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A CASE STUDY: EVALUATION OF RESULTS AND SELECTION OF ADDITIONAL STUDIES

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A standard battery of tests (bacterial reverse mutation assay, *in vitro* chromosomal aberration test and *in vivo* micronucleus test in Japan) is recommended for genotoxic study of chemicals. However, additional tests could be selected, especially when negative results are obtained from the standard battery or are expected from knowledge of the chemical's structure. Here I summarize a case study of "omeprazole".

Omeprazole is a proton pump inhibitor (H^+ / K^+ ATPase inhibitor) (Fellenius *et al.*, 1981) and is used as an anti-ulcer drug (Arnold and Koop, 1989). Life-long administration of omeprazole induced carcinoids of enterochromaffin-like (ECL) cell origin in the fundus in rats (Ekman *et al.*, 1985; Havu, 1986) and mice (Betton *et al.*, 1988). This unwanted side effect is thought to reflect the fact that pharmacological blockade of acid secretion results in hypergastrinamia, that long-standing hypergastrinamia gives rise to hyperplasia of ECL cells, and that carcinoids arise from the ECL cells (Hakanson and Sundler, 1990). Gastric carcinoid tumors in patients with Zollinger-Ellison syndrome on long-term omeprazole was once reported (Goldfain, *et al.*, 1989). Genotoxicity of omeprazole is important to examine considering its carcinogenicity. The results of the short-term genotoxicity tests (bacterial reverse mutation assay, *in vitro* chromosome damage test, *in vitro* mouse lymphoma assay and

the micronucleus test in mice) were all negative (Ekman *et al.*, 1985; Evans, 1990). However, these results could be expected, because omeprazole genotoxicity tests were performed at neutral pH; the compound is converted though into active sulphenamide (Fig. 1) in acidic conditions (Beil *et al.*, 1987). It is difficult to isolate sulphenamide and test it in ordinary genotoxicity tests because of its instability.

We gave omeprazole to rats by gastric tube,

Fig. 1. Structure of omeprazole and its activation in acid.

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made omeprazole active in the stomach and examined the genotoxicity in the stomach mucosa. We used unscheduled DNA synthesis (UDS) and DNA single strand scission as markers. For UDS, test groups of 5 male F344 rats, 8 weeks old, were given omeprazole, then killed after 4 h. The stomach pyloric mucosa was removed and cultured in the presence of ^3H -thymidine for 2 h with or without hydroxyurea (inhibitor of replicative DNA synthesis). DNA was extracted from the tissue and the incorporation of ^3H -thymidine into DNA was measured using a liquid scintillation counter; UDS and replicative DNA synthesis were then calculated. Omeprazole (30 and 100 mg/kg bw) induced UDS (Fig. 2) (Furihata *et al.*, 1991; Evans, 1991; Furihata and Matsushima, 1991); however, some lots of omeprazole did not induce UDS. We conclude that omeprazole's ability to induce UDS in the rat stomach mucosa is equivocal. For

DNA single strand scission tests, groups of 8 male F344 rats, 8 weeks old, were examined. Five rats were given omeprazole, a positive control was given 4-nitroquinoline 1-oxide and 2 negative controls were given H_2O and examined 2 h after administration. The stomach pyloric mucosa was removed and treated in lysis solution and fragmented DNA was eluted from 2μ filters at pH 12.1. Omeprazole at doses of 30-500 mg/kg bw induced DNA single strand scission in some experiments but not in some other experiments. We examined a total of 45 rats with omeprazole. The result was statistically significant ($p < 0.01$) by the Cochran-Armitage binomial trend test; however, a clear dose-relationship was not observed. We conclude that omeprazole is equivocal in induction of DNA single strand scission in the rat stomach mucosa (Fig. 3, Furihata *et al.*, 1996). Mereto *et al.* (1993) reported that omeprazole did not induce DNA

Fig. 2. UDS in the stomach pyloric mucosa in male F344 rats 4 h after oral administration of omeprazole (Furihata *et al.*, 1991). Points show results of individual rats. Bars show mean at each dose. The same marker shows the result of the same rat in the presence and absence of HU at each dose. Values in the presence of HU at a dose of 100 mg/kg bw were significantly different from those at 0 dose by Student's t-test ($p < 0.05$).

Fig. 3. DNA single strand scission in the stomach pyloric mucosa of male F344 rats 2 h after omeprazole administration (Furihata *et al.*, 1996). Points show results of individual rats. Bars show mean at each dose. C means historical controls. Dashed line suggests a border line of control group (mean + 2.6 SD). 4NQO is a concurrent positive control. Results of omeprazole are significantly different from control by Cochran-Armitage binomial trend test ($p < 0.01$).

fragmentation in gastric mucosa and liver of Sprague-Dawley rats at a dose of 100 mg/kg body wt. However, the results may be ascribed to a lower sensitivity of their assay, due to pretreatment before alkaline elution or temperature or elution flow rate differences.

Interaction of ^{14}C -omeprazole with DNA was observed in rat stomach pyloric mucosa (36 pmol omeprazole/mg DNA) 1 h after administration (Phillips *et al.*, 1992). However, DNA adducts could not be confirmed. The author suggested non-covalent interaction.

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