"Above all do no harm – but whose responsibility is it?"

ABR THOMSON, MD, FRCP

On February 17, 1990, the Lancet published an article entitled "Genotoxicity studies of gastric acid inhibiting drugs" (1), followed by a comment (2), an editorial (3), and several subsequent letters to the editor (4-9). Two days later a report appeared in a major American newspaper, commenting on this material. The problem is unfortunately before us, with patients wishing us to comment on an issue which is technically very complex. This editorial acknowledges its responsibility to inform readers and to provide physicians with a balanced view of the potentially confusing issue of genotoxicity. Let us focus on the question at hand: "What is unscheduled DNA synthesis (UDS)?" Let us centre our attention on the scientific evidence for possible genotoxicity of potent ulcer-treating therapies.

An historical perspective is essential. Glaxo Group Research Ltd invested several years of major effort to test loxetine, a potent noncompetitive unsurmountable H2 receptor antagonist with long lasting inhibition of gastric acid secretion. When loxetine was given orally to rats in high doses for an extended period of time, there was an increase in enterochromaffin-like (ECL) cells in the mucosa of the gastric fundus with the eventual appearance of carcinoid tumors (10,11). These findings led to the abandonment of loxetine development. However, during the process of product development, the potential genotoxicity of loxetine in the stomach was investigated by attempting to measure the induction of UDS within the gastric mucosa, using methods that are still under debate (12). This assay was intended to measure DNA repair synthesis by assessment of the unscheduled incorporation of tritiated thymidine into DNA, followed by the removal by protease digestion of a superficial layer of the stomach, and scintillation counting of 3H-thymidine, and expression of the results as disintegrations/min/µg DNA.

Scheduled S-phase DNA synthesis is a normal event occurring during cell replication. This contrasts sharply with UDS, which reflects the repair of DNA damage. Scheduled DNA synthesis is maximal around the isthmus neck junction of the gastric glands, but is detectable up to five cell positions from the surface. There is a correlation between the carcinogenic potency of a chemical compound and its capacity to induce UDS, at least in the liver (13). It must be appreciated that the UDS assay has been applied to numerous organs, including liver, kidney, nasopharynx and stomach (14). It is also important to recognize that the rat stomach has two distinct areas: squamous and glandular. The stomach UDS assay detects known gastric glandular carcinogens such as 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) and the forestomach carcinogen epichlorhydrin. Curiously, the assay does not detect all forestomach carcinogens, such as aristolochic acid (AA). The reason for this lack of detection is unknown; possibly detection requires nitroreduction by bacteria which only exist in significant numbers in the forestomach. It was striking, however, that after 14 to 16 h of exposure of the rat's stomach to orally administered MNNG, increased 3H-thymidine disintegrations/min/µg DNA were detected with a statistically significant response at 12.5 mg/kg; the highest response was obtained after a dose of 50 mg/kg, with induction of UDS at even higher doses. Even after shorter periods of exposure (4 h) the UDS assay was positive for MNNG. Using indomethacin, a nongenotoxic gastric irritant, UDS was negative. Thus, the UDS assay was positive
for two of three known rat gastric carcinogens, but it remains debated whether these data were due at least in part to S-phase activity.

In some experiments using the UDS assay, the uptake of \(^{3}\)H-thymidine occurs in both the presence and the absence of hydroxyurea (15). Hydroxyurea blocks the majority of S-phase cell division (16). Since the stomach has a high rate of cell turnover, any variability in the hydroxyurea block might result in inconsistencies in radiolabelled S-phase cells which could potentially lead to misinterpretation of the results. Hydroxyurea was not used in these studies of gastric UDS (1). The author of this method (12) acknowledged that the assay might need to be improved to allow for superior cell isolation techniques to yield cells of acceptable morphology enabling the autoradiographic detection of UDS which, although more time consuming, is preferable to the detection of UDS by the more indirect scintillation counting method. This would allow the selective assessment of UDS induction in the various cell types which are present in the glandular stomach and any potential S-phase induction, eg, as for AA.

This is an important consideration since the replicated cells incorporate large amounts of \(^{3}\)H-thymidine, and any contamination of the superficial layers (removed by protease digestion) would potentially invalidate test results. Thus, it is essential to prove that there is a lack of contamination, and that there is a lack of change in the depth of cell removal in the different test groups. This is particularly important since all antisecretory drugs may result in hypergastrinemia which will itself increase DNA replication. In the letter submitted to the Lancet by Ekman (2) it was noted that the extensively folded structure of the gastric epithelium, and the presence of cell division in the neck region of the gastric glands, makes it very unlikely that the technique achieved adequate separation.

The point is well taken that the adequacy of the separation method and the lack of contamination must be established, since even minor contamination of the cell preparation with dividing cells would invalidate the procedure. These authors also note that "errors inherent in the isolation method are compounded if normal cell division is stimulated, by gastrin for example." The rats were orally lavaged with loxotidine or omeprazole 14 h before the \(^{3}\)H-thymidine and it is possible that "omeprazole's long duration of action would, however, almost certainly mean that serum gastrin was increased throughout the time period of the experiment." Serum gastrin measurements were not obtained in these studies, but comparable hypergastrinemia would likely result from loxotidine treatment, which was negative in the UDS assay.

As noted by NA Wright (17), Any method which relies on selective separation of non-dividing surface cells to identify unscheduled DNA synthesis requires stringent controls to exclude contamination with deeper, dividing cells. This is particularly important in situations where there is a stimulus to cell proliferation, as occurs with compounds such as MNNG and with drugs causing hypergastrinemia. The proliferation results in cells that are undergoing scheduled DNA synthesis appearing closer to the surface, where they are even more likely to contaminate the non-dividing surface layer. Any such contamination invalidates claims to selective demonstration of the unscheduled DNA synthesis.

Thus, it remains to be proven that the UDS assay is absolutely infallible for the detection of genotoxicity in the rat stomach exposed to potent antisecretory drugs.

At issue is not the previous extensive and carefully performed negative genotoxicity tests for omeprazole, nor is the issue of accepted hyperplasia of oxyntic mucosal cells, including 'hyperplasia' of the endocrine ECL cells, and development of gastric carcinoids in rats (11). These occur at a dose of 1.7 mg/kg in one in 50 animals, ie, 2% and at a higher frequency with higher doses (16). These carcinoids develop as a result of hypergastrinemia, and not as a direct effect of the antisecretory drug; this phenomenon has been accepted as 'the gastrin mechanism'. Nonetheless, the UDS assay bears important testimony to the care with which premarketing testing of omeprazole was undertaken. At issue is not whether morphology should be done in animals receiving indomethacin, in whom UDS was negative, or whether serum gastrin measurements should have been performed. Of course it would have been of interest if hydroxyurea were used to inhibit S-phase synthesis incompletely; of course it would be of interest to know whether UDS was present less than 14 h after the administration of omeprazole; of course it would be of interest to know whether UDS was present with repeated dosing; and of course it would be of interest to know whether UDS was present over a wide range of doses of omeprazole or in other species. But again we return to the question of the reliability of this UDS assay. The number of rats tested in the UDS assay was modest and there was variability of DNA recovery. And although a dose-response effect was claimed with increasing \(^{3}\)H-thymidine disintegrations/min with increasing doses of omeprazole ranging from 10 to 30 mg/kg (10, 15, 20, 30), it was interesting that the assay was negative for UDS at omeprazole doses of 40 and 80 mg/kg (18).

For the scientific community it is important to know if there was an induction of UDS. If so, is it the result of omeprazole, a produg, or the active breakdown product sulphenamide? Or is the assay simply inappropriate or inaccurate? Sulphenamide might be an alkylating agent, but it is destroyed at neutral pH and is relatively poorly available for crossing membranes to exert a damaging effect. Thus, it is unclear what the mechanism of action of omeprazole might have been in causing the alleged UDS. Omeprazole has been subjected to an extensive series of tests for genotoxicity, ranging from mutational assays in bacteria (Ames test), mutation and chromosome damage to mammalian cells in vitro, and chromosome damage in mouse bone marrow and erythroid precursor cells exposed in vivo – to DNA damage.
in the liver nuclei of rats also exposed in vivo (19). While a total of six categories of genetic toxicology studies have been performed on omeprazole, the mouse micronucleus test and an in vivo bone marrow chromosome aberration test were borderline or weakly positive. HJ Evans (Professor at the MRC Human Genetics Unit in Edinburgh, Scotland) has assessed all the genotoxicity tests as 'negative'.

The unsigned editorial of the February 17, 1990 article in the Lancet (3) notes that work to validate the new method of measuring DNA damage in the stomach should be carried out with all speed, and further investigation into the action of omeprazole on acid-secreting cells should be done.

Such work is in progress. The editor goes on to note that it is regrettable that research on basic mechanisms of toxicity is conducted by a few groups and universities who are struggling for funds, and by groups in the industry who cannot be expected to expend the same effort on clearing up the mechanisms of action of their competitors' drugs as they would put into the investigation of their own compound. The question of omeprazole's safety should have been evaluated independently by a national licensing body.

Can the results of the UDS test be duplicated in an independent laboratory? Astra scientists were unable to reproduce the effect of omeprazole 30 mg/kg in the UDS assay. At issue is the question of omeprazole's safety, as suggested by the Lancet editorial "Should this compound have been evaluated independently by national licensing bodies?" This compound has been evaluated independently by numerous national licensing bodies; but should they do the actual assays? If so, should they do this for all compounds. What is the next step? Studies were initiated by Glaxo in the process of examining the safety of their product lomotidine and further development of the compound was discontinued when it was found to produce carcinoid tumours. It is not the responsibility of Glaxo to prove the safety of omeprazole! It now becomes the responsibility of Astra to follow up the findings of Burlinson et al (10), and to prove or disprove their data. This effort has been mounted in a major undertaking, and the data suggest that there is no genotoxicity by the UDS assay, as performed by their scientists and independent researchers.

What should we do now, as clinicians? I have the option of using one of the many safe and efficacious therapies for the treatment of patients with peptic disorders (antacids, anticholinergics, H2-receptor antagonists, 'cytoprotectives'), or a proton pump blocker. Since the UDS assay is subject to some controversy, the original findings have not been confirmed and there is not a typical dose-response, I hesitate to caution patients who are on or about to go on Losec that a high dose of omeprazole, given to rats, produced, in studies using a technique which is not uniformly accepted, data in which "a genotoxic mechanism of action cannot be discounted." Patients should continue to receive omeprazole where properly indicated for the treatment of diagnosed acid-peptic disorders. The Health Protection Branch has determined the compound to be safe, and they will continue to monitor the situation closely. I personally will continue to do clinical studies with omeprazole, and to alert readers of The Canadian Journal of Gastroenterology to new and important matters relevant to the Art and Science of Medicine. That is my responsibility as a gastroenterologist, a teacher, a scientist, and an editor. May I suggest that your responsibility is to stay informed, to consider the arguments objectively, and to reach your own informed decision?

What will be the responsibility of the Canadian Association of Gastroenterology? Or the Canadian Medical Association? Or the Canadian Medical Protective Association? And our own Health Protection Branch? We have good people in the Health Protection Branch and in our universities, and they need to continue to be provided with the means to assess safety profiles independently under circumstances where modern molecular biology can potentially be applied to an understanding of the safety of the drugs we give to our patients. And if the UDS assays are inconclusive, then I am confident that the Health Protection Branch will review the situation independently and do additional studies if indicated. Thus, I am confident that the Health Protection Branch will continue to provide the highest possible level of monitoring to ensure the safety of Canadians using omeprazole and all other new compounds.

Is there a lesson here for our politicians? My suggestion would be that we tell our MPs, "Now is not the time to reduce funding for medical research and development in Canada. If our patients are to enjoy the benefits of First World technology, they must also enjoy the benefits of similar levels of safety."

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