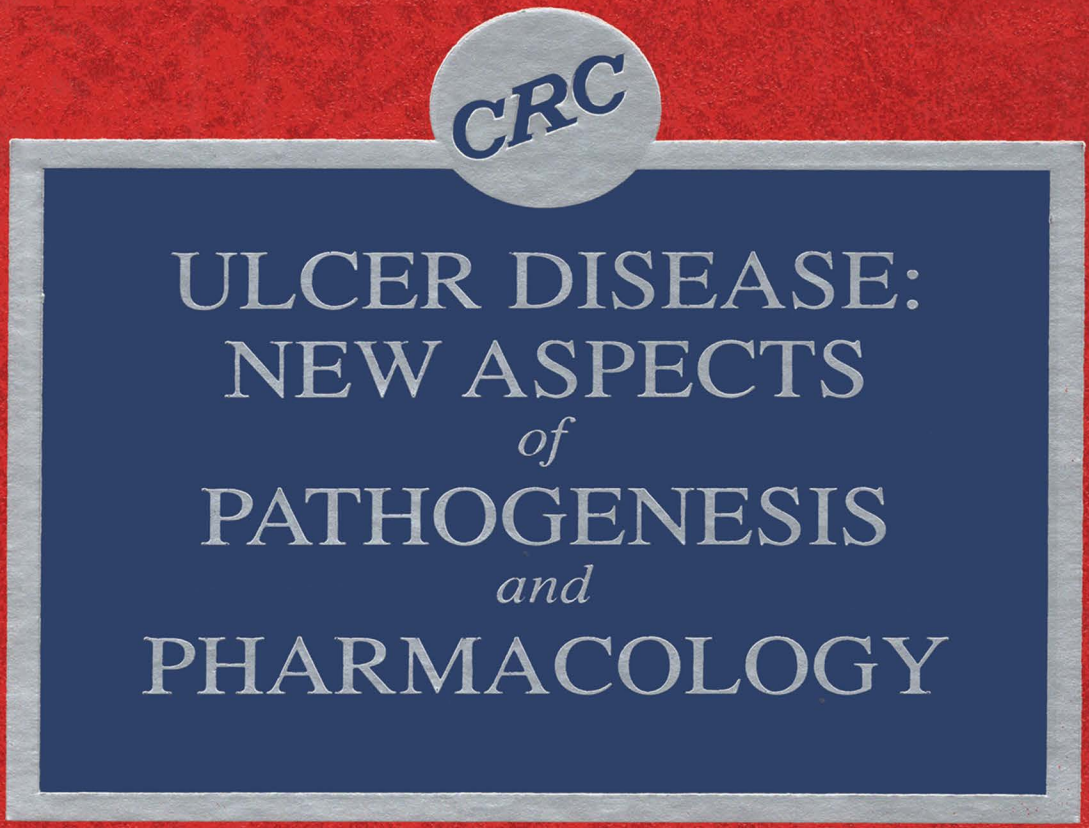

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DRUGS AND PEPTIC ULCER

Volume I

Therapeutic Agents for Peptic Ulcer Disease

Volume II

Pathogenesis of Ulcer Induction Revealed by Studies in Human and Animals

CANCER OF THE ESOPHAGUS

ANIMAL MODELS FOR INTESTINAL DISEASE

ULCER DISEASE: NEW ASPECTS OF PATHOGENESIS AND PHARMACOLOGY

Ulcer Disease: New Aspects of Pathogenesis and Pharmacology

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PREFACE *

Ulcer is a defect caused by chemical, physical, or infectious agents in the epithelial lining (e.g., skin, mucous membranes). Gastric and duodenal ulcers as interpreted in this book refer to a complex group of disorders for which the only common element seems to be the mucosal defect resistant to healing.[1,2] It is, thus, appropriate to use the designation ulcer disease, since the pathogenesis of gastric and duodenal ulcers involves not only local alterations in the gut but central changes in brain and neuroendocrine system as well.

These nonspecific acute or chronic ulcers are preferentially localized in the acid-producing part of the stomach, antrum, and anterior or posterior wall of the duodenum. Nevertheless, their pathophysiology is poorly understood, and the lesions are often simplistically labeled "peptic ulcers". It is, however, unfortunate and misleading to label a disorder by only one, mostly historic pathogenetic factor, especially when gastric acid and pepsin hypersecretion, but especially peptic activity, play a more and more doubtful role in duodenal and gastric ulceration. Furthermore, only about 50% of duodenal ulcer patients secrete more than normal gastric acid, and a substantial number of gastric and duodenal ulcer patients are hyposecretors.[3] Until classification based on etiologic factors will be available for ulcer disease, it is more appropriate to rely on classification based on the well-elucidated localization of ulcers than to overemphasize a single and doubtful pathogenetic factor such as pepsin.

The incidence and prevalence of gastric and duodenal ulcers are different in various areas of the world, but it is generally estimated that about 10% of the population suffers from this disease at least once during their lifetime.[3] The etiology and pathogenesis of ulcer disease are complex and poorly elucidated. Drugs and stressors, such as burn, brain damage, and trauma, account for about 20 to 25% of all cases, while the etiology of ulcer disease is not known in about 70 to 80% of cases. If the plethora of descriptive studies on the pathogenesis and mostly empirical pharmacologic interventions are critically evaluated, one cannot escape from the surprising realization that we may not know more about the etiology and pathophysiology of ulcer disease than those of malignant tumors. Ulcer research is so confusing that doubts have been raised about whether we can equate etiology with pathogenesis.[4] Nevertheless, there is no successful treatment or prevention of a disease without pharmacology based on etiology. Furthermore, virtually everybody agrees that ulcer pathophysiology is a complex multifactorial or pluricausal disorder.[2,5-7]

Needless to say, to study the role of multiple etiologic and pathogenetic factors, animal models of ulcer disease are needed.[8,9] Clinical studies with ulcer patients are essential to determine the factors influencing the healing and recurrence of ulcers in the stomach and duodenum, but the early pre-ulcerogenic biochemical, functional, and structural alterations generally can be investigated only in animal models.[9-11] It is, thus, both from results of clinical studies and work with animal models that the major pathogenetic factors were recently reviewed and presented graphically in Figure 1.[2] Historically, most of the emphasis has been placed on "secretion"; i.e., neutralization or inhibition of secretion of gastric acid. In addition, new studies delineate the role of gastric and duodenal bicarbonate secretion. Elucidation of its regulation will yield important new therapeutic approaches to ulcer disease. Motility in the stomach has long been recognized as a possible pathogenetic factor in duodenal ulceration, but a recent review of the literature indicated that duodenal motor abnormality, contributing to abnormal acid emptying and a "misplaced mix" of duodenal bicarbonate and acid might be another factor in duodenal ulceration that can be pharmacologically modulated.[2] New results with models of duodenal ulceration are certainly promising in this respect.[12,13]

* Parts of this introductory overview originate from a foreword of a recently published book: Szabo, S. and Mòzsik, Gy., Eds., *New Pharmacology of Ulcer Disease. Experimental and Therapeutic Approaches,* Elsevier, New York, 1987.

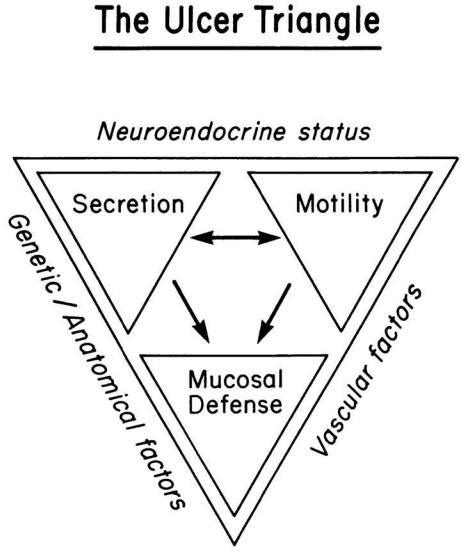

FIGURE 1. Major endogenous pathogenetic factors in ulcer disease. (Modified from Szabo, S., *Lab. Invest.*, 51, 121, 1984.)

Mucosal defense has been investigated with increasing vigor, especially after the introduction of the concept of gastric "cytoprotection".[14,15] Numerous publications and symposia have dealt with this new property of prostaglandins: to prevent at nonantisecretory doses the chemically induced and grossly visible hemorrhagic erosions in the rat stomach.[15] We now know that sulfhydryls, carotenoids, colloidal bismuth, and sucralfate also exhibit gastric mucosal protection without inhibiting gastric secretion. Thus, pharmacologic emphasis has been placed on strengthening "mucosal defense" despite the lack of agreement on a definition of mucosal defense.

Other new pathogenetic elements and new targets which can be pharmacologically modulated are being investigated, such as bicarbonate secretion, vascular factors, and neuroendocrine elements. These may replace the previous two factors as well as others (for example, acid secretion and motility). From the pathogenetic factors illustrated (Figure 1) only the genetic and anatomical determinants (e.g., position of the duodenum between certain ligaments, liver and pancreas that contribute to the drainage of gastric juice and localization of duodenal ulcers) cannot be influenced pharmacologically.[16] All the other etiologic and pathogenetic factors may be considered for new preventive or therapeutic targeting. If we accept that the pathophysiology of ulcer disease is pluricausal, why cannot its pharmacology be multifactorial? This does not mean that several drugs should automatically be prescribed for each patient but new drugs with multiple mechanisms of action (e.g., in addition to acid neutralization or decreased secretion, the drugs might stimulate bicarbonate secretion, stabilize motility and the vascular tree).

A revolution in ulcer pharmacology was created with the introduction of histamine H_2-receptor antagonists. These drugs, however, have side effects, some patients are resistant to them,[17] and certain factors such as cigarette smoking may neutralize the effect of at least some of the members of this class of drugs.[18] More disappointing, however, is the recognition that the effect of H_2-receptor antagonists is restricted: duodenal and gastric ulcer healing rate that, in placebo-treated patients varies from 30 to 60%, is only pushed up to 80 to 95% in most of the studies, and when the drug is discontinued the ulcer reappears in more than half of the patients within 1 year.[17,19] Similar temporary successes were seen in the pre-

penicillin era of antibiotics when the drugs were not strictly and causally designed for certain infectious diseases.

With such a need for additional information about the pathogenesis and pharmacology of ulcer disease, it is thus appropriate to present in a selected form the highlights of the new data presented at the 5th International Conference on Experimental Ulcer (Boston, MA, May 16 to 18, 1985). The abstracts of all the about 200 presentations were published in a journal,[20] and a critical evaluation of the results and concepts was also prepared and published.[21] Subsequently, we asked some of the invited speakers and authors of free communications to prepare a detailed and updated account of their work. The new information about the latest development in the pathogenesis and pharmacology of ulcer disease thus is derived from this material.

S. Szabo
C.J. Pfeiffer

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Chapter 1

MECHANISMS OF GASTROINTESTINAL DAMAGE BY NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

K. D. Rainsford

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I. INTRODUCTION

Gastrointestinal (GI) side effects are among the most frequent of the untoward effects from nonsteroidal anti-inflammatory drugs (NSAIDs).[1-4] These conditions range in severity from being rather discomforting or painful (e.g., nausea, vomiting, epigastric pain, diarrhea) to those which are potentially life-threatening (e.g., hemorrhage and ulceration).[1,3,4] The exact contribution of NSAIDs to the occurrence of peptic ulcer and other GI pathology is very debatable, for a wide variety of environmental agents or conditions can cause or contribute to the cause of these pathological states. Thus, in addition to NSAIDs, the following agents can cause or contribute to upper GI pathology, namely:

1. Corticosteroids[5]
2. Ethanol (''alcohol'')[6-8]
3. Nicotine (from cigarette smoke)[8-10]
4. Caffeine-containing beverages[8-11]
5. Certain metal ion or salt supplements[11] (e.g., KCl, Fe compounds)[3,12]

Because patients taking NSAIDs may be exposed to these agents, it is difficult to know (1) what the exact contribution is from these drugs in relation to that of NSAIDs in all such side effects, and (2) if there is potentiation of synergism of NSAIDs with the other known ulcerogenic agents (e.g., ethanol, cigarette smoking) or environmental factors.[2]

There have, however, been some attempts to estimate the incidence of GI ulceration associated with use of NSAIDs in arthritis therapy, but these vary somewhat according to the basis upon which the assessments are determined. Thus, estimates published recently include those derived from population studies such as data collected by the U.K. Committee on the Safety of Medicines,[4] the U.S. Food and Drug Administration,[13] and the Swedish Health Department.[4] Each of these sets of data may have a different basis for assessing the percentage incidence of severe GI complications from NSAIDs, but two things they all agree on are that (1) the NSAIDs are a major factor contributing to the incidence of GI hemorrhage and ulceration, and (2) GI side effects are among the most frequent and severe of all the side effects of NSAIDs.

Other approaches employed in studying the association of NSAID ingestion with GI complications include (1) determinations on the admissions of arthritic patients to hospital for treatment of peptic, gastric, or duodenal ulcer,[7,8] (2) endoscopic studies in arthritic patients,[9] or (3) clinico-epidemiological survey data[10,11] on the incidence of upper GI lesions recorded from those individuals who have taken NSAIDs. A whole range of variables must be considered in determining the percentage incidence and risk of such ulcer formation, hemorrhage, and perforation. Again, there are some general points which can be concluded from these clinical studies, namely:

1. Upper GI ulceration and hemorrhage are frequent events in arthritic patients taking NSAIDs. Estimates of the incidence in patients referred to hospitals for investigation into the incidence of NSAID-associated peptic ulcer disease range from 16 to 82%.[7-10]
2. The incidence of upper GI ulceration and hemorrhage is particularly high among patients over 65 years of age, especially women,[6-8,11] a feature which has been associated with impaired drug handling in these aged patients (see Section II.A).
3. The frequency of upper GI disease is as common following use of non-aspirin drugs as that from the salicylates themselves.[10]
4. Patients with rheumatoid arthritis (RA) appear equally susceptible to the ulcerogenic effects as those with osteoarthritis (OA) (osteoarthrosis).[10] The latter is a surprising

finding in view of the fact that the range of drugs, dosages, and types of acute inflammatory drugs employed often vary considerably. Thus, gold salts, D-penicillamine, oral corticosteroids, and cytostats are employed quite frequently in RA, whereas they are, with the exception of corticosteroids, rarely prescribed for OA patients. Moreover, the gold salts (gold thiomalate, Auranofin) and D-penicillamine have a different pattern of GI side effects[15,16] than seen with the acid nonsteroidals.[1,2] Also, the dosage of acidic NSAIDs as employed in OA is more variable when compared with that in RA because, in the former condition, NSAIDs are essentially taken when required to relieve occasional painful episodes.

II. FACTORS AFFECTING GI DAMAGE

A. Drug Pharmacokinetics and Relation to the Site of Pathology

Historically, aspirin, indomethacin, and phenylbutazone were frequently implicated as causes of upper GI ulceration and hemorrhage. More recently however, other drugs, notably piroxicam, indoprofen, and Osmosin®, the osmotic slow-release (GITS) formulation of indomethacin, have been a major cause for concern for their propensity to induce injury to the lower intestinal tract. These and some other NSAIDs either cause injury in this region by pronounced enterohepatic recirculation resulting in re-exposure of the intestinal mucosa to the drug or because the formulated design releases the NSAID in the intestinal tract, rather than in the stomach.[3,17] The lower region of the intestinal tract appears particularly vulnerable to the effects of those NSAIDs which accumulate in the intestine because of the combined nature of the existence of bacterial flora, the presence of bile salts, and other physiological characteristics[18] (see later in this section). Assessment of the incidence of NSAID-related intestinal injury in man has been hampered because it is difficult to diagnose the early signs of injury there, before the consequences are manifest. This is because (1) there may be few, if any, overt symptoms which would enable a specific diagnosis to be made, and (2) conventional endoscopic procedures do not allow exploration of those deeper regions of the bowel, i.e., the jejunum through to the proximal colon.

It has become evident that the mechanisms of mucosal injury in the stomach may be quite different to those in the lower intestinal tract[9] (Table 1). This may have its basis in the differences in physiologic and anatomic properties of these regions. For example, the presence of acid and pepsin in the stomach markedly contributes to gastric mucosal injury therein, whereas these agents are unlikely to be involved in NSAID injury in the lower intestinal tract because of their absence in appreciable quantities there. However, in contrast to the stomach, bile salts and enteric bacteria are abundant in the lumen of the lower intestinal tract and both contribute considerably to injury in this region. These factors may have less influence in the stomach except from bile reflux, or the overgrowth of *Campylobacter* and other bacterial species accompanying achlorhydria.[9] Hence, a variety of intrinsic physiologic, as well as environmental factors, contribute to the ulcerogenic potential of a particular NSAID in specific regions of the GI tract (see Table 1).

The NSAIDs which are notably injurious in the lower intestinal tract (i.e., phenylbutazone, fenamates, phenylacetic and phenylpropionic acids)[17-20] have certain common physicochemical properties (e.g., high lipophilicity, molecular weight ~300) which account for their enterohepatic recirculation. These drugs are secreted in the bile duct as conjugates of glucuronic acid and once in the intestinal lumen are hydrolyzed by bacterial derived β-glucuronidases, thus releasing "active" drug (i.e., active in respect of an inhibitor of the synthesis of mucosal-protective prostaglandins, or other biochemical actions).[17,18] Evidence in favor of this hypothesis has been derived from studies in which (1) antibiotic treatment has been shown to prevent intestinal ulcers from drugs such as indomethacin in rats, (2) germ-free animals devoid of intestinal bacteria show reduced intestinal ulceration from those NSAIDs

Table 1
ENVIRONMENTAL FACTORS INFLUENCING THE ULCEROGENIC ACTIONS OF NSAIDs IN THE UPPER AND LOWER GI TRACT

Factor	Region	
	Stomach/duodenum	Lower intestine
Age, sex	+[a]	?+
Ethanol consumption	+	?0
Nicotine (cigarettes)	+	?0
Acid stimulation (e.g., by cholinomimetics[a])	+	0
High fat diet[b]	+	+
Inflammatory[b] disease	+	+
Physical stress[b] (cold, restraint)	+	0
Deficiency of gluconeogenic substrates/precursors[b]	+	+
Protein deficiency[b]	+	+
Ascorbate deficiency[b]	+	?
Bile salts	+	+

Note: + denotes factor is positively implicated such that when evident will enhance ulcer development. 0 denotes factor not implicated. ? denotes questionable involvement.

[a] Pronounced in elderly females
[b] Principally implicated from studies in laboratory animals

Adapted from Rainsford, K. D., *Side Effects of Anti-inflammatory Drugs*, Vol. 2, MTP Press, Lancaster, England, 1987, 3.

with propensity to elicit mucosal lesions in this region, and (3) there is a direct correlation between intestinal ulcerogenic actions and extent of enterohepatic recirculation with several NSAIDs.[17-22]

The pharmacokinetic features which determine upper GI injury are complex and at this present stage of knowledge somewhat indefinite. Some general conclusions can be drawn and these are summarized in Table 2. Some of these factors may also apply to the ulcerogenicity of NSAIDs in the lower intestinal tract, as well as the upper region of the GI tract. Thus, the long plasma half-life of drugs, such as phenylbutazone may, where there is impairment of drug elimination, lead to enhanced drug accumulation, resulting in more of the drug in the circulation through the GI mucosa.

B. Disease and Physical Stress States

Chronic inflammatory conditions (e.g., adjuvant arthritis induced in rats) markedly enhance the susceptibility of the GI mucosa to NSAID-induced damage. It is possible that this arises from defective liver and intestinal detoxification of these drugs and from reduction in the defensive properties of the GI mucosa (e.g., depressed prostaglandins, mucus secretion). Defects in metabolism of NSAIDs are manifest in both arthritic animals and man,[23] but it is not known exactly how this contributes to the enhanced damage produced by NSAIDs in the GI tract of arthritic animals or even man.

Significant reduction occurs in the biosynthesis of sulfated mucus glycoproteins in both the fundic and jejuno-ileal mucosa of adjuvant arthritic rats compared with their normal (nondiseased) animals.[18] Oral administration of aspirin, which reduces gastric mucus synthesis in normal animals,[24] causes a further reduction of gastric sulfomucin biosynthesis in arthritic rats which could be due to the enhanced gastro-irritancy of aspirin in these diseased animals. In contrast, aspirin fails to cause a statistically significant reduction in the jejuno-

Table 2
SUMMARY OF PHARMACOKINETIC FACTORS OF NSAIDs AFFECTING THEIR MUCOSAL ULCEROGENICITY IN SPECIFIC REGIONS OF GI TRACT[17,18,20]

1. Absorption — dependent upon:
   - A. Dissolution (from tablet, capsule) or release (from sustained-release preparations)
   - B. Intrinsic rates of absorption of pure drug related to aquo/liposolubility
   - C. Specific cell accumulation (e.g., aspirin, salicylate) in parietal cells
2. Uniqueness of Drug Metabolism
   - A. Mucosal metabolism of specific drugs, e. g., aspirin by esterases to less irritant salicylate, competition with acetylation by aspirin
   - B. Liver or other metabolism:
     Pro-drugs: sulindac → sulindac sulfide interconversion; fenbufen → biphenylacetic acid
     Glucuronide conjugation determines "free" drug concentrations
3. Distribution
   - A. Extent of enterohepatic recirculation: where more pronounced, this favors propensity for intestinal damage
4. Elimination
   - A. Plasma or elimination half-life: long half-life to drugs result in longer exposure of GI mucosa; Where renal eliminations impaired (as in elderly), these drugs accumulate in systemic circulation

ileal sulfomucin synthesis in normal compared with arthritic rats.[18] The lack of effects of aspirin on mucus synthesis in the intestine is related to the fact that it does not induce lesions in this region of the GI tract.

In contrast to the situation with aspirin, indomethacin, and diclofenac reduce sulfated-mucus synthesis in the jejuno-iteal region of normal rats[24] and both drugs produce intestinal lesions in nondiseased animals[18,22,57] which are enhanced in arthritic rats.[19,26,27] The pronounced enterohepatic recirculation of indomethacin[17,20] and diclofenac[25] in rats could account for the inhibition of mucus synthesis which is not observed with those NSAIDs without this pattern of intestinal absorption.[24] The enhancement of lesion severity seen in arthritic rats given indomethacin[19,26] and diclofenac[27] may also be due to the additional depression in the synthesis of sulfomucins by these drugs in the intestinal tract of arthritic animals.

Recently, the gastro-ulcerogenic effects of some NSAIDs have been investigated in carrageenan-inflamed rats, a model of an acute inflammation.[28] While there are statistical and other limitations in the interpretations of the data from those studies reported in Reference 28 (see criticisms Reference 29), the results do show that carrageenan enhances the gastro-ulcerogenesis of some NSAIDs, but not all NSAIDs. The mechanism of this selective enhancement of the gastro-ulcerogenic effects of some NSAIDs is not known, although it has been suggested[29] that the systemic generation of prostaglandins elicited by the carrageenan may override the ulcerogenicity of "reversible" prostaglandin synthesis inhibitors (e.g., piroxicam) compared with that from irreversible inhibitors (e.g., aspirin).

Physical stress states have also been shown to enhance NSAID-induced GI damage though the pattern of drug-stress interactions is quite different to that in disease states. Exposure to cold stress conditions concomitant with orally or parenteral dosing of NSAIDs markedly enhances the susceptibility of the fundic and antral mucosa to the ulcerogenic effects of these drugs.[27,30-32] In contrast, exposure to cold stress has no added effect on NSAID-induced damage in the jejuno-ileal region.[27] This shows that there is a high degree of specificity for the cold stress synergy in the stomach.

Another approach employed in exploring the importance of stress, and for that matter other environmental states, on the development of gastric lesions by NSAIDs has been to examine the effects of administering certain pharmacological agents which mimic stress effects or which modulate gastric functions in the stomach (e.g., acid/pepsin secretion) in combination with NSAIDs. Thus, Angeel et al.[33] have shown that nicotine enhances the

ulcerogenic actions of aspirin. The mechanism presumably involves some nicotinic receptor activation in vagal-controlled acid/pepsin secretion though the authors have not fully explored these effects.

Likewise, the author has shown that muscarinic activation with bethanechol chloride (acetyl β-methyl choline) enhances the gastro-irritancy of a range of NSAIDs in both mice[34,35] and rats[31] (unpublished studies). In these studies, the pronounced dose-response observed in mice with more potent ulcerogens (e.g., aspirin, indomethacin, piroxicam) was contrasted with the lower response obtained with less known ulcerogenic NSAIDs (e.g., azapropazone, nabumetone).[35] The enhanced gastro-ulcerogenicity of NSAIDs correlated well with the dose-responses observed with these NSAIDs in the cold-stress model. The bethanechol model is of interest in relation to the mechanism of NSAID-like physical stress interactions. Previous studies[31] showed that muscarinic antagonists markedly reduced aspirin plus stress-induced gastric ulceration as well as that from aspirin or severe stress alone. Among the advantages of this model are the facts that (1) it enables quantitation of gastro-ulcerogenicity of NSAIDs without having to employ the stressing procedures which have now become ethically unacceptable, and (2) the pharmacologic agent can be employed specifically to mimic the effects of a stressing procedure and so obviate the many profound systemic influences of cold stress, some of which could be very difficult to interpret, such as the reduced peripheral organ blood flow from reduced cardiac output in the cold.

III. CONCEPTS OF THE MECHANISMS OF GI ULCERATION

Table 3 gives a summary of the principal biochemical, cellular, and physiological actions of NSAIDs which are considered at present to be important in the pathogenesis of mucosal injury in the GI tract.

It should be noted that although many of the NSAIDs exhibit similar biochemical and cellular effects shown in Table 3, these drugs differ considerably in potency as inhibitors of "perturbers" in these systems. Such variations in inhibitory potency may also account for differences in the GI ulcerogenicity of NSAIDs, together with the variations of pharmacokinetic properties of these agents as previously discussed (Section II.A).

A. The Role of Chemical Structure

It is obvious that there are wide differences in the gastric and intestinal ulcerogenicity of different NSAIDs which are due to their chemical structure. Some aspects of the structure of NSAIDs relative to their ulcerogenic actions have been studied and reviewed previously (see References 36 to 41). It is possible that lipophilicity and pKa of NSAIDs affects the gastric ulcerogenic actions of NSAIDs by (1) influencing absorption/distribution of the drug, (2) affecting interactions with biochemical systems (e.g., enzymes) involved in normal defense processes, and (3) causing direct injury to surface mucosal cells. Evidence of the latter can be seen in the surface irritant actions of acidic NSAIDs visualized by electron microscopy. These effects are markedly reduced by esterification of the acidic group[36,39-41] implying that irritancy is largely due to the acidic groups of these drugs. Other chemical features also contribute to the gastro-ulcerogenicity of NSAIDs. Thus, the position of certain electron withdrawing substituents on the second aromatic ring of the bi-aryl type of NSAIDs (which form the principal type of acidic drugs) clearly has considerable influence on their gastro-ulcerogenicity.[37] A classic example of this type is seen in comparisons of the gastro-irritancy of some phenyl acetates. Here diclofenac and its congeners is among the more ulcerogenic, whereas fenclofenac is the least gastro-irritant.[37] In relation to anti-inflammatory and analgesic actions — a practical therapeutic aspect of the use of these drugs — diclofenac is also the more potent analgesic agent of the two. When anti-inflammatory actions are considered, fenclofenac has influences on different parts of the inflammatory system[42] com-

Table 3
SUMMARY OF MAIN PHYSIOPATHOLOGIC AND BIOCHEMICAL FEATURES IN THE PATHOGENESIS OF GI MUCOSAL INJURY BY ACIDIC NSAIDs

Factor	Principal consequences
Acidity of drug (organic acid)[a,36-40]	Loss of membrane integrity
Sloughing physical changes to changed surface mucus layer[54,68] and inhibition of mucus biosynthesis (at enzyme level)[24,50]	Impaired mucus protective actions[50,64]
Inhibition of PGI_2 and PGE_2[44-46,51] synthesis	Altered blood flow → anoxia[62] Platelet-vessel adhesion promoted Microvascular injury[67,71] Reduced "cyto"-protection by: decreased mucus production[64] decreased HCO_3^- secretion[51,64,69]
Release of lysosomal hydrolases[63]	Local cellular autolysis
Pepsinogen activation[58]	
Cholinergic activation[35]	Acid/pepsin secretion enhanced in stomach[58]
Histamine release from mast cells[52]	Promotes acid secretion, vasodilation (stomach)
Enhanced oxyradical production[65,72,73]	Localized tissue destruction
Reduced sulfhydryls[66]	Possible loss of reductive protection by mucosal biomolecules against oxyradical damage and pertubed eicosanoid metabolism
Exhanced motility (amplitude)[52]	Altered GI transit,? relation to prostaglandin control of smooth muscle function[52]
Inhibition of ATP production[59,61]	Reduced capacity to resist cell injury from mucus and other synthetic reactions
Altered cAMP levels (? from phosphodiesterase inhibition)[55,56]	Altered cell metabolism including effects on acid and mucus secretion (stomach)

Note: It should be emphasized that many of the probable consequences of the above-mentioned drug actions are rather speculative at the present state of knowledge. Summary based on references denoted in superscript; see also reviews References 3, 17, 18, and 44.

[a] See also text.

pared with that of diclofenac[43] so that the question of potency depends on what part of the immunoinflammatory system is being influenced by the drug. Clearly any structure-function analysis must include relationships to therapeutic actions and this introduces many complex features to comparisons of this kind.

B. Inhibition of Prostaglandin Production

One of the major biochemical effects of the NSAIDs, the inhibition of prostaglandin (E_2/I_2) synthesis has been considered by some to be a major factor in the gastric ulcerogenicity of NSAIDs.[44] However, other studies[45,46] have shown that such relationships do not hold for all NSAIDs. A classic situation is seen with aspirin which, when given parenterally or orally, has been shown to inhibit the production of PGI_2 *in vivo* without inducing gastric lesions in rats.[46] Furthermore, other NSAIDs given orally in sufficient doses to achieve inhibition of prostaglandin (PG) synthesis (as assessed from *in vitro* potencies, and drug absorption data) do not always cause reduction of fundic prostaglandin E_2 (PGE_2) content coincident with the time-course of development of mucosal damage in rats.[18,45] Some other NSAIDs (e.g., oxaprozin, chloroquine), when dosed orally, reduce the content of PGE_2 in rat mucosa without causing significant signs of gastric injury assessed visibly or by scanning electron microscopy.[18]

Some understanding of the role played by inhibiting gastric PG production in gastric injury comes from studies in which drugs such as oxaprozin have been examined for their gastro-

ulcerogenic effects in some animal models in which the gastric mucosa is specifically sensitized towards the irritant actions of this drug and other NSAIDs.[18,48,49] These NSAIDs, given orally or parenterally, reveal appreciable gastro-irritancy in rats exposed to physical or disease stress states, whereas in the unstressed, but fasted, animals little or no mucosal injury is evident.[49]

Initially, inhibition of PG production by the NSAID might, therefore, be envisaged as a "priming" effect. The additional exposure to stressful conditions may, depending on their type and consequent effects, enhance the gastro-irritant actions of the NSAIDs by affecting blood flow, energy (ATP) metabolism, acid/pepsin secretion, mucus synthesis, or mucosal resistance factors.[18] The precise contribution of each of these biochemical events may vary with the different stress conditions.[18] Summarizing, it appears that the priming from inhibition of PG synthesis event could influence PG dependent processes e.g., blood flow, acid and mucus-bicarbonate secretion, with the secondary effects being due to stress states or nutritional variables. This hypothesis might be a useful view for envisaging some major part of the gastro-ulcerogenesis from NSAIDs.

C. Inhibition of Mucus Secretion and Synthesis

Correlations have been noted between the gastro-ulcerogenic actions of NSAIDs and their ability to inhibit the biosynthesis *in vivo* of gastric mucus glycoproteins.[24] It is possible that mucus secretion is regulated by the E-type-PGs and it is possible that this association is due to effects of NSAIDs on PG production. However, *in vitro* studies show that NSAIDs can directly inhibit those enzymes involved in the biosynthesis of mucus and related glycoproteins.[50] Thus, it appears that the effects of NSAIDs on mucus synthesis could be related to both their actions as inhibitors of PG production and actions on the biosynthetic steps in mucus synthesis.

D. The Consequences of Inhibiting Prostaglandin Synthesis

Current concepts indicate that the main consequence of inhibiting PG E_2/I_2 synthesis and production is to reduce the levels of these endogenous "cytoprotective" agents.[51] There is considerable criticism over what the term "cytoprotection" really means and whether it is an appropriate term to describe the apparent protection of the GI mucosa from injury by NSAIDs and other agents.

An alternative, or possibly an additional explanation for the effects of inhibiting PG cyclooxygenase is that this could lead to diversion of arachidonate through the lipoxygenase pathway leading to enhanced production of the vasoactive leukotriene $C_4/D_4/E_4$ together with a peroxidative-derived oxyradical species which has been claimed to have potential cell destructive effects.[47] This hypothesis has been examined by determining the effects of 5-lipoxygenase (5-LO) inhibitors and leukotriene (LT) C_4/D_4 antagonists for their potential to modify the gastro-ulcerogenic effects of NSAIDs. The results in the bethanechol-treated mouse model (described above) showed that the gastric irritant effects of NSAIDs, when given orally or parenterally, could be prevented by coadministration of a range of 5-LO inhibitors and LT antagonists.[47] Hence, it does appear that enhanced production of 5-LO products could explain some of the consequences of inhibiting PG cyclooxygenase by NSAIDs inhibition.

IV. CONCLUSIONS

The NSAIDs individually vary in their propensity to cause GI ulceration and hemorrhage. This depends on a number of factors including the drug pharmacokinetics, interaction with various environmental factors (stress conditions, ethanol etc.), and the spectrum of their biochemical and cellular actions on the gastrointestinal mucosa. As shown in Table 3, there

are a variety of biochemical and cellular events implicated in the pathogenesis of mucosal injury by acidic NSAIDs. The antirheumatic drugs, such as gold salts, D-penicillamine, etc., appear to have actions quite different to the classical PG synthesis inhibiting acidic anti-inflammatory agents. Within the latter group, several factors are common and list of biochemical/cellular actions in Table 3 unifies to some extent this group, except that the quantitative contribution of each of these factors appears to vary considerably from drug to drug. This appears to account for the differences in the GI ulcerogenicity of NSAIDs. Future prospects may be envisaged in exploring the chemical properties of those NSAIDs which show little or minimal biochemical or cellular changes which are characteristic of an ulcer-producing profile for a drug.

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Chapter 2

CORRELATIONS BETWEEN FREE RADICALS AND MEMBRANE-DEPENDENT ENERGY SYSTEMS IN ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE IN RATS

Gy. Mózsik, G. Sütő, Á. Vincze, and T. Zsoldos

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I. INTRODUCTION

Free radicals have been implicated in the pathogenesis of tissue damage caused by physical agents (e.g., ionizing radiation), chemicals (e.g., carbon tetrachloride), paraquat, and ischemia followed by perfusion.[1-3] Lipid peroxidation is considered one of the biochemically measurable processes by which free radicals cause membrane damage, cell damage, and tissue injury.[1,2] Numerous compounds, including sulfhydryl groups, vitamins A and E, and levamisol scavenge free radicals, decrease lipid peroxidation, and prevent organ injury. Most work related to free radicals has been carried out with cell damage in the liver.[4-6]

Peptic ulcer disease represents a multicausal, multifactorial disease.[7,8] Many structurally unrelated chemicals damage the gastric mucosa in experimental animals and humans. The most widely used chemicals in animal models are 0.6 *M* HCl, 0.2 *M* NaOH, hypertonic NaCl, 96% ethanol,[9] and drugs such as aspirin, indomethacin, and other nonsteroidal anti-inflammatory agents,[10] reserpine,[11] and epinephrine.[12] Gastric mucosal erosions or ulcers develop during stress caused by burns, sepsis, trauma, as well.

The list of agents that prevent acute gastric mucosal lesions is also very diversified. Namely, the lesions are prevented not only by classic antisecretory drugs, such as anticholinergic and antimuscarinic agents, proton-pump inhibitors, or histamine H_2-receptor antagonists, but also non-antisecretory agents, such as vitamin A, lutein, beta-cryptoxanthin, beta-carotene,[13-17] RGH-2961,[18] RGH-5909,[19] disodium chrommoglicate,[20] Pelsonin,[21] lipid soluble scavengers,[22] and "cytoprotective" doses of prostaglandins (PGs). Some possible role(s) of free radicals have recently been reviewed in different ulcer models.[23]

The time periods which are needed for the development of mucosal lesions differ in various experimental models, e.g., 1 h as in ethanol, NaOH, HCl, hypertonic NaCl-induced model, etc. If the biochemical measurements, including the energetics and free radical parameters, were carried out at the end of experiments, we would not be able to ascertain which changes in those parameters are causes for ulcer development, or which are consequences of ulcer development. Czeglédi et al.[24] indicated significant changes in superoxide dismutase activity of rat gastric mucosa after 4 h of pylorus-ligation plus aspirin treatment animals, depending on the time periods after surgery. Many biochemical studies have indicated that changes in membrane-dependent energy systems represent etiological events in the gastric mucosa related to development of mucosal injury.[25-26] One of the most important factors is the time dependence between the changes of membrane-dependent energy systems and ulcer development.[27] A close time correlation is also needed to evaluate relationships between free radicals and ulcer development. Some correlations can be suggested between changes in membrane-dependent energy systems, free radicals, and ulcer development.[28] The experimental evaluations of these correlations are complex, because peptic ulcer disease has a multifactorial background.[7,8,29,30]

In this investigation, the changes in free radicals, superoxide dismutase, glutathione-peroxidase, glutathione content, catalase, malondialdehyde, and energy system parameters, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, cyclic adenosine monophosphate, lactate, adenylate pool, "energy charge", and ratio of ATP/ADP were studied in ethanol-induced gastric mucosal damage. The observations were carried out at 0, 1, 5, 15, 30, and 60 min after administration of 96% ethanol. The aims of our observations were

1. To follow the macroscopic appearance of mucosal damage at different times after ethanol administration
2. To evaluate the free radical mechanism related to macroscopic development of gastric mucosal lesions
3. To study changes in the gastric mucosal biochemistry related to ulcer development

4. To evaluate possible correlations between free radicals and membrane-dependent energy systems before and after macroscopic appearance of gastric mucosal lesions.

II. MATERIALS AND METHODS

The observations were carried out on both sexes of CFY strain rats (LATI, Godollo, Hungary) weighing 180 to 210 g body weight. The animals were fasted for 24 h before experiments, and they received water *ad libitum.*

The observations were carried out in the morning. Gastric mucosal damage was produced by intragastric administration of 96% ethanol. Saline solution (1 ml) was given intragastrically to control animals.

The animals were killed at 0, 1, 5, 15, 30, and 60 min after administration of ethanol. The number and severity of gastric mucosal lesions were noted. The severity of gastric mucosal lesions was estimated by a semiquantitative scale. The number and severity of gastric mucosal lesions were calculated as means ± SEM per rat stomach.

The biochemical examinations were carried out on rat gastric fundic mucosa at different times after ethanol administration.

The tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and lactate were enzymatically measured (Boehringer, Ingelheim, F.R.G.). The mucosal level of cyclic adenosine monophosphate (cAMP) was measured by RIA (Becton Dikinson, Orangeburg, U.S. Adenylate pool (ATP + ADP + AMP), values of $ATP{\cdot}ADP^{-1}$ and "energy charge" (ATP + 0.5 ADP). $(ATP + ADP + AMP)^{-1}$[31] were calculated (Figure 1).

The gastric mucosal superoxide dismutase (SOD) activity was measured by the method of Misra and Fridovich,[32] using a modification by Matkovics et al.[33] Catalase activity was determined by the method of Beers and Sizer.[34] The glutathione peroxidase (GSH-Px) activity was measured by the method of Sedlak and Lindsay,[35] while the content of reduced glutathione (GSH) was estimated by the method of Fong et al.,[37] after a modification of Zsoldos et al.[38] (Figure 2).

The protein content was measured by the method of Lowry et al.[39] The biochemical results were calculated with respect to 1 mg mucosal protein (means ± SEM).

The unpaired Student's t test was used for statistical analysis of results, except of ulcer severity, where the Mann-Whitney's test was applied.

III. RESULTS

A. Macroscopic Appearance of Ethanol-Induced Gastric Mucosal Damage

No macroscopic appearance of mucosal lesions was obtained at 1 min after ethanol administration, but it was found at 5 min (about 50% severity as detected at 1 h after ethanol administration).

B. Biochemistry of the Gastric Mucosa

The tissue level of cAMP was increased at 1 and 5 min, thereafter its level decreased. No significant change was obtained in the tissue level of ATP in the first 5-min period, and tissue level decreased at 15 and 30 min, while it was increased at 60 min, after ethanol administration. The tissue level of ADP was decreased at 1, 5 and 15 min, and its value returned to normal 1 at 30 and 60 min. The AMP level was decreased after administration of ethanol (Figure 3).

Adenylate pool (ATP + ADP + AMP) was decreased in all times (Figure 4).

No elevation was obtained in the tissue level of lactate. The value of $ATP{\cdot}ADP^{-1}$ was increased at 1 and 5 min, thereafter decreased only at 15 and 30 mins (Figure 5).

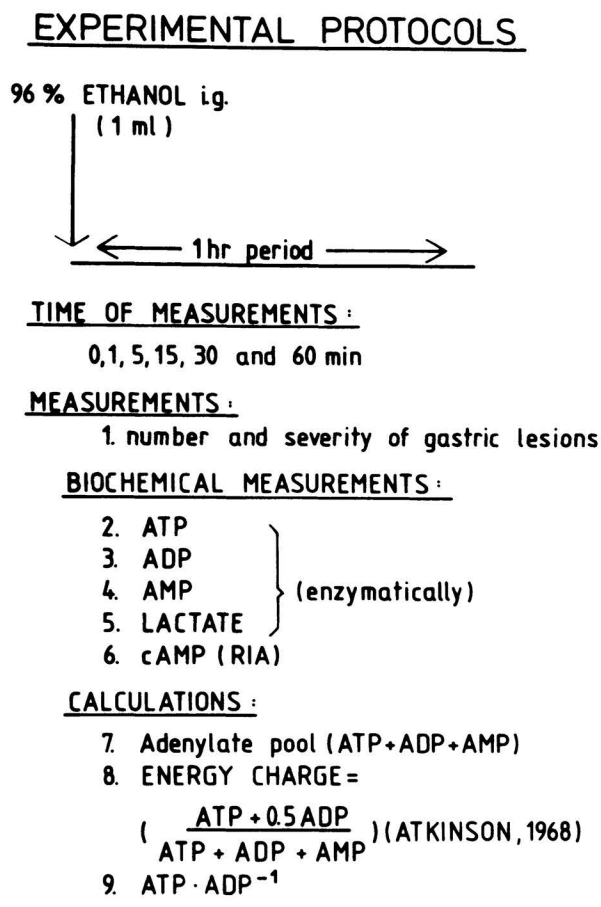

FIGURE 1. Experimental protocol for biochemical evaluation of cellular energy status in gastric mucosa treated with 96% ethanol given intragastrically. Time dependence after ethanol application.

C. Free Radical System in the Gastric Mucosa

The catalase activity was increased only at 1 min after ethanol administration, while thereafter its value returned to normal (Figure 6).

Glutathione peroxidase (GSH-Px) activity was increased at all times, especially from 15 min after ethanol administration (Figure 7).

The reduced glutation (GSH) content was increased at 5, 15, and 30 min, and its value decreased at 60 min (Figure 7).

The SOD activity was increased from 15 min, while the MDA content increased from 30 min after ethanol administration (Figure 8).

D. Correlations between the Gastric Mucosal Biochemistry, Free Radicals, and Development of Ethanol-Induced Gastric Mucosal Damage

Different biochemical changes, including decreased extent of ATP-ADP transformation, increased extent of ATP-cAMP transformation, decreased extent of cAMP-AMP transformation, increased activity of catalase, GSH-Px, increased value of $ATP \cdot ADP^{-1}$, were found at 1 min after administration of ethanol, which preceded the macroscopic appearance of gastric mucosal damage (Table 1). No significant changes were obtained in tissue levels of ATP and lactate at 1 min after ethanol administration. From 15 min, GSH-Px activity and

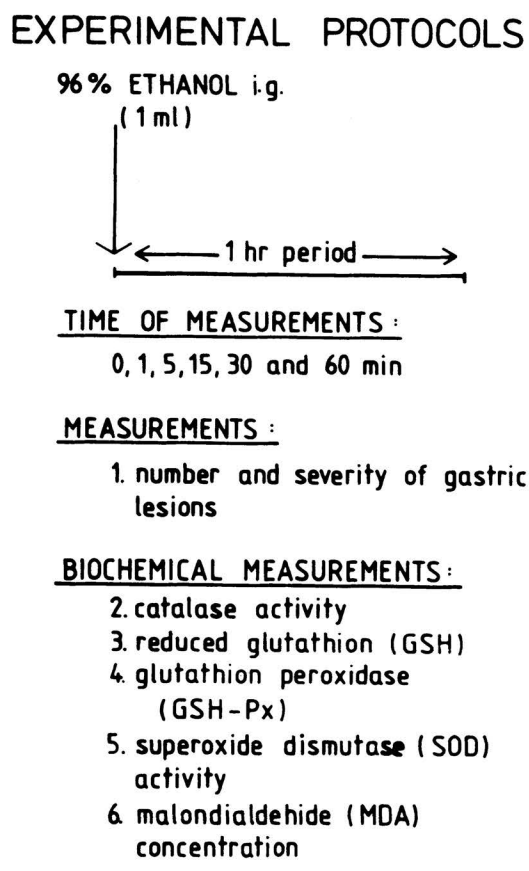

FIGURE 2. Experimental protocol for biochemical evaluation of free radical mechanisms in gastric mucosa treated wtih 96% ethanol given intragastrically. Time dependence after ethanol application.

SOD activity was increased. In that time, the extent of ATP-ADP transformation was increased also, in association with the decreased extent of ATP-cAMP transformation and of cAMP-AMP transformation. Interestingly, gastric mucosal damage appeared macroscopically before the increase of MDA content in the gastric mucosa (Table 1).

IV. DISCUSSION

Energy is stored in phosphorylated adenosine compound (ATP) for the living cells. Energy is liberated when the ATP is split by membrane ATPase into ADP and adenylate cyclase into cAMP, in presence of Mg^{2+}. Both enzymes are located in the cell membranes. The liberated energy is partly used for regulation of up-hill mechanisms of cells, like gastric H^+ secretion, and movements of Na^+, K^+, Cl^- across the cell membrane. The interrelationships between the membrane-bound ATP-dependent energy systems, gastric H^+ secretion, and ulcer development and its prevention have been reviewed.[25,26,40]

Previously, the tissue levels of ATP, ADP, AMP, adenylate pool (ATP + ADP + AMP), ratio of $ATP \cdot ADP^{-1}$, lactate, cAMP were measured at 1 h after administration of ethanol.[41,42] Szabo et al.[43,44] reported the appearance of vascular reactions in the gastric mucosa at 3 and 6 min after administration of ethanol, and Leung et al.[45] and Pihan et al.[46] observed a significant decrease of mucosal blood flow in this early state. When the tissue level of lactate

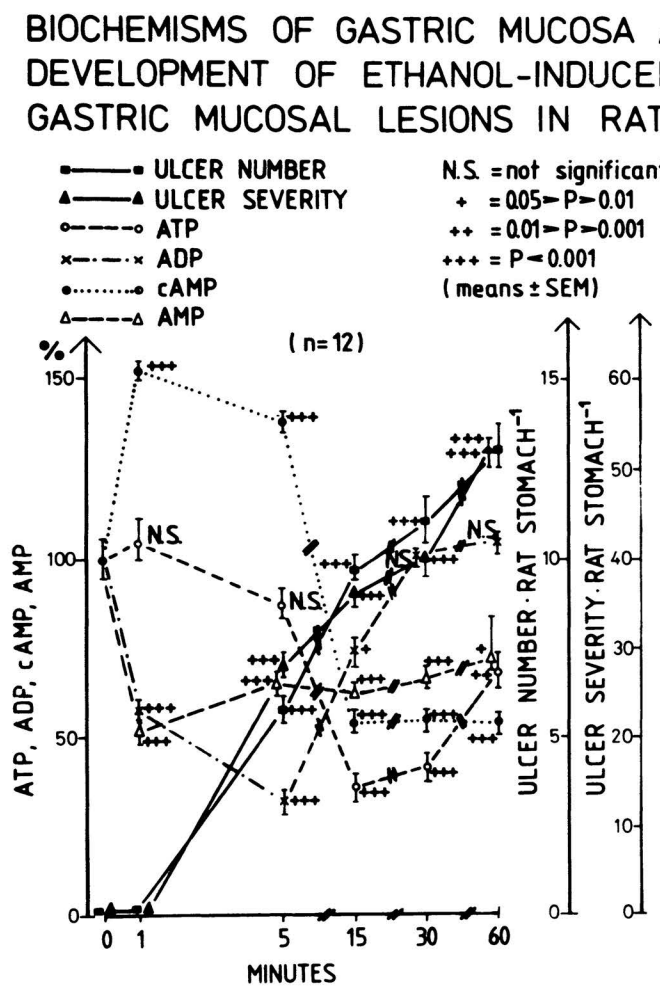

FIGURE 3. Correlations between changes in tissue content of ATP, ADP, AMP, and cAMP with ulcer development in gastric mucosa in rats treated with intragastric administration of 96% ethanol.

was measured at 1 h after administration, no elevation was found.[41] This contradiction was biochemically evaluated in this study when the biochemical examinations were carried out at 0, 1, 5, 15, 30, and 60 min after ethanol administration, in association with the macroscopic appearance of gastric mucosal lesions.

The following main biochemical results were obtained in the cellular energy systems:

1. ATP content was unchanged at 1 and 5 min, and its value decreased at 15 min, and thereafter gradually increased (30 and 60 min)
2. The tissue level of ADP decreased at 1, 5, 15, and 30 min, and its value returned to normal at 60 min
3. The ratio of ATP·ADP^{-1} was increased at 1 and 5 min, after which its value decreased
4. cAMP level was increased at 1 and 5 min and its value decreased thereafter
5. The tissue level of lactate was unchanged during the 1 h period

The extent of phosphorylation and/or dephosphorylation can be estimated by the formula of Atkinson.[31] The ratio of (ATP + 0.5 ADP) · (ATP + ADP + AMP)$^{-1}$ is referred to

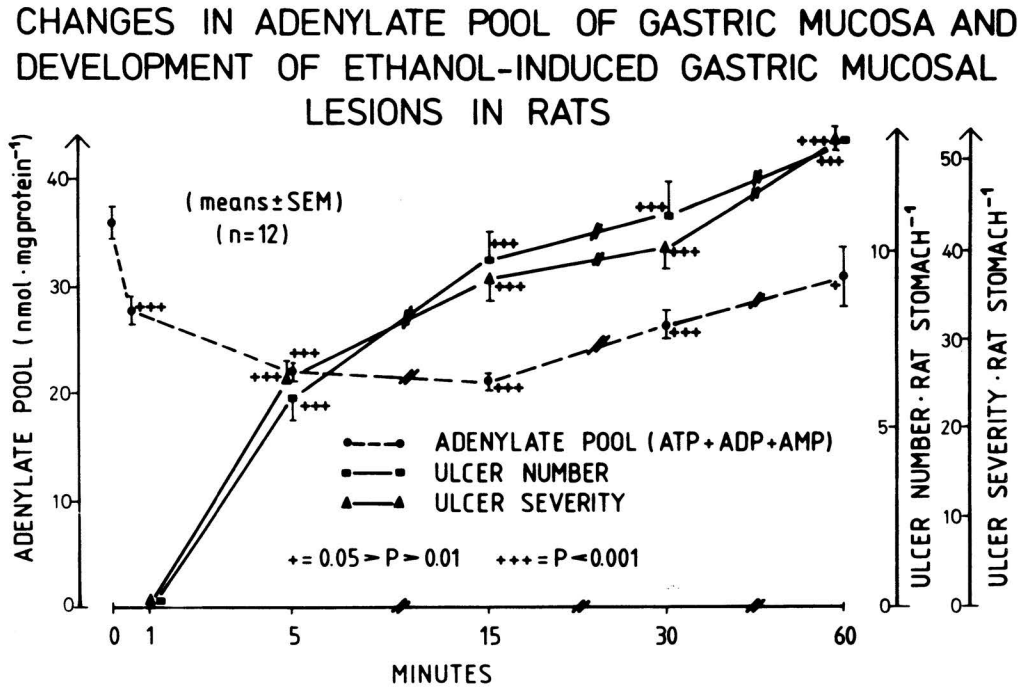

FIGURE 4. Correlations between changes of adenylate pool (ATP + ADP + AMP) in the gastric mucosa and the development of ethanol-induced gastric mucosal damage.

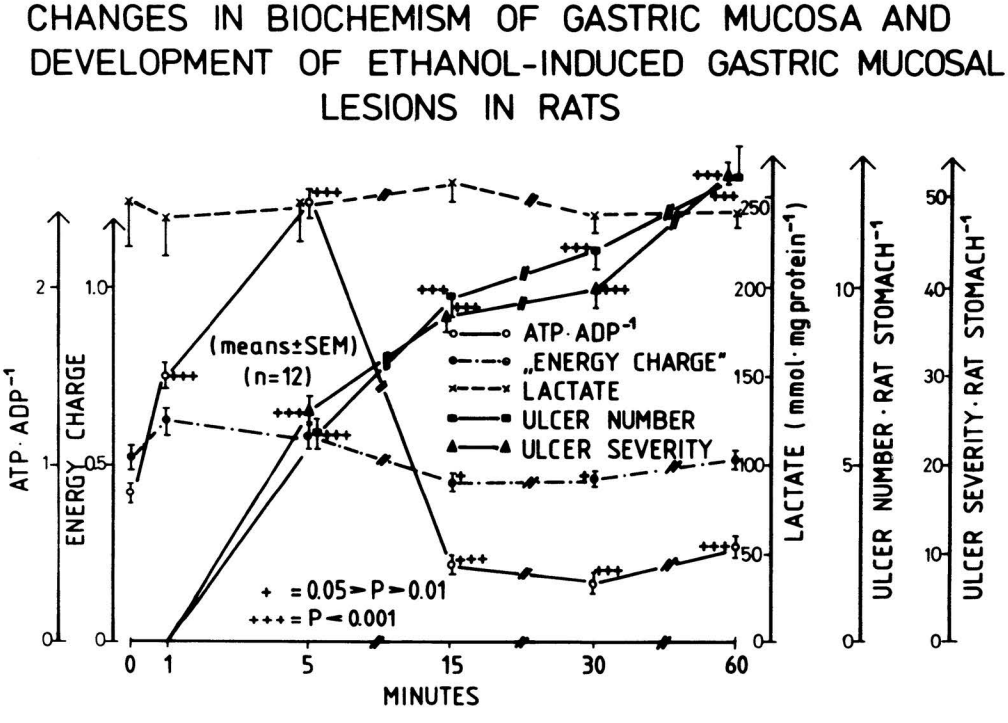

FIGURE 5. Correlations between tissue content of lactate, values of "energy charge", and of ATP—ADP^{-1}, with development of ethanol-induced gastric mucosal damage.

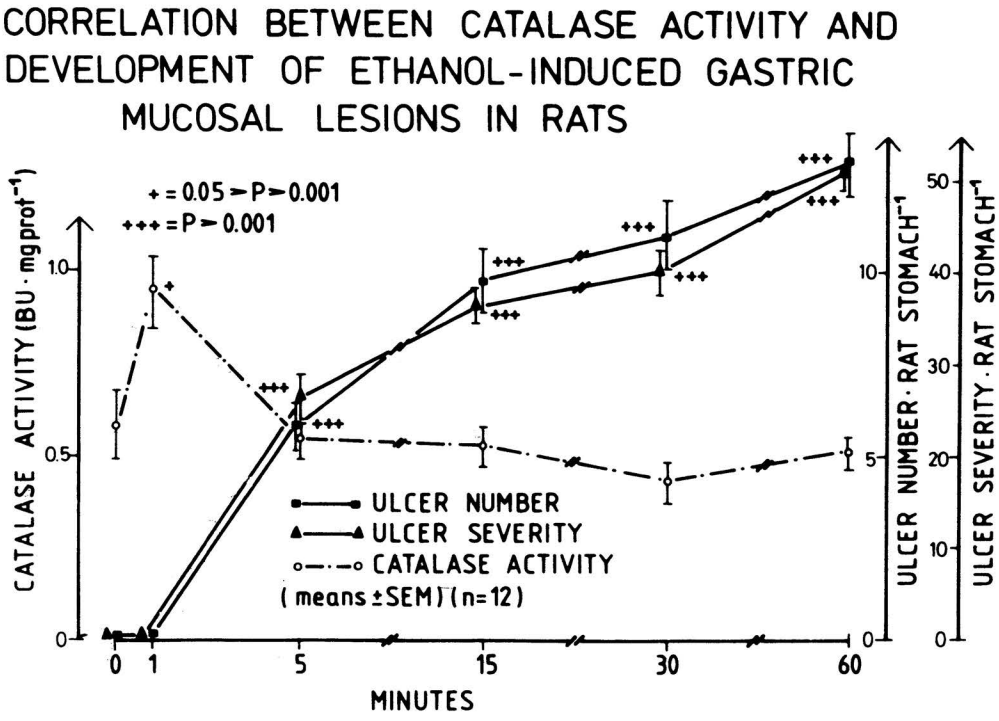

FIGURE 6. Correlations between the change of catalase activity in the gastric mucosa and development of ethanol-induced gastric mucosal damage.

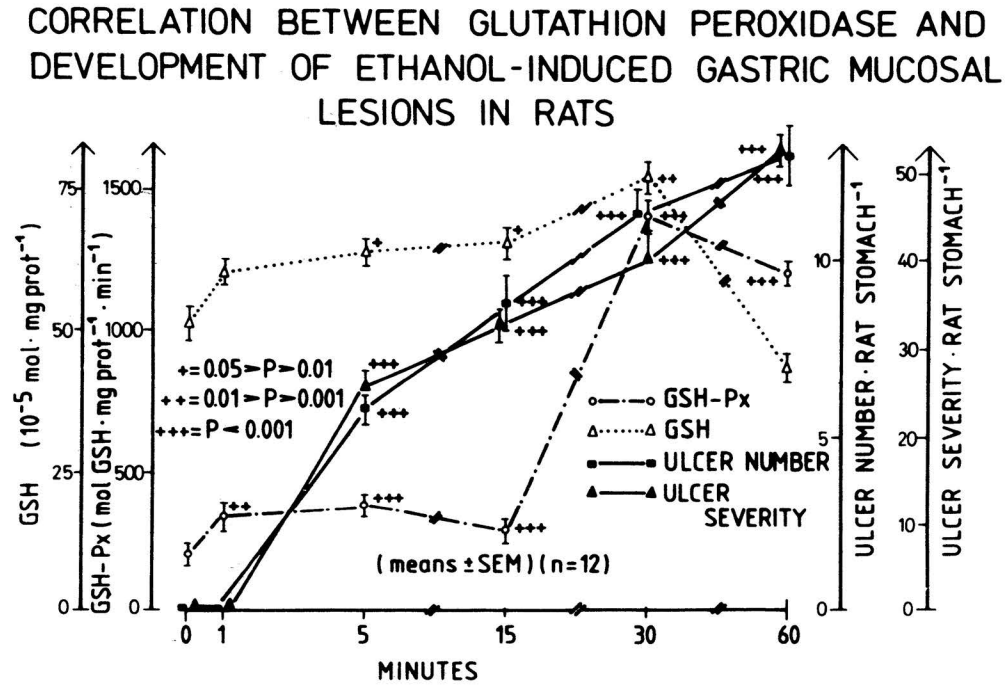

FIGURE 7. Correlations between the glutathione peroxidase (GSH-Px) and content of glutathione in the gastric mucosa with development of gastric mucosal damage produced by ethanol.

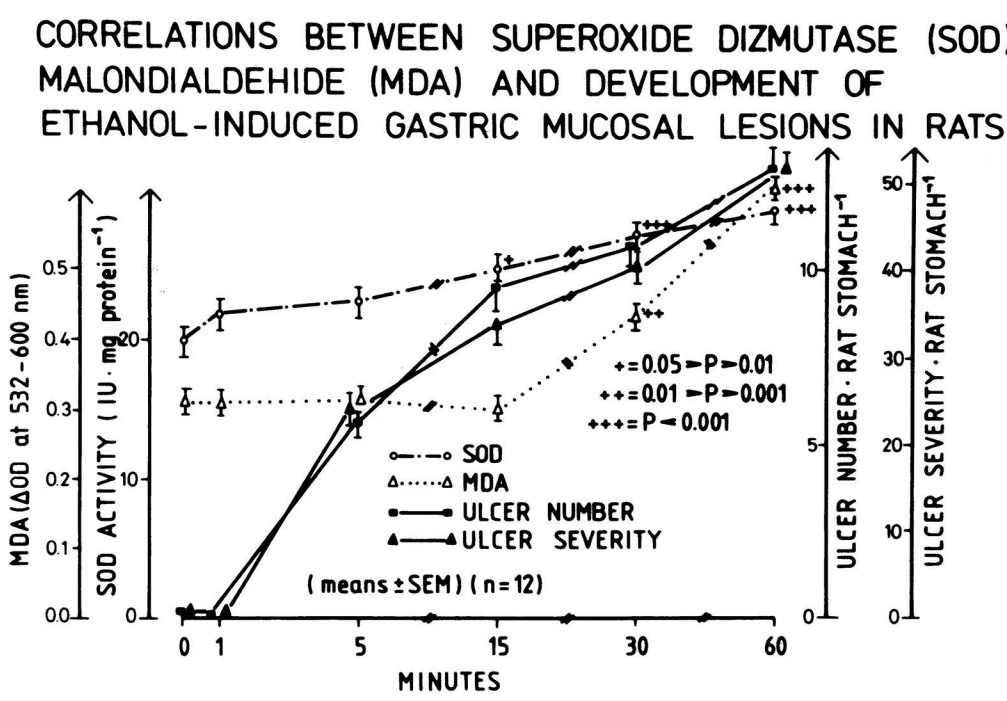

FIGURE 8. Correlations between the superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in the gastric mucosa and development of ethanol-induced gastric mucosal damage.

Table 1
CORRELATIONS BETWEEN THE CHANGES IN CELLULAR ENERGY SYSTEMS, FREE RADICALS, AND DEVELOPMENT OF ETHANOL-INDUCED GASTRIC MUCOSAL LESIONS IN RATS

Examined parameters	Time after ethanol administration (min)				
	1	5	15	30	60
Number of ulcers	—	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Severity of ulcers	—	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Cellular ATP	NS	NS	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
Cellular ADP	↓ ↓ ↓	↓ ↓ ↓	↓	NS	NS
ATP·ADP^{-1}	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	↑ ↑ ↑
ATP-ADP transformation	↓ ↓ ↓	↓ ↓ ↓	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Cellular cAMP	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
ATP-cAMP transformation	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
cAMP-AMP transformation	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
Adenylate pool	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
"Energy charge"	NS	NS	↓	↓	NS
Tissue level of lactate	NS	NS	NS	NS	NS
Catalase activity	↑	NS	NS	NS	NS
Glutathione peroxidase (GSH-Px)	↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Glutathione (GSH)	NS	↑	↑	↑ ↑	NS
Superoxide dismutase	NS	NS	↑	↑ ↑ ↑	↑ ↑ ↑
Malondialdehyde	NS	NS	NS	NS	↑ ↑ ↑

Note: NS, not significant; p values: control at 0 min vs. results at different times, ↑ (↓), $p < 0.05$; ↑ ↑ (↓ ↓), $0.01 > p > 0.001$; ↑ ↑ ↑ (↓ ↓ ↓), $p < 0.001$; ↓, decrease, ↑, increase.

as "energy charge". The value of "energy charge" is about 1, when all adenosine compounds can be found in phosphorylated form. This value is close to 0 when all adenosine compounds are in the dephosphorylated form. The extent of phosphorylation was decreased at 15 and 30 min after ethanol administration.

The biochemical results indicated that the gastric mucosal lesions appeared in consequence to active metabolic events of the gastric mucosa and not in consequence of tissue damage. There are many steps of physiological "detoxication", including reactions of tissues against free radicals, such as catalase, glutathione peroxidase, superoxide dismutase, and glutathione. The malondialdehyde appears as a consequence of lipid peroxidation. The glutathione peroxidase and catalase are competitive enzymes for breakdown of H_2O_2.

It is known that some enzymes located intracellulary (cytochrome oxidase, NADPH-oxidizing enzyme, mixed oxidase systems, etc.) superoxide anions are formed from molecular oxygen:[44]

$$2O_2 + 2H^{\cdot} \xrightarrow{\text{oxidases}} 2O_2^{\tau} + 2H^+$$

The free radical thus formed, even under normal conditions in the cell, must by any means be disposed of. The function of the superoxide dismutase is to defend against the superoxide anion generated by some enzymes in cells metabolizing oxygen, as follows:

$$2O_2^{\tau} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2^{\cdot}$$

The general function of SOD is to protect oxygen-metabolizing cells against the potentially toxic effects of superoxide radical anions generated during enzymic activity.

Following this, other oxygen radical intermediates will also be produced ($^{\cdot}OH, _2O_2$) as a consequence of the reactions between the formed H_2O_2 and primordial O_2:[48]

$$O_2^{\tau} + H_2O_2 \rightarrow {}^1O_2 + {}^{\cdot}OH + HO^-$$

The latter two radicals play a role mostly in the peroxidative transformations of membrane lipids, which are ultimately responsible for membrane injury. The task is to bind these toxic free radicals. When the natural factors (SOD, glutathione peroxidase, catalase, and other peroxidases) are not sufficient for the elimination of the toxic, free radicals, exogenous manipulations (administrations of scavangers) may also become necessary. The other protecting mechanism — the GSH-GSSH system — should also be taken into consideration in this respect, since it is capable of reverting the redox state to the normal level.[48]

Table 1 summarizes the possible correlation between the biochemistry and free radical system in relation to macroscopic appearance of ethanol-induced gastric mucosal damage. Significant changes in tissue levels of ATP, cAMP, AMP, increased extent of ATP-cAMP transformations, increase of ATP—ADP^{-1}, "energy charge", and increased activity of catalase and glutathione peroxidase were found in the gastric mucosa before the macroscopic appearance of ethanol-induced mucosal damage. The time period from 0 to 5 min after ethanol administration differs from that of 15 to 60 min, for both biochemistry and free radical findings. The increase of catalase and glutathione peroxidase activity was associated with an increased extent of ATP-cAMP transformation, while increase of SOD activity were associated with the increased extent of ATP-ADP transformation. It can be concluded that the free radical mechanisms appear in consequence to increased (not decreased!) cellular energy metabolism. In addition, no hypoxemic circumstance was detected at the time of increased free radical mechanisms.

Because many biochemical reactions could be detected in the gastric mucosa which

preceded the macroscopic appearance of gastric mucosal lesions, the etiologic role of biochemical reactions could be suggested. The time dependence is only one of the factors for suggestion, but no close correlations were found between the changes in gastric mucosal biochemistry and development of mucosal damage.

V. SUMMARY

We found that (1) gastric mucosal lesions appeared in macroscopic form at 5 min after ethanol administration; (2) no change of tissue level of ATP was obtained at 1 and 5 min, thereafter its value decreased; (3) cAMP levels were increased at 1 and 5 min, thereafter their level decreased; (4) the tissue level of ADP, AMP, and adenylate pool decreased; (5) no increase in tissue level of lactate was found; (6) the values of $ATP \cdot ADP^{-1}$ increased at 1 and 5 min, while those decreased thereafter; (7) catalase activity was increased only at 1 min, GSH-Px increased from 1 min to 60 min, and SOD activity increased from 30 min; and (8) GSH content was increased from 15 min, while MDA content from 30 min.

We conclude that (1) no hypoxemic damage of gastric mucosa was induced by ethanol administration; (2) the increase in free radicals was associated with increased energy liberated from membrane-bound ATP dependent energy systems; (3) different biochemical changes preceded the macroscopic appearance of ethanol-induced mucosal lesions; and (4) an early (0 to 5 min) and a late (15 to 60 min after alcohol) time period was found in changes of biochemical parameters in the gastric mucosa.

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Chapter 3

GASTRIC MOTILITY AND OVERALL BLOOD FLOW DURING COLD RESTRAINT IN THE RAT

Thomas Garrick, Felix W. Leung, Sally Buack, Ken Hirabayashi, and Paul H. Guth

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