Cell/Tissue Injury and Cytoprotection/Organoprotection in the Gastrointestinal Tract

Mechanisms, Prevention and Treatment

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The ‘Gastric Cytoprotection’ Concept of Andre Robert and the Origins of a New Series of International Symposia

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Abstract

Andre Robert’s concept of ‘gastric cytoprotection’ was based on wrong premises (in part because of the initial lack of histologic evaluation). It, nevertheless, stimulated extensive research that led to better understanding of cell/tissue injury, discovery of new pathways of gastroprotection and new drug developments (e.g. misoprostol, NSAID-NO, NSAID-PL). It was also the origins of the International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection of which Robert one of the ‘founding fathers’. These meetings improved the communications and exchange of ideas among dozens of top investigators, in addition to stimulating hundreds of young researchers to ‘join the club’ and investigate mechanisms, pathogenesis, manifestations and prevention of drug-induced gastric mucosal lesions.

The historic article of Andre Robert and the accompanying editorial on ‘gastric cytoprotection’, published in 1979 [1, 2], opened not only a new frontier in gastrointestinal (GI) research and sparked enormous interest, but also created a lot of controversies. Namely, the prevention of chemically (e.g. intragastric gavage of 1 ml of 100% ethanol, 0.6 N HCl, 0.2 N NaOH) or physically (e.g. hot water) induced gastric hemorrhagic mucosal lesions (HML) in fasted rats by trace amounts of prostaglandins (PG) was almost ‘too nice to be true’ for a lot of idle investigators who rushed to repeat and expand Robert’s experiments. As the late Judah Folkman used to say: ‘I don’t know what hundreds of researchers are doing, but when new and original results are published, they rush to repeat, refute or expand the new findings’ [3]. Of course, he was thinking about the explosion of angiogenesis research in the 1990s that was similar to the new wave of GI investigations in the 1980s and early 1990s.

The hundreds and later thousands of abstracts submitted to the annual meetings of the American Gastroenterological Association (AGA) and subsequent full papers
prove that Robert’s experiments were mostly reproducible, i.e. pretreatment of rats with PG in doses that do not inhibit gastric acid secretion prevented the chemically induced HML. Furthermore, additional original articles were also published, e.g. Paul Guth’s lab was the first to show that ‘gastric cytoprotection’ is not unique to PG, since other drugs (e.g. cimetidine and probanthine in non-antisecretory doses) also prevented the experimental HML [4]. While reading this article in the Harvard Medical Library one late evening in December 1979, it occurred to one of us (Sz.S.) that if several compounds of different structure exert ‘gastric cytoprotection’, there must be common endogenous mechanisms, e.g. hormones and/or antioxidant sulfhydryls (SH). While most of my (Sz.S.) hypotheses were wrong, this was one of few times when I was right. Thus, a rapid series of experiments in rats demonstrated that the PG- or cimetidine-induced prevention of ethanol- or acid-caused gastric HML were dependent on endogenous glucocorticoids, since the prevention was lost in adrenalectomized (but not in thyroidectomized or ovariectomized) rats and it is restored by administration of cortisone, but not by mineralocorticoids [5]. Before the publication of this article in *Gastroenterology*, our abstract, based on preliminary results, was selected for opening plenary session presentation at the annual AGA meeting [6]. Our findings, published in Science in 1981 [7] that gastrototoxic chemicals depleted endogenous glutathione (GSH) in rat gastric mucosa were also reinforced that pretreatment with SH-containing chemicals [e.g., L-cysteine, N-acetylcysteine (NAC, or Mucomyst), cysteamine, methionine or dimercaprol (BAL)] also prevent the ethanol- or acid-induced HML in rats. Furthermore, injection of SH alkylators like N-ethylmaleimide (NEM) or iodoacetamide before the administration of PG counteracted their protective effect on chemically induced HML [7].

Here we review the surprising twists in the evolution of ‘gastric cytoprotection’ and the resulting controversy that led to the creation of ‘International Symposia on Cell Injury and Cytoprotection’ that was later modified to ‘International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection’.

**Evolution of the Concept of Gastric Cytoprotection**

*Initial Investigations*

One of the biggest surprises of the early years of ‘gastric cytoprotection’ research is that this prevention is not only relative (e.g. it depends on endogenous modulators such as glucocorticoids and SH antioxidants), but it is limited in morphologic scope. Namely, we were surprised to see in our lab in the early 1980s that SH containing compounds prevented only the hemorrhagic component and the deep necrosis in the gastric mucosa, but not the superficial cellular damage. These findings were in agreement and parallel with those of Susumu Ito and Eric Lacy who used PG-pretreated rats in the next building (Anatomy Department of Harvard Medical School) and published
several landmark articles on the histologically limited nature of PG-induced ‘gastric cytoprotection’ [8, 9].

Further histologic investigations revealed that after pretreatment of rats with almost any ‘cytoprotective’ drug (e.g. PG, SH, cimetidine), the ethanol-induced gastric superficial mucosal lesions remained nonhemorrhagic because the minimized tissue damage never involved the subepithelial vascular endothelial cells (fig. 1). The surviving epithelial cells in the gastric pits then rapidly migrate and cover the superficial

Fig. 1. a Desquamation of superficial gastric mucosal cells in NAC-pretreated rats 3 min after intra-gastric administration of 96% ethanol; note the congested subepithelial capillaries with stasis of red blood cells, but no hemorrhage. b Histologic section of a similar rat stomach 1 h after alcohol, showing complete restoration of superficial gastric epithelial cells. HE. ×100. c Schematic illustration of similar events in the gastric mucosa after pretreatment with a gastroprotective drug, compared with normal gastric mucosa (left).
mucosal defect by ‘restitution’ (fig. 2) [8, 9]. Hence, ‘cytoprotective’ drugs prevent the early vascular injury and the subsequent bleeding, but not the deaths of thousands of epithelial cells on the top of gastric mucosa [10]. Our lab had been the first to investigate the time sequence of ethanol- or acid-induced gastric mucosal lesions. Using specific vascular tracers, we demonstrated that endothelial damage is visible by light and electron microscopy (EM) within 1 min after intragastric gavage of ethanol or acid, followed by early hemorrhage in about 3–5 min [10, 11]. This is in sharp contrast with the Robert-type initial ‘gastric cytoprotection’ experiments when rats were killed 1 h after a gastrototoxic chemical to grossly evaluate the HML [1, 2, 4].

After these light and EM investigations the GI research community realized that the initial ‘gastric cytoprotection’ results of Robert and most of others [1, 2, 4, 12] were based only on macroscopic photographs of grossly visible HML. Subsequently, most of the scientific journals required histologic analysis and scoring of gastric lesions before accepting the claim of ‘protection’. Yet, even in these evaluations the ‘prevention’ refers only to the absence of hemorrhagic lesions and not to the deaths of a huge number of superficial epithelial cells whose place is covered by rapidly migrating surviving epithelial cells and within 1 h the restitution makes the mucosa almost completely repaired. Hence, this is not cell or cytoprotection, but tissue or organ protection since the main structures and functions of the stomach remain intact. We and the Konturek lab suggested that ‘gastric cytoprotection’ is more a concept rather than prevention of cellular injury in the gastric mucosa and we should instead refer to organoprotection or gastroprotection or vasoprotection (fig. 3), since the basis of PG-induced
Gastric Cytoprotection

Protection is the prevention of vascular endothelial damage [10, 13]. These surprising shifts in paradigm led to the change in the title of these symposia to 'International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection'.

Human Studies and Clinical Relevance

It is ironic that Robert’s initial goal was to demonstrate the clinical relevance of PG that was developed by the Upjohn pharmaceutical company as a new antiulcer drug [3]. He was indeed the main GI basic science investigator at Upjohn, working mostly on the antisecretory effect of PG and the prevention or treatment of experimental gastric and duodenal ulcers [14, 15]. He was invited to a symposium in the late 1970s to present new results on the potential antiulcer effects of PG for clinical use [3] and almost by accident his coworkers found that very low doses of PG prevented not only the gastric erosions produced by aspirin-like drugs, but also the alcohol-induced HML in rats [1–3].

The human relevance of prostaglandin cytoprotection was first demonstrated in 1981 by one of us (A.T.). In these direct endoscopic and histologic studies of duodenal and gastric mucosae (two separate studies) healthy volunteers were pretreated by a local administration of either saline or 16,16-dimethyl PGE2 (1 μg/kg) followed 15 min later by 40% ethanol (duodenum) or 60% ethanol (stomach) administered locally.

Fig. 3. Sequence of vascular events and related mucosal changes with (a) or without gastroprotective pretreatment (b) that provided the basis for the evolution of the concept ‘gastric cytoprotection’.
onto mucosa via an endoscopic spraying tube. Macroscopic mucosal assessment and quantitative histologic assessment of mucosal biopsies obtained 15 and 30 min after ethanol demonstrated that 16,16-dmPGE2 dramatically reduced injury of duodenal and gastric mucosa by ethanol, predominantly preventing occurrence of hemorrhagic erosions. These studies were presented at the CURE meeting in September 1980, at the International Meeting on Protective Action of Prostaglandins on Gastrointestinal Mucosa in Santa Monica, California, January 1981, at the AGA annual meeting in May 1981 [16], and subsequently published as full papers [17, 18]. In the same year, Domschke’s group in collaboration with Andre Robert also showed in indirect studies (measurement of gastric potential difference and epithelial exfoliation) protective action of PGE2 on human gastric mucosa [19]. Inspired by Andre Robert’s work, I (A.T.) asked the question whether cytoprotection by PG is limited to gastric epithelial cells or may also apply to other epithelial cells such as hepatocytes. Indeed, in 1980 and 1981 we demonstrated for the first time that 16,16-dmPGE2 protects rat liver against injury by several hepatotoxic agents, including carbon tetrachloride, alcohol, d-galactosamine and others. Interestingly, 16,16-dm-PGE2 afforded almost complete protection to all hepatocytes [20–23].

In subsequent studies performed in 1982–1987, we showed that arachidonic acid (PG precursor), sucralfate and aluminum containing antacids protect gastric mucosa against ethanol injury and that this protection is mediated, at least in part, by enhanced mucosal generation of PG [24–27]. In 1987, the cellular and ultrastructural features of PG-induced protection of human gastric mucosa against ethanol injury with focus on mucosal microvessels were examined [28, 29]. These studies demonstrated that human gastric mucosal microvasculature is an important target site for alcohol injury and PG protection, indicating that PG changes cytoskeletal ultrastructure of endothelial cells in a manner that may render them more resistant to alcohol injury [29]. While protection of the endothelial cell lining the mucosal microvasculature represents an example of a broader phenomenon of protective action of PG on various cells, the crucial strategic role of the microvasculature makes preservation (protection) of its integrity of special importance for the gastric mucosa. This fully confirmed and expanded the previously proposed critical role of vascular factors in gastroprotection [10, 11]. In a separate study, we demonstrated that 16,16-dmPGE2 also protects isolated human gastric glands (mainly parietal and chief cells) against indomethacin and ethanol injury, and indicated the cytoskeleton as one of the targets of injury and protection [30].

In 2002, one of us (A.T.) showed that a trophic and mucosal growth-promoting action of PGE2, demonstrated by Konturek’s and Halter’s groups in earlier studies, but without a clear underlying mechanism, is mediated by transactivation by PGE2 of epidermal growth factor receptor [31]. This indicates that PG may serve in GI mucosa as growth signaling molecules.

The clinical application of PG for the protection of gastroduodenal mucosa was fueled by the false assumption that mucosal protection will also translate to acceleration
of healing of gastric and duodenal ulcers. Namely, several multicenter studies demonstrated only a weak or marginal healing action of PG on gastric and duodenal ulcers, especially in comparison with proton pump inhibitors. However, a PGE1 synthetic analog misoprostol (Cytotec) was effective in prevention of gastric ulcers induced by chronic NSAID use, as demonstrated in osteoarthritis patients by Graham et al. [32]. This seminal, double-blind, placebo-controlled study in 420 patients provided the first clear demonstration that NSAID-induced gastric ulcers are preventable by PG supplementation and were the basis for FDA approval of misoprostol (Cytotec) in 1988 for reducing the risk of gastric ulcers in patients treated chronically with NSAIDs.

Central Modulation of Gastroprotection

It was very surprising that the prevention of strictly localized gastric HML would have a central component. Earlier reports, however, in the late 1970s and in 1980 established that the direct electrical stimulation of the vagus nerve increases PG release in the isolated rat stomach preparation [33]. These seminal observations were indicative that the synthesis and release of endogenous gastric PG may be under vagal control. The 1980s saw the explosion of knowledge on the identification of specific neuropeptides regulating gastric function through the modulation of the autonomic pathways as initiated by one of us [34]. In particular, the three amino acid peptide, thyrotropin-releasing hormone (TRH) located in the brain medulla was established to play a physiologic role in the stimulation of vagal outflow to the stomach through activation of TRH₁ receptor located in the dorsal motor nucleus of the vagus (DMN) providing a strong rationale to examine the potential gastroprotective action of brain TRH. Initial experiments in rats demonstrated that the stable TRH agonist, RX-77368 injected into the cisterna magna at a low dose (1.5 ng/rat) which did not influence gastric acid secretion, prevented HML induced by orogastric administration of 60% ethanol (5 ml/kg) [35]. The centrally initiated action of RX-77368 was demonstrated by the observation that direct microinjection of the peptide into the DMN resulted in similar gastroprotection while the peptide microinjected nearby but outside the DMN or peripherally had no effect [36]. The gastroprotection against HML induced by 60% intragastric ethanol conferred by intracisternal injection of RX-77358 was associated with increased PGE2 levels measured in the effluent of dialysis fibers implanted into the corpus submucosa in anesthetized rats and abolished by indomethacin supporting a role of gastric PGs in gastroprotection [35, 37]. Furthermore, central injection of TRH analog-induced gastric PG2 release was abolished by vagotomy and atropine further establishing the recruitment of brain-medullary TRH receptor activating vagal cholinergic-PGE2 pathways to increase the resistance of gastric mucosa to injury [35, 37, 38]. Mozsik and Miller independently discovered that in vagotomized rats PG pretreatment did not prevent the chemically induced gastric HML [12, 39, 40]. Importantly, endogenous brain medullary TRH mediates the vagal-dependent adaptive gastroprotection whereby a mild gastric irritant protects against a strong gastric irritant [41, 42]. The activation of brain medullary TRH₁ receptor contributes
to the cephalic phase of vagal stimulation of gastric secretion [43, 44], implying that the cephalic phase, through activation of medullary TRH, also triggers these PG-dependent gastric mucosal protective mechanisms. Conversely, a deficient cephalic phase may render the gastric mucosa more vulnerable to aggressive factors in the absence of such vagally recruited gastroprotective mechanisms including PGE2 [45].

These investigations opened a new field of research to delineate the central vagal regulation of gastric mucosa by neuropeptides through modulation of gastric PG release. In particular work, Gyires et al. [46, 47] established that other neuropeptides including nociceptin, nocistatin, β-endorphin, and deltorphin or stimulation of alpha-2-adrenoreceptors can also induce gastroprotection through central vagal-PG-dependent pathways. These studies were indicative that differential neurocircuits were most likely recruited by different states (cephalic phase, stress or other peripheral sensory input reaching the brain) to maintain gastric mucosal integrity through brain regulation of gastric PG synthesis and release [reviewed in 45].

Studies by Peter Holzer and the Szolcsányi-Mozsik team discovered another neural component of ‘gastric cytoprotection’, i.e. long-term pretreatment with capsaicin (desensitization) also interfered with PG-induced gastroprotection [48, 49]. However, a single dose of capsaicin 30 min before ethanol also reduced the gastric HML, most likely because of the ‘mild irritant’ or ‘adaptive ‘cytoprotection’ effect of this chemical.

Molecular and Cellular Mechanisms of Gastroprotection

After the very surprising initial experiments were reproduced and expanded by several GI investigators, the search for mechanisms began in earnest. Obviously, it was not reduction in gastric acid secretion, since by the initial definition of ‘gastric cytoprotection’ [1, 2, 4], the doses of PG and cimetidine were below the antisecretory doses, not to speak about SH and other drugs that don’t influence or actually increase gastric acid secretion. The next suggestion was on enhanced mucus and bicarbonate secretion by PG [50, 51]. This became a classic example of flawed study design and data interpretation, i.e. just because PG exerts gastroprotection and enhance mucus secretion, it does not mean that the PG-induced gastroprotection is due to enhanced mucus secretion. This is the standard ‘true-true, but unrelated’ trap, i.e. if we speak in an illuminated room, it does not mean that we could not talk when the light was off. The flawed connection between enhanced mucus secretion and gastroprotection was reinforced by simple pharmacologic experiments that demonstrated that the SH containing mucolytic NAC (Mucomyst) also exerts cytoprotection while gastroprotection by PG and virtually any gastroprotective drug is counteracted by SH alkylating chemicals (e.g. N-ethylmaleimide, iodoacetamide), implicating the role of endogenous SH antioxidants like GSH [7, 52, 53].

A creative, chemically well-defined refinement of the ‘mucus-bicarbonate barrier’ was published in Science by Lenard Lichtenberger outlining that PG increase the hydrophobicity (i.e. water-repellent feature) of the gastric mucus layer, thus decrease
or delay the absorption of water-soluble damaging chemicals [54]. He ascribed this to the phospholipid component of mucus layer and his subsequent research over the last about 20 years led to the clinical testing of ‘safe aspirin’-like drugs after attaching phosphatidylcholine radicals of phospholipids (PL) to nonsteroidal anti-inflammatory drugs (NSAID) [55–57]. Since some of these NSAID-PL drugs have been approved by FDA for clinical tests, a small pilot clinical study has demonstrated a statistically significant reduction of gastroduodenal toxicity (without interfering with antiinflammatory action) of high dose (2,400 mg/day) ibuprofen-PC versus standard ibuprofen in osteoarthritic patients [55]. A 7-day clinical trial comparing regular dose (325 mg/day) aspirin vs. equivalent dose of aspirin-PC (PL2200) showed >70% reduction in gastroduodenal ulcers and/or meaningful erosions [56]. A previous 4-day cross-over clinical study comparing aspirin vs. aspirin reported a ~70% reduction in gastric erosions with PC-NSAID [57].

Another early suggestion was a direct cell protection by PG that turned out to be of limited value since pretreatment of isolated gastric epithelial cells by PG offered at best about 20–30% protection again low concentrations (5–15%) of ethanol [58, 30, 59]. This is no match for the very toxic 75–100% ethanol or 0.6 n HCl or 0.2 n NaOH that essentially melts the membranes of any cell instantaneously. Hence, the likely protective mechanism(s) must be very complex and probably operating at the tissue level.

The first multi-component, tissue-level protection came out of the recognition that early vascular injury plays a critical role in the development and prevention of chemically induced gastric HML. Actually, this was just a logical, textbook-like extrapolation of the consequences of vascular endothelial damage which is known to lead to platelet aggregation that usually attaches to injured endothelial cell, along with leukocytes, leading to reduced flow of oxygen-carrying erythrocytes, blood flow stasis in small and large blood vessels, with resulting hypoxia in surrounding tissue and death of these cells (necrosis) (fig. 4). Indeed, mucosal blood flow measurements in anesthetized rat stomach showed that after exposure to 50–75% ethanol of or 0.6 n HCl, blood flow slowed down and stopped in 1–2 min, in agreement with the previously
demonstrated endothelial damage in 1–3 min [60,10]. In PG- or SH-pretreated rats the blood flow never stopped and HML lesions did not develop [22]. This was in agreement with the findings of Paul Guth who just did functional studies, i.e. measured gastric mucosal blood flow in rats (without doing morphologic studies to demonstrate early endothelial damage) and also found that PG ‘maintain mucosal blood flow’ [61]. This is so far the most accepted mechanistic explanation for the prevention of chemically induced HML by gastroprotective drugs and it resulted in the ‘appearance’ of mucosal blood vessels/flow in the illustrations of gastroprotection in modern review articles [62, 40] (fig. 5) and book chapters (vs. old graphs that showed only epithelial cells and mucus layers).

It is encouraging to see endothelial cells and maintenance of mucosal blood flow as the main targets and mechanism of gastroprotective drugs, but very few investigators asked: How this vasoprotection comes around? It was shown that the attached leukocytes release free radicals that expand the initial endothelial damage [63, 64], although released vasoconstrictor endothelins (ET) contribute to the chemically induced endothelial injury [65–67] that is also prevented by ET antagonists [65]. The vasodilator NO-producing [45, 68] and NO-releasing drugs [69] also exert gastroprotection against alcohol or aspirin-like drugs [27]. Yet, as original as these results may be, they are not the ‘full story’, especially since white blood cells attach only to damaged endothelium (hence, what causes the initial injury which is aggravated by released free radicals?). Furthermore, although the ET/NO balance may be an important contributory mechanistic factor, why are the endothelial cells not damaged after pretreatment with gastroprotective drugs?

The answer may be emerging now, as a follow-up to one of our previous in vitro experiments in Boston that demonstrated that cultured endothelial cells are about twice more sensitive to ethanol than cultured epithelial cells [70, 71]. These experiments were recently expanded and showed that cultured endothelial cells were more sensitive to ethanol than fibroblasts and epithelial cells [unpubl. data]. We thus reasoned that at the tissue level a slightly increased vascular permeability (e.g. after all, PG are mediators of inflammation that always starts with increase in vascular permeability) may dilute the perivascular concentration of toxic chemicals (after they kill and penetrate between superficial epithelial cells) for a few minutes and delay their absorption [72] while the protective gastric emptying moves the toxic chemicals from the stomach to the duodenum. The intragastric luminal dilution by intact and fragmented mucus and bicarbonate probably also contribute to this ‘histodilutional barrier’ (fig. 6) or defense [73]. So far this is most unifying and comprehensive mechanistic explanation for gastroprotection that also includes so wide range of prevention scenarios as Robert’s original ‘adaptive cytoprotection’, i.e. prevention of concentrated ethanol-induced HML by pretreatment of rats with 1 ml of diluted (20% ethanol). As far as we know nobody offered mechanistic explanation for this ‘adaptive cytoprotection’, other than stimulation of endogenous PG synthesis, thus initiating the vascular phase, i.e. increased microvascular permeability, of mild inflammation. Our
Hormonal regulation
- Gastrin, CCK
- Ghrelin, growth factors and cytokines
- Adrenal corticosteroids

Mucus

Muscularis mucosa

Submucosal artery

Submucosal vein

Prostaglandins (PGE2 and PGI2) maintain and enhance all mucosal defensive mechanisms working synergistically with nitric oxide

Mast cells

Sensory nerves

Fig. 5. Modern illustration of gastroprotection. From Laine et al. [62].
suggestion is supported by the fact that low doses of injected histamine (which is the main mediator on increased vascular permeability in the early stages of inflammatory reaction) that induce a slightly increased vascular permeability, created a perivascular edema (fig. 6) in rats, prevented the 75% ethanol-induced HML [73]. Our explanation is also supported by previous experiments of Robert that showed that ‘cytoprotection by PG occurs in spite of penetration of absolute ethanol into the gastric mucosa’ [74], i.e. just a delay of absorption for a few minutes because of perivascular dilution and gastric emptying [72] seems to make the ‘die or survive’ difference for thousands of surface gastric mucosal cells. Thus, ‘gastric cytoprotection’ is nothing more than an initial endogenous, spontaneous mild inflammatory (e.g. in ‘adaptive cytoprotection’) or induced (e.g. by PG or other drugs) self-defense. Yet, Andre Robert deserves a lot of credit for calling our attention to this natural phenomenon, as Pavlov did with his discovery of spontaneous and conditional reflexes. Subsequent clinical studies indeed demonstrated that in sucralfate-pretreated patients the absorption of stomach-damaging anti-inflammatory drugs was delayed for 3–5 min, i.e. in the critical early stages when vascular injury may or may not occur [75].

**Fig. 6.** a–c Toluidine blue-stained ‘thick’ (1 μm) sections of a rat stomach. a From a control rat. b, c After injection of small doses of histamine that created a prominent subepithelial edema, due to slightly increased vascular permeability that results in gastroprotection again ethanol. d Graphical illustration of histodilutional barrier.
Origins and Development of Related International Symposia

The initial publications on ‘gastric cytoprotection’ sparked interest not only in the mechanisms of these unexpected protective actions of PG, but also in the progression of cell injury and mechanisms of cell damage. Actually, by the 1970s there were a substantial number of publications on cell injury and organ protection that was triggered by the first successful kidney transplantation in 1953 in Boston, followed by first liver, lung and heart transplants in the subsequent two decades. These transplantations needed not only substantial surgical skills, but great basic science and practical knowledge on preserving and transporting the transplants from donors to the receiving patient as well as on controlling organ rejection by immunologic modulation. In addition to organ preservation, the emerging field of cardiac ischemic and reperfusion damage leads to the recognition of reversible and irreversible stages of cell injury (table 1) [76].

Mechanisms of Cell Injury and the Origins of Symposia

In the rapidly progressing stage of basic and clinical sciences in the 1970s and 1980s, two mechanistic explanations of cell damage dominated the field of experimental pathology and molecular biology: the critical role of intracellular calcium (John Farber) and phospholipase activation (Sten Orrenius). These disputes coincided with the arguments about if, in addition to PG (Andre Robert), antioxidant SH chemicals (Sz.S.) also offer gastroprotection. This symposia series is thus the product international cooperation and interdisciplinary communication between basic science and clinical investigators who demonstrated that controversies and scientific disputes are best resolved by open communication and rapid display of new data. The fifth member of the initial Standing Committee has been K.H. Usadel who was the first clinical investigator to demonstrate the (initially controversial) protective role of somatostatin in pancreatitis and chemically induced hepatic damage. After the untimely death of Andre Robert in 1991, Andrzej Tarnawski was asked to join the Standing Committee (table 2). He demonstrated in direct, quantitative endoscopic and histologic studies the human relevance of PG protection of

<table>
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<th>Stages of cell/tissue injury</th>
<th>Reversible stage</th>
<th>Irreversible stage</th>
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<td>Cell membrane and endoplasmic reticulum</td>
<td>Blebbing, vacuolization</td>
<td>Mitochondrial and nuclear damage</td>
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<td>Important for organ transplantation and cardiac resuscitation</td>
<td>Necrosis or apoptosis</td>
<td>Necrosis is followed by acute/chronic inflammation, e.g. gastroduodenal ulceration, ulcerative colitis</td>
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Table 2. The Standing Committee of the International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection

Original (‘founding fathers’)
John L. Farber, MD, Philadelphia, Pa., USA
Sten Orrenius, MD, PhD, Stockholm, Sweden
Andre Robert, MD, PhD, Kalamazoo, Mich., USA
Sandor Szabo, MD, PhD, MPH, DSc(h.c.), Boston, Mass., USA
Klaus Henning Usadel, MD, Heidelberg, Germany

Updated (after the death of Andre Robert)
John L. Farber, MD, Philadelphia, Pa., USA
Sten Orrenius, MD, PhD, Stockholm, Sweden
Sandor Szabo, MD, PhD, MPH, DSc(h.c.), Boston, Mass., Irvine/Long Beach, Calif., USA
Klaus Henning Usadel, MD, Heidelberg/Frankfurt, Germany
Andrzej Tarnawski, MD, PhD, DSc, Irvine/Long Beach, Calif., USA

gastroduodenal mucosa, provided first demonstration of liver protection by PG and showed the cytoprotective mode of action of arachidonic acid (precursor of PG), sucralfate and antacids.

The name of symposia, mostly on the suggestions of Andre Robert [3] and one of us (Sz.S.), was also chosen to include both new information of cell injury and protection: 'International Symposia on Cell Injury and Cytoprotection'. The first formal meeting was held in 1986 at the University of Heidelberg in Germany and the second symposium was organized in 1989 at Harvard Medical School in Boston (fig. 7): Andre Robert attended both symposia and very actively participated not only with presenting his new findings but also in formal and unofficial discussions by strongly defending the initial definition of 'gastric cytoprotection'. After it became obvious in the late 1990s that most of the protection is not at the cellular but at the tissue level, the name of the symposia has been changed to 'International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection' and has been used at the subsequent symposia held in various parts of the world (table 3).

Objectives, Scope and Format of the Symposia
The objectives of this international symposia series are to provide a lively forum for experts to present and integrate new findings on the mechanisms, manifestations, diagnosis, and consequences of cell and tissue injury. One of the goals is to review emerging concepts, e.g. that reversible or irreversible injury to a few or numerous cells not only that it does not necessarily lead to tissue damage, but actually results in mucosal protection (e.g. in stomach 'cytoprotection' or gastroprotection).

A further goal is to apply the new knowledge about cell and tissue injury to the pharmacologic prevention of tissue damage in organ systems. Our specific areas of
focus include new developments in the molecular mechanisms of cell and tissue injury in the GI tract. Although the traditional focus of these symposia is the upper GI tract, at the recent symposia new developments in ulcerative colitis pathogenesis, prevention and treatment were also presented. Some symposia included topics on mechanisms of cell injury and protection in the liver, pancreas, kidney and heart as well as new concepts in the emerging fields of regenerative medicine. These presentations on damage and protection in various organs are meant to lead to cross-fertilization of ideas and to stimulate creative, innovative thinking.

The scope of the symposium involves primarily molecular and cell biologic aspects of cell injury and protection, with emphasis on biochemical and genetic mechanisms of both damage and protection, i.e. to extrapolate from new mechanistic findings in cell injury to new possibilities of cytoprotection. A major emphasis is given to pharmacologic methods of cytoprotection and organoprotection as well as to morphologic
manifestations of cell and tissue injury, mechanisms of mucosal protection and ulcer healing. The latter scope and topics actually represent a deviation from the original goals of Andre Robert who under ‘gastric cytoprotection’ accepted only prevention and not the treatment of existing GI lesions; he used to say [3] that ‘antiulcer drugs are meant for treatment’.

The format of these international symposia has usually included a few minisymposia on various topics. In this format after major presentations by a few invited internationally known experts, short presentations scheduled selected from abstracts submitted (usually by young) investigators. Poster sessions have also been organized at all the symposia so far. The presence of prominent and emerging experts, young and old, is not only a good learning experience, but it also stimulates cross fertilization of ideas and original thinking. The internationally known experts included several Nobel Laureates, the last being Barry Marshall who gave an impressive presentation of his Nobel lecture at the 4th International Symposium in Long Beach in 2006. At this symposium, George Sachs, recipient of the Garner Award, also presented his new findings on the mechanisms of Helicobacter pylori-induced gastric cell damage. The opportunity is also assured by the limited size of participation and duration of the symposia. It was also one of the original organizational suggestions of Robert not to accept more than about 100 participants who should disperse after three days because ‘otherwise we will get to each other nerves’ [3]. Actually, the six symposia so far demonstrated that on the third day mostly intellectual exhaustion or inertia sets in after the intensive, often spirited discussions following certain presentations or during social events. Namely, the good atmosphere was also assured by the well-organized social functions, in good part because of modest, but now declining support, mostly from pharmaceutical companies. Other sources of support


| First series |

| Second series |
| 3rd International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection, 2000, Long Beach, Calif., USA (S. Szabo) |
| 4th International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection, 2006, Long Beach, Calif., USA (S. Szabo and A. Tarnawski) |
| 5th International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection, 2008, Yalta, Ukraine (T. Beregova) |
| 6th International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection, 2011, St. Petersburg, Russia (L. Filaretova) |
| 7th International Symposia on Cell/Tissue Injury and Cytoprotection/Organoprotection, 2012, Honolulu, Hawaii, USA (K. Takeuchi) |
included local universities, city and state governmental services as well as nonprofit organizations such as the IUPHAR GI Pharmacology Section that provided limited but well appreciated support to the last two symposia and some satellite meetings.

**Symposia Outcomes, New Developments and Suggestions for the Future**

The concept and excitement around ‘gastric cytoprotection’ led to a series of partially related or independent discoveries, some of which originated from the Symposia or have been reinforced or expanded there. The first positive outcome was that some contemporary scientific disputes calmed down or became more civil. For example, from the late 1980s, we cannot see much if any published or oral arguments about the primary role of calcium and phospholipases in cell injury, in part because now accepted that both are essential in the pathogenesis of cell damage [76]. Furthermore, disputes about the initially controversial role of somatostatin in organoprotection have been settled down and this short peptide is now clinically one of the few options for the treatment of acute pancreatitis. Because of these controversies a new action of somatostatin has been discovered: although the peptide inhibits, in addition to growth hormone, a large number of other hormones and neuropeptides including gastrin that results in inhibition of gastric acid secretion, we were surprised to see that somatostatin exerts so far the only stimulatory action in the body, i.e. phagocytosis, most likely because of stimulation of macrophages [77]. This may be beneficial in the various organoprotective actions of somatostatin, especially since it reduces experimental septic shock and mortality in rats [unpubl. results]. Last but not least, nobody argues about the supremacy of PG in gastroprotection anymore, since it is accepted that SH and numerous other drugs may also prevent chemically induced gastric HML.

The attempts to reproduce ‘gastric cytoprotection’ in vitro reinforced the distinction between reversible and irreversible stages of cell injury [76] (table 1) and may have led to recognition of new mechanisms and markers. Namely, the previously discussed modest (about 20– 25%) in vitro cytoprotection by PG against low concentrations of toxic chemicals apparently prevented only the reversible stages of ethanol-induced cell damage, since most of these studies used only outer cell membrane damage indicators such as trypan blue exclusion or LDH (lactate dehydrogenase) release [58, 30] Yet, for total cell viability assessment one has to use indicators of mitochondrial viability (e.g. by activity of mitochondrial succinic dehydrogenase) and intactness of nuclear membrane (e.g. by fluorescent ethidium or propidium bromide that may reach the nuclear DNA only after major disruption of nuclear membrane that is not compatible cell survival) [76, 78]. The lesson from these in vitro cytoprotection experiments is that it is difficult to obtain a large number of freshly isolated viable gastric mucosal cells [78, 79]. Furthermore, for full assessment of cell viability, the modern study design should include not only markers of cell membrane damage (e.g. trypan blue exclusion and LDH release), but indicators of mitochondrial and nuclear viability (e.g. the mentioned fluorescent probes), since no cell will survive if the nucleus is damaged [76, 78, 59].
One of the most impressive discoveries relates to the recognition that although exogenous glucocorticoids in large doses cause gastric erosions and ulcers in experimental animals or in patients [80], but in small doses are gastroprotective [5, 81–83]. Glucocorticoids released in response to stress or other ulcerogenic stimuli may also be gastroprotective [81–83]. These seminal observations of Ludmila Filaretova creatively expanded the previously demonstrated essential role of adrenal glands, especially glucocorticoids for the gastroprotective effect of PG and SH compounds [5]. Now, mostly due to her extensive investigations, it is generally accepted that these steroids, like oxygen, pH, and histamine exert biphasic biologic effects, i.e. in optimal doses are essential for our normal physiologic functions, but in high or low concentrations are either incompatible with life or cause cell and tissue damage [84].

Another new development in the field of gastroprotection, in part reinforced and expanded by these Symposia, is the recognition of new receptors, or new functions for receptors as well as discovery of new drugs. The lab of Koji Takeuchi extensively and systematically investigated the role of PG receptors and identified PG EP receptor subtypes that mediate the actions of PG on gastric acid secretion, GI motility and gastroprotection [85–87]. In an elegant study using rats and knockout mice, he demonstrated that adaptive gastric cytoprotection is mediated by PG EP1 receptors [86]. His lab also showed that upregulation of COX-2 is a key element to NSAID-induced GI damage [88].

ET receptors were also identified as being responsible for the initial vascular injury in the pathogenesis of gastric mucosal injury and prevention [65–67]. Clinically most relevant are the potential new developments that grew out of the extensive research following the seminal publications of Robert and coworkers [1, 2]. The first attempt, shortly after the discovery of the role of SH in gastroprotection [7], has been to put an –SH radical on aspirin, especially since pretreatment of rats with NAC or methionine 1 min before intragastric administration of aspirin substantially reduced the HML [89]. However, the clinical development of this approach has been abandoned after one of the pharmaceutical companies discovered that SH-aspirin had been patented a few decades ago, along with other possible radicals that were attached to aspirin, without any biologic data or rationale, yet the company was afraid to get in a new, similar compound because of potential patent challenges [3]. Fortunately, Lichtenberger and Wallace were lucky because they could patent and develop PL- and NO-aspirin, respectively, new drugs, some of which are under clinical evaluation now [54–57, 69]. Another new discovery in Wallace’s lab indicates that hydrogen sulfide (HS) also exerts gastroprotection and attachment of this HS moiety to aspirin-like drugs increase the gastric safety of these widely used drugs [90].

Despite these new developments and advances in basic sciences, the benefits of these Symposia may be further enhanced. One of the almost obvious improvement and standardizations seems to be to include presentations on cell injury and protection in other organ systems (e.g. liver, kidney, pancreas, lung, heart) to enhance intellectual cross-fertilization and recognize common or different pathways of cell injury and protection. As mentioned earlier, this was done at some, but not all Symposia. The
abstracts of only some of the Symposia were published in good international journals, followed by selected full papers after peer review in journals or books – yet these publications would assure that not only the about 60–100 participants of the Symposia would benefit from the new data presented, but the wide international research community as well. Based on the abstracts submitted and long presentations at the last four Symposia, we should probably yield to modern trends and include new results not only on the prevention of acute lesions, based on the strict definition of Robert [3], but on the progression and healing of ulcerative and inflammatory lesions in the GI tract and in other organ systems. Indeed, ‘organoprotection’ may be interpreted as total pathogenesis of lesions and organ disorders, starting with initiation, followed by progression and spontaneous or accelerated, pharmacologic healing. This may lead to the unification and synthesis of these Symposia with the ‘International Symposia on Ulcer Healing’ that we also initiated about 20 years ago (so far three have been held).

Definitions of ‘Gastric Cytoprotection’ and Gastroprotection
Andre Robert was strict to define ‘gastric cytoprotection’ as the prevention of chemically (e.g. alcohol, acid, base, hypertonic solutions) or physically (e.g. thermal) induced gastric injury by non-antisecretory doses of PG [1, 2]. At these Symposia and other meetings he was always on alert to verify in all presentations that the doses of PG or H2 receptor antagonists used in ‘gastric cytoprotection’ experiments were below the minimal antisecretory amounts that he also called antiulcer doses [3]. This vigilance to assure that gastric acid does not play a role in gastroprotection led to division of gastrotoxic chemicals as ‘acid sensitive’ (e.g. aspirin-like drugs) and ‘acid-independent necrotizing agents’ (e.g. concentrated alcohol, base, hypertonic solutions).

The modern definition of gastroprotection is more complex and it includes the results of multidisciplinary studies in a large number laboratories all over the world. We could summarize it as the preservation of subepithelial endothelial cells and microcirculation that assures that the surviving gastric foveolar cells can migrate (restitution) and proliferate (regeneration) to replace the lost surface epithelial cells. Thus, it’s a complex protection at tissue, mucosal levels when a large number of superficial epithelial cells are ‘sacrificed’ to protect the millions of deeper mucosal cells and structures. Apparently, direct cytoprotection (e.g. in vitro) plays a limited role in gastroprotection. A more detailed, integrative and mechanistic definition of ‘gastric cytoprotection’: prevention (by PG, PL, SH, NO, etc.) of chemically induced hemorrhagic gastric erosions by mechanisms other than inhibiting acid secretion whereby preservation of microcirculation is a key element.

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