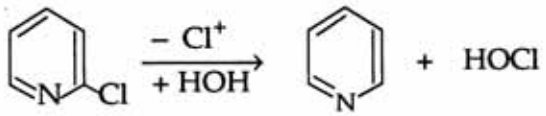
Acta Biochimica Polonica
Vol. 40 No. 1 1993

Contribution of N-oxidation and ·OH radicals to mutagenesis of 2-chloropyridine in *Salmonella typhimurium**

Bożena Chłopkiewicz, Mścisław Wójtowicz, Jadwiga Marczewska, Dorota Prokopczyk and Jadwiga Koziorowska

Drug Institute, Chełmska 30/34, 00-725 Warsaw, Poland

Pyridines and substituted pyridines are important chemicals used in industry. Metabolic N-oxidation of this class of compounds has been demonstrated [1], however, the role of *N*-oxides in the interaction of pyridines with biological systems is still unclear [2]. 2-Chloropyridine has been found mutagenic in a *Salmonella* assay [3]. The present study describes experiments designed to explain the possible role of N-oxidation by determining mutagenic activity of 2-chloropyridine and its *N*-oxide using two different experimental protocols. In parallel experiments, the presence of *N*-oxides was determined after incubation of 2-chloropyridine with fraction S9. We have also taken into account the possibility that, due to the dissociation of the chlorine atom, 2-chloropyridine may generate reactive oxygen species. We propose the following hypothetical chain of reactions:



$$2\,HOCl \longrightarrow 2\,HCl + 2\,O$$

$$2\,O + e^- \longrightarrow O_2^{\cdot-}$$

$$O_2^{\cdot-} + H_2O_2 \longrightarrow O_2 + OH^- + {}^{\cdot}OH$$

Mutagenicity of 2-chloropyridine and its *N*-oxide was evaluated in *Salmonella typhimurium* TA100 using the plate incorporation assay [4] or the same assay but with a preincubation procedure.

Table 1 shows that 2-chloropyridine is mutagenic exclusively in the presence of fraction S9. However, the preincubation of 2-chloropyridine with fraction S9 for 10, 20 or 30 min before plating abolishes completely mutagenicity of this compound. No mutagenicity was observed with 2-chloropyridine *N*-oxide, a metabolite of 2-chloropyridine, irrespective of the assay conditions applied.

To elucidate the effect of preincubation of the mutagenicity of 2-chloropyridine, experiments were performed to evaluate by thin-layer chromatography the presence of metabolites in the

Table 1
Evaluation of mutagenicity of 2-chloropyridine

Dose (μg/plate)	Revertants/plate				
	Plate incorporation		Preincubation		
	− S9	+ S9	+ S9		
			10'	20'	30'
500	−	138 ± 12.7	−	−	−
1000	−	240 ± 41	−	−	−
2500	−	360 ± 38	−	−	−
5000	−	575 ±118	−	−	−
7500	−	609 ± 59	−	−	−

Each entry presents the average of 4 tests ± standard deviations. As positive controls *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (2 μg/plate, −S9, 1980 ± 73 revertants per plate) and 2-aminoanthracene (1 μg/plate, +S9, 850 ± 68 revertants/plate) were applied. Regardless of the protocol used there were 120 - 200 spontaneous revertant colonies per plate. Revertant values represent counts corrected for spontaneous mutants

*This work was supported by U.S. - Polish MSC Joint Fund II

Table 2
*Thin-layer chromatographic identification of metabolites in the mixtures of S9, cofactors and 2-chloropyridine**

Conditions	Spots	R_F values
30 min preincubation in water bath with shaking	2-chloropyridine *N*-oxide	0.55 detected
	pyridine *N*-oxide	0.33 detected
30 min preincubation in water bath with shaking and 48 h in incubator	2-chloropyridine *N*-oxide	0.55 detected
	pyridine *N*-oxide	0.33 not detected
48 h in incubator	2-chloropyridine *N*-oxide	0.55 detected
	pyridine *N*-oxide	0.33 not detected

*Thin-layer chromatography of the sample and standards was performed on precoated Merck silica gel 60 F_{254} plates with the solvent system n-penthanol-acetone-water (5:5:1, by vol.). R_F of 2-chloropyridine in this system is 0.96

mixtures containing S9, cofactors and 2-chloropyridine.

The presence of 2-chloropyridine *N*-oxide was detected irrespective of the conditions of incubation (Table 2). The intensity of the spots corresponded to 2 - 3 μg of the compound. A small amount of pyridine *N*-oxide was detected exclusively when the preincubation procedure was applied, that is in the mixtures incubated in the water bath with shaking for 30 min at 37°C.

The possible contribution of reactive oxygen species was determined by evaluating the mutagenicity of 2-chloropyridine in the presence of reduced glutathione, radical scavengers or pyridine *N*-oxide.

Table 3
Effect of chosen compounds on the mutagenicity of 2-chloropyridine (7500 μg/ml)

Compound	Dose/plate	Net revertants/plate* +S9
None (control)	–	601 (100)
Glutathione	20 μM	32.5 (5.2)
Superoxide dismutase	900 U	615
Catalase	3000 U	639
Thiourea	35 mM	143 (21.2)
D-Mannitol	50 mM	236 (35.5)
Hydroquinone	5 μM	672
Pyridine *N*-oxide	200 μg	197

*In parentheses, % of control

Glutathione and the $^{\bullet}OH$ scavengers such as mannitol or thiourea suppressed totally or partially the mutagenicity of 2-chloropyridine (Table 3). The mutagenic actvity in TA100 was not suppressed either by catalase or superoxide dismutase or the singlet oxygen scavenger hydroquinone. None of the radical scavengers was able by itself to induce any alterations in the number of revertants.

The mutagenicity of 2-chloropyridine in the presence of pyridine *N*-oxide indicates that the effects produced by the latter compound were similar to those observed in the presence of glutathione or $^{\bullet}OH$ scavengers. Pyridine *N*-oxide itself was shown to be nonmutagenic (see above).

In the present study we have shown that glutathione abolishes totally the mutagenic activity of 2-chloropyridine and that its mutagenicity can be effectively suppressed by scavengers of hydroxyl radicals and is not inhibited by the addition of superoxide dismutase or catalase. However, our results do not permit to conclude whether the mutagenicity of 2-chloropyridine was caused by $^{\bullet}OH$ radicals or whether other species generated intracellularly were involved, because superoxide dismutase or catalase added to the medium cannot scavenge $O_2^{\cdot}$ or H_2O_2 generated within the cells. In this study we have also confirmed the assumption that N-oxidation of pyridines may protect the cells from the effects of reactive oxygen species [5]. In this respect biological properties of pyridine *N*-oxide may be compared to those of nitric oxide which can protect

cells from the toxic effect of reactive oxygen species by scavenging ·OH radicals [6].

REFERENCES

1. Covan, D.A., Damani, L.A. & Gorrod, J.W. (1987) *Biomed. Mass Spectrum.* **5**, 551 - 556.
2. Namboodiri, K., Osman, R., Weinstein, H. & Rabinowitz, J.R. (1987) *Mol. Toxicol.* **1**, 131 - 141.
3. Claxton, L.D., Dearfiels, K.L., Spanggord, R.S., Riccio, E.S. & Mortelmans, K. (1987) *Mutation Res.* **176**, 185 - 198.
4. Maron, D.M. & Ames, B.N. (1983) *Mutation Res.* **113**, 173 - 215.
5. Gorrod, J.W. (1985) *Drug Metab. Disposition* **13**, 283 - 286.
6. Kanner, J., Harel, S. & Granit, R. (1991) *Arch. Biochem. Biophys.* **289**, 130 - 136.