate      Glutathione      Transport*

### 1. INTRODUCTION

During recent years, Na-mercaptoethane sulfonate ( $\text{HS}-\text{CH}_2-\text{CH}_2-\text{SO}_3^- \text{Na}^+$  (Mesna) has proven to be a valuable drug in the protection against urothelial toxicity in patients treated with oxazaphosphorine cytostatics (ex. cyclophosphamide) [1,2]. A series of clinical and experimental studies have been performed, and it is now well established that Mesna after oral or parenteral administration is circulating in its chemically inert disulfide form (Dimesna) and does not penetrate into living tissues, except the kidney [3,4]. Here, however, it is extracted from the renal vasculature and undergoes an intracellular reduction mechanism whereby the disulfide bond is split and the reactive thiol form (Mesna) is excreted to the tubular lumen where it is available for binding and detoxifying acrolein and other irritating metabolites of oxazaphosphorine cytostatics. The biochemical reaction-pathway for Dimesna reduction has been studied in vitro and seems to involve the two cytosolic enzymes thiol transferase and glutathione reductase with reduced glutathione (GSH) and reduced pyridine nucleotide (NADPH) as cofactors [4].

Among the various cellular functions ascribed to GSH, an involvement with membrane integrity and transport processes has been suggested [5,6]. Thus, the glutathione status of the kidney may be of relevance to the pharmacodynamics of Dimesna. The group of patients who will potentially receive this drug are likely to be in a poor nutritional state, which may upset their whole-body glutathione status [7]. This study therefore aims to investigate the relationship between GSH status and the transport and reduction of Dimesna in the kidney, as well as to study the polarity and specificity of Dimesna uptake into renal epithelial cells.

### 2. MATERIALS AND METHODS

Male Sprague-Dawley rats (180–230 g) were kept in stainless steel cages with free access to tap water and pelleted rat food. Kidney perfusion was performed as in [8]. To obtain a non-filtering kidney the perfusate concentration of albumin was increased to 10%, thereby creating an osmotic pressure of 55 mm Hg, and the perfusion pressure simultaneously decreased to 50 cm  $\text{H}_2\text{O}$  [9]. Isolation of renal tubular epithelial cells was performed as in [10]. The cytosolic fraction from a kidney

cortex homogenate was recovered as the  $105\,000 \times g$  supernatant.

Double-distilled water was used for all solutions. Mesna, [ $^3\text{H}$ ]Mesna, Dimesna and [ $^{14}\text{C}$ ]Dimesna were kindly supplied by Asta-Werke AG, Degussa Pharma Gruppe (Bielefeld). Anthglutin was a generous gift from Dr M. Tanaka, Sankyo Laboratories (Tokyo). All other reagents were of at least reagent grade and were purchased from local sources.

Mesna was analyzed as total thiol as in [11] or as in [12] as modified in [13]; in cellular systems after subfraction of glutathione as measured by high-performance liquid chromatography [14]. Dimesna was assayed as reducible disulfides after treatment with sodium borohydride as in [13].

Cellular uptake of radiolabelled Mesna and Dimesna was determined by incubation of isolated kidney cells with the labelled compound, separation of the cells from the medium by rapid centrifugation in a Microfuge, washing once with fresh medium and lysis of the cells with distilled water, whereupon cellular radioactivity was counted in a Beckman LS 100 liquid scintillation counter.

Metabolic incubations were routinely performed in rotating round-bottom glass flasks at  $37^\circ\text{C}$  under continuous gassing with carbogen (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ), or 100%  $\text{N}_2$ . Incubation medium was a modified Krebs–Henseleit bicarbonate buffer (pH 7.4) supplemented with 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid).

### 3. RESULTS AND DISCUSSION

Previous investigations [15] have demonstrated that after oral as well as parenteral administration, Mesna undergoes rapid oxidation in plasma and circulates as the chemically inert disulfide (Dimesna). Thus, the various cells and tissues in the body are exposed to Dimesna only, and since most cells do not take up low-molecular disulfides (cf. [4]) Dimesna remains in the extracellular space. In the kidney, however, Dimesna is taken up and undergoes a reductive activation which is a prerequisite for its local uroprotective effect. This work is an extension of earlier investigations on whole-body handling and metabolism of Mesna/Dimesna and focusses on the renal transport and metabolism functions.

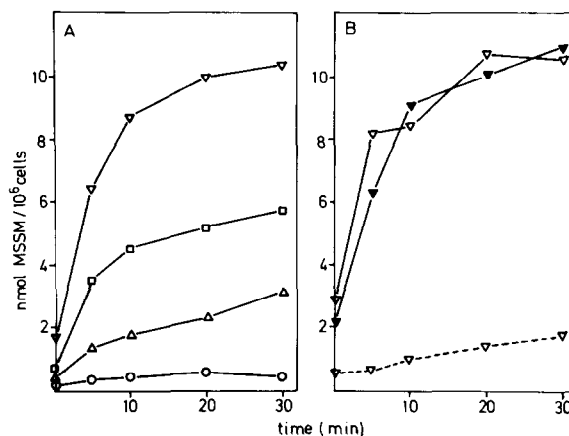


Fig. 1. Uptake of [ $^{14}\text{C}$ ]Dimesna into isolated rat kidney cells. (A) Cells incubated with 100  $\mu\text{M}$  radiolabelled Dimesna under continuous carbogen gassing at various temperatures: ( $\circ$ — $\circ$ )  $4^\circ\text{C}$ ; ( $\Delta$ — $\Delta$ )  $20^\circ\text{C}$ ; ( $\square$ — $\square$ )  $29^\circ\text{C}$ ; ( $\nabla$ — $\nabla$ )  $37^\circ\text{C}$ . (B) Cells incubated with 1 mM radio-labelled Dimesna under continuous carbogen gassing at  $37^\circ\text{C}$ : ( $\nabla$ — $\nabla$ ) control; ( $\blacktriangledown$ — $\blacktriangledown$ ) plus Anthglutin 1 mM; ( $\nabla$ --- $\nabla$ ) cells preincubated with 10  $\mu\text{M}$  FCCP for 10 min prior to addition of Dimesna.

Being a salt of a weak acid, Dimesna is highly water-soluble and transmembranal uptake must be expected to require an active, energy-requiring transport mechanism. The existence of such a function is supported by the effect of incubation temperature on uptake rate which is illustrated in fig. 1A. Further support for this hypothesis is derived from the dramatic effect on Dimesna uptake observed upon treatment of the cells with 10  $\mu\text{M}$  carbonyl cyanide *p*-trifluoromethoxyphenylhydrazide (FCCP) (fig. 1B) which lowers cellular ATP level to 20% of control value [16].

A great variety of active transport mechanisms for various compounds are operating across the renal tubular epithelium, one of which is suggested to be coupled to the activity of the brush border glycoprotein enzyme  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), which is prominent in kidney and intestines [6]. To investigate whether  $\gamma$ -GT is involved in Dimesna uptake in renal cells, the potent  $\gamma$ -GT inhibitor Anthglutin [17] was added to the incubate. As is seen from fig. 1B, no effect on Dimesna uptake was observed despite a 90% inhibition of  $\gamma$ -GT activity, which refutes a quantitatively important coupling.

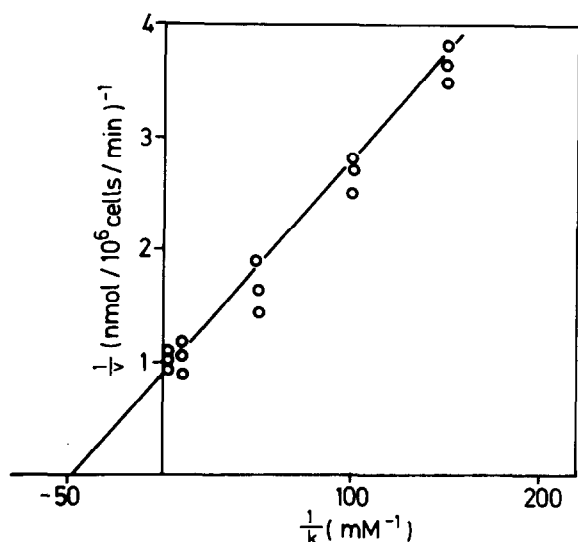


Fig. 2. Kinetics of Dimesna uptake in isolated rat kidney cells. Incubations were performed at 37°C under continuous carbogen gassing as described in section 2. The cell concentration in the incubate was  $1 \times 10^6$ /ml and the initial incubation vol. was 5 ml.

In fig. 1B the perfusate concentration of Dimesna is 10-times that in fig. 1A, but the rates of cellular uptake are almost identical. This led us to study the kinetics of Dimesna uptake in isolated kidney cells, and the results are shown in fig. 2. From the data plotted in this figure – which are calculated from the first 5 min of incubation when the uptake rate is still linear – an apparent  $K_m$  for the Dimesna transport mechanism is computed to  $\sim 22 \mu\text{M}$  and the  $V_{\max}$  to  $\sim 1.4 \text{ nmol} \cdot 10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}$ . These values are within the range of transport capacity for other compounds taken up in isolated kidney cells [17].

The influence of other low molecular thiols and disulfides on Dimesna uptake was studied at concentration ratios ranging from 1:1 to 1:100 (table 1). Only in the case of reduced glutathione at high excess was an inhibitory effect observed, suggesting that the transport mechanism for Dimesna is quite selective. Whether the inhibitory effect of GSH was due to competition for binding sites, or to other mechanisms is still an open question – at least in the liver it is shown that extracellular GSH augments the uptake of methotrexate, probably by

Table 1

Influence of various low-molecular thiols and disulfides on Dimesna uptake into isolated rat kidney cells ( $\text{pmol MSSM} \cdot 10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}$ )

Additions to incubate	Uptake rate
None	$105 \pm 30$
GSSG, 10 $\mu\text{M}$	$116 \pm 42$
GSSG, 1000 $\mu\text{M}$	$100 \pm 21$
Cystine, 10 $\mu\text{M}$	$98 \pm 19$
Cystine, 500 $\mu\text{M}$	$92 \pm 15$
GSH, 10 $\mu\text{M}$	$98 \pm 26$
GSH, 1000 $\mu\text{M}$	$70 \pm 12$
Cysteine, 10 $\mu\text{M}$	$113 \pm 30$
Cysteine, 1000 $\mu\text{M}$	$104 \pm 19$
N-acetylcysteine, 10 $\mu\text{M}$	$99 \pm 12$
N-acetylcysteine, 1000 $\mu\text{M}$	$104 \pm 23$
Mesna, 10 $\mu\text{M}$	$112 \pm 15$
Mesna, 1000 $\mu\text{M}$	$94 \pm 8$

The concentration of [ $^{14}\text{C}$ ]Dimesna was 10  $\mu\text{M}$  in all incubations. Experiments performed at 37°C under carbogen atmosphere. Results given as means  $\pm$  SD of 3 separate expts

Table 2

Influence of renal cellular GSH content on rates of uptake and reduction of Dimesna

Cellular GSH content ( $\text{nmol}/10^6 \text{ cells}$ )	Rate of Dimesna uptake ( $\text{nmol} \cdot 10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}$ )	Rate of thiol formation ( $\text{nmol} \cdot 10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}$ )
$29 \pm 4$	$0.82 \pm 0.12$	$1.24 \pm 0.38$
$18 \pm 4$	$0.86 \pm 0.19$	$0.62 \pm 0.18$
$8 \pm 2$	$0.78 \pm 0.08$	$0.19 \pm 0.04$

Incubations were performed at 37°C under  $\text{N}_2$  atmosphere. Initial Dimesna concentration was 100  $\mu\text{M}$ . Cell concentration was  $2 \times 10^6 \text{ cells/ml}$

interfering with the balance of SH- and S-S-groups in the plasma membrane, and it is suggested that this redox state may be involved in the control of membrane permeability [5]. However, in vivo the effect of GSH on Dimesna uptake is likely to be marginal since both plasma and urinary GSH

concentrations are in the micromolar range [18].

The reaction sequence previously proposed for intracellular reduction of Dimesna involves cytosolic GSH as a co-factor [4]. Consequently, cells deficient in GSH would be expected to have a lower Dimesna-reducing capacity, which is confirmed by the data presented in table 2. Partial GSH depletion achieved by preperfusing the kidneys with 4  $\mu$ M diethylmaleate for 2 min diminishes cellular GSH content by ~30%, and after 5 min preperfusion the content is ~25–30% of the normal [19]. These cells retain unchanged viability for at least 2 h and neither the capacity to take up amino acids [19] nor Dimesna (table 2) seems to be impaired. Dimesna reduction rate, however, is dramatically decreased along with the cellular GSH concentration, reflecting the limited supply of a necessary co-factor in the reaction sequence [4]:

- (i)  $\text{MSSM} + \text{GSH} \rightarrow \text{MSH} + \text{GS-SM}$
- (ii)  $\text{GS-SM} + \text{GSH} \rightarrow \text{MSH} + \text{GSSG}$
- (iii)  $\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+$

The rate of Dimesna reduction could be restored completely by incubating the partly GSH-depleted cells with an amino acid mixture containing glutamate, glycine and cyst(e)ine, thereby replenishing the GSH pool.

Thus, adequate levels of renal cellular GSH is a prerequisite for the uroprotective action of Dimesna. Normally, the biological halflife of GSH is short in the kidney, ~30 min [20], and resynthesis is dependent on a supply of precursor amino acids, whereof the intracellular level of cysteine [19] appears to be the limiting factor, and in the clinical situation the importance of an adequate dietary intake of sulphur-containing amino acids must be remembered. Work in [4] indicated that the all-over renal handling of Dimesna consists of glomerular filtration, tubular reabsorption, intracellular reduction and subsequent secretion of Mesna to the tubular lumen, and of any Dimesna exceeding the reductive capacity to the peritubular capillaries. Prompted by the observed influence of extracellular GSH on Dimesna uptake and the fact that plasma GSH is actively extracted in the kidney [8,21,22] experiments were undertaken to establish whether Dimesna could also be absorbed from the basolateral side of the tubular epithelium. Filtering and non-filtering kidneys were perfused for 20 min

with [ $^{14}\text{C}$ ]Dimesna-containing medium, then with a Dimesna-free perfusate for 10 min, and the radioactivity retained in the tissue was compared. In the non-filtering kidney, only traces of radioactivity were found, whereas the filtering kidney exhibited about 100-fold higher counts/weight unit. Thus, it seems that the main route for Dimesna uptake in the kidney is across the luminal brush border and that glomerular filtration rate may be a limiting factor in the activation of Dimesna as a local protector against oxaphosphorine toxicity.

## REFERENCES

- [1] Brock, N., Stekar, J. and Pohl, J. (1979) *Naturwissenschaften* 66, 60–61.
- [2] Brock, N., Pohl, J., Stekar, J. and Scheef, W. (1983) *Eur. J. Cancer Clin. Oncol.*, in press.
- [3] Brock, N., Pohl, J. and Stekar, J. (1981) *Eur. J. Cancer Clin. Oncol.* 17, 1155–1163.
- [4] Ormstad, K., Orrenius, S., Låstbom, T., Uehara, N., Pohl, J., Stekar, J. and Brock, N. (1983) *Cancer Res.* in press.
- [5] Leszczyńska, A. and Pfaff, E. (1982) *Biochem. Pharmacol.* 31, 1911–1918.
- [6] Orłowski, M. and Meister, A. (1970) *Proc. Natl. Acad. Sci. USA* 67, 1248–1255.
- [7] Tateishi, N. and Higashi, T. (1978) in: *Functions of Glutathione in Liver and Kidney* (Sies, H. and Wendel, A. eds) pp. 3–7, Springer, Berlin, New York.
- [8] Ormstad, K., Låstbom, T. and Orrenius, S. (1982) *Biochim. Biophys. Acta* 700, 148–153.
- [9] Maack, T. (1980) *Am. J. Physiol.* F71, 238–245.
- [10] Jones, D.P., Sundby, G.B., Ormstad, K. and Orrenius, S. (1979) *Biochem. Pharmacol.* 28, 929–935.
- [11] Saville, B. (1958) *Analyst* 83, 670–672.
- [12] Ellman, G.L. (1959) *Arch. Biochem. Biophys.* 82, 70–77.
- [13] Stekar, J. (1982) *Ärzt. Labor.* 28, 187–191.
- [14] Reed, D.J., Babson, J.R., Beatty, P.W., Brodie, A.E., Ellis, W.W. and Potter, D.W. (1980) *Anal. Biochem.* 106, 55–62.
- [15] Pohl, J., Brock, N., Schneider, B. and Wetzelsberger, K. (1981) *Exptl Clin. Pharmacol.* 3 (suppl. 1) 95–101.
- [16] Orrenius, S., Thor, H., Rajs, J. and Berggren, M. (1976) *Forensic Science* 8, 255–263.
- [17] Minato, S. (1979) *Arch. Biochem. Biophys.* 192, 235–240.
- [18] Wendel, A. and Cikryt, P. (1980) *FEBS Lett.* 120, 209–211.

- [19] Ormstad, K., Jones, D.P. and Orrenius, S. (1980) J. Biol. Chem. 255, 175–181.
- [20] Sekura, R. and Meister, A. (1974) Proc. Natl. Acad. Sci. USA 71, 2969–2972.
- [21] Bartoli, G.M., Häberle, D. and Sies, H. (1978) in: Functions of Glutathione in Liver and Kidney (Sies, H. and Wendel, A. eds) pp. 27–31, Springer, Berlin, New York.
- [22] Häberle, D., Wahlländer, A. and Sies, H. (1979) FEBS Lett. 108, 335–340.