Nimesulide May Be More Efficient Than Allopurinol in Protecting Pancreas From Acute Ischemia/Reperfusion Injury in an Animal Model

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Abstract
Objective: To determine the influence of allopurinol and nimesulide in the protection of the pancreas from acute ischemia–reperfusion (I/R) injury. Materials and Methods: A total of 30 rabbits were divided into 3 groups, group A: acute I/R only; group B: allopurinol (30 mg/kg) was administered intravenously 10 minutes before ischemia; group C: nimesulide (50 mg/kg) was given intraperitoneally 20 minutes before ischemia. Neopterin and superoxide dismutase (SOD) levels were examined. Pancreatic biopsies were obtained for electron microscopy study. Results: The mean neopterin concentrations in group A are 3.56 ± 3.41, 7.74 ± 3.59, and 8.94 ± 2.86 ng/mL, respectively, in the stabilization, ischemia, and reperfusion phases; group B: 3.40 ± 3.03, 7.45 ± 8.89, and 10.64 ± 7.47 ng/mL; and group C: 3.41 ± 2.71, 5.67 ± 2.76, and 4.34 ± 2.87 ng/mL. The mean SOD concentrations in group A are 4.25 ± 1.79, 4.48 ± 1.60, and 5.57 ± 1.15 ng/mL; group B: 4.32 ± 0.81, 5.08 ± 1.10, and 4.45 ± 1.31 ng/mL; and group C: 4.10 ± 0.99, 5.23 ± 1.60, and 3.72 ± 1.30 ng/mL. Histopathology showed the least deterioration in group C. Conclusion: Nimesulide is more efficient than allopurinol in protecting pancreas from acute I/R injury.

Keywords
allopurinol, nimesulide, pancreatic I/R injury

Introduction
Ischemia/reperfusion (I/R) injury plays a pivotal role in acute pancreatitis. This injury is not caused only by I/R but also involves the activation of pancreatic enzymes. Pancreatic I/R injury is often described after operations of the abdominal aorta, thromboembolism of pancreas-supplying arteries, cardiac bypass surgery, pancreas transplantation, and hemorrhagic shock.1,2 Once acute pancreatitis has been diagnosed, treatment is focused on maximizing tissue organ perfusion and pain control. The intense inflammatory response that accompanies pancreatitis can cause increased vascular permeability and dramatic fluid losses from the intravascular space.3,4

The relationship between pancreatic ischemia and acute pancreatitis has been established experimentally.5 Furthermore, prolonged ischemia of the pancreas followed by reperfusion with oxygenated blood causes morphological changes, such as swelling of the endothelial cells, interstitial edema due to increased capillary permeability, and, finally, tissue necrosis.6

The metabolites produced during I/R injury, such as superoxide, hydrogen peroxide, and hydroxyl radical, cause cellular injury by direct action and by secondary activation of polymorphonuclear neutrophils (PMNs). Various substances that compete with the production or action of reactive oxygen species (ROS) most likely protect organs that are under I/R conditions.7,8

Allopurinol, an inhibitor of the enzyme xanthine oxidase (XO), displays anti-inflammatory properties in vivo.9-11 Nimesulide, one of the most widely used anti-inflammatory drugs,10,11 scavenges and inhibits the production of ROS.12-14 However, nimesulide has...
not been studied in the context of I/R. Therefore, the aim of this study was to investigate the actions of allopurinol (intravenously [iv]) and nimesulide (intraperitoneally) in preventing pancreatic tissue damage after I/R.

Materials and Methods

Experimental Protocol

A total of 30 rabbits (White New Zealand, 4-8 months old and weighting 2-3 kg) were used in this study. The animals were divided randomly into 3 groups: control (group A, n = 10), I/R plus iv infusion of allopurinol (30 mg/kg) 10 minutes before ischemia (group B, n = 10), and I/R plus intraperitoneal administration of nimesulide (50 mg/kg) 20 minutes before ischemia (group C, n = 10).

Rabbits were maintained for 2 days in comfortable cages (poly methyl methacrylate, Plexiglas, Evonik Industries AG, Essen, Germany) and were exposed to iso atm ospheric hyperoxia (density of tidal air above 21%). The oxygen cylinder was made of a rubber tube and was introduced through a small hole in the bottom of the cage. Rabbits were fed freely with commercially available rabbit chow and tap water. All animal experiments were conducted according to the Guidelines for Animal Experimentation approved by the University Ethics Committee.

The technique was divided into 3 phases:

1. As a stabilizing step, the animal was anesthetized to stabilize hemodynamic parameters (phase I).
2. Pancreatic ischemia (phase II).
3. Pancreatic reperfusion (phase III).

Anesthesia was induced with intramuscular ketamine-hydrochloride (35 mg/kg Ketalar; JMP Pharmaceuticals, Parsippany, New Jersey) plus xylazine hydrochloride (5 mg/kg Rompun; Bayer, Leverkusen, Germany). After a lapse of 5 minutes, the animal was under anesthesia with spontaneous respiration. A small venous catheter (22-24G Abbocath; Abbott Laboratories, North Chicago) was introduced into a vein in the ear. Then, the animal was placed supine on the operating table and was submitted a tracheotomy with endotracheal tube placement (No. 3.5-4). The tube was connected to a small animal ventilator (type V5 KG, Narco Bio-Systems, Inc, Houston, Texas), which provided 100% oxygen. The tidal volume was 15 mL/kg per body weight and the breath rate was 25 to 35/min, depending on the depth of anesthesia. The femoral vessels (artery and vein) were dissected through a short longitudinal incision in the right thigh. The common femoral artery was used for monitoring the vital signs through a venous catheter (18-20 G Abbocath; Abbott Laboratories), which was connected to a monitor (Criticon, Dinamap plus monitor 9700 series, DRE, Louisville). The right femoral vein was used for the blood samples and the administration of substances. During the experiments, animals received lactated Ringer solution through the ear vein, and the mean infusion rate was 15 to 25 mL/kg per h. The animal was kept in a heated room and under a lamp to reduce heat loss during the surgery. The duration of the first (stabilization) phase was approximately 20 to 25 minutes.

A midline abdominal incision was made. The small intestine was reflected to the left, and the superior and inferior pancreaticoduodenal arteries with vessels of the splenic artery were exposed. The arteries were occluded using microvascular clamps (Scalcan, St Paul, Minnesota). In this phase, 150 IU/kg of heparin was administered iv to prevent thrombosis.

After 30 minutes, the clamps were removed. The reperfusion period was 30 minutes. During the period of reperfusion, minor hemodynamic instability and bradycardia were treated by increasing the infusion rate of crystalloids and administering atropine, respectively. At the end of the experiment, the animals were sacrificed by iv administration of KCl. The pancreas was totally removed and fixed with formaldehyde for histological examination.

Sham operation involved the same technique and exposure without clamping the arteries. During the experiments, we recorded the mean arterial pressure (MAP = diastolic + [1/3 systolic – diastolic]) in mm Hg and the heart rate at 5 minutes before ischemia, 5 minutes before reperfusion, and 5 minutes before the end of the experiment (during reperfusion). At the same time intervals, blood samples were taken for superoxide dismutase (SOD) and neopterin determination.

Five-milliliter vials of allopurinol in powder form (Sigma-Aldrich Co LLC, Athens, Greece) were utilized for iv administration. The powder was dissolved in a minimum volume of 0.1 N NaOH, and 0.1 N HCl was added to make the final pH approximately 11. The final solution was soluble in normal saline (N/S: 0.9% solution NaCl) and had a concentration of allopurinol of 50 mg/mL. Finally, 0.6 mL of solution was further diluted in 5 mL N/S and injected slowly iv into the animal.

Because no available formulation of nimesulide for parenteral administration exists, a commercial formulation of granules for per os use (Mesulid, Boehringer Ingelheim Ellas A.E., Elliniko, Athens, Greece) was dissolved in N/S 0.9% and homogenized for 60 seconds using a Polytron homogenizer.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 13 (SPSS Inc, Illinois). Statistical significance was set at a value of $P < .05$. The normality of distribution of the values was tested with the Kolmogorov-Smirnov test. Due to fluctuations in the measurements of neopterin in group B, these values were logarithmically transformed to better approximate a normal distribution and to normalize the fluctuations, so that the Levene test became non significant ($P > .1$).

Repeated-measures analysis of variance (general linear model) was used to test the differences within the groups, with Bonferroni correction and with Dunnett post hoc analysis.
Table 1. Mean Concentrations of Neopterin ± SD (in ng/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Phase I: Stabilization</th>
<th>Phase II: Ischemia</th>
<th>Phase III: Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Control group</td>
<td>3.56 ± 3.41</td>
<td>7.74 ± 3.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.94 ± 2.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group B: Allopurinol</td>
<td>3.40 ± 3.03</td>
<td>7.45 ± 8.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.64 ± 7.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group C: Nimesulide</td>
<td>3.41 ± 2.71</td>
<td>5.67 ± 2.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.34 ± 2.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.
<sup>a</sup> P < .05 compared to the stabilization phase.
<sup>b</sup> P < .05 in this particular phase compared to the control group.

Table 2. Statistical Significance of Differences in Neopterin Levels: Effect of Clustering

<table>
<thead>
<tr>
<th>% (P&lt;sub&gt;post hoc&lt;/sub&gt;)</th>
<th>A versus B</th>
<th>B versus C</th>
<th>A versus C</th>
<th>P&lt;sub&gt;ANOVA&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I: stabilization</td>
<td>+4.7% (P = .965)</td>
<td>-0.3% (P = .980)</td>
<td>+4.4% (P &lt; 1.000)</td>
<td>.897</td>
</tr>
<tr>
<td>Phase II: ischemia</td>
<td>+3.9% (P = .889)</td>
<td>+31.4% (P = .996)</td>
<td>+36.5% (P = .730)</td>
<td>.652</td>
</tr>
<tr>
<td>Phase III: reperfusion</td>
<td>-15.9% (0.862)</td>
<td>+145.1% (P = .331)</td>
<td>105.9% (P = .005)</td>
<td>.029</td>
</tr>
<tr>
<td>Total</td>
<td>0.893</td>
<td>0.946</td>
<td>0.364</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ANOVA, analysis of variance.

Table 3. Mean Values of SOD ± SD (in ng/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Phase I: Stabilization</th>
<th>Phase II: Ischemia</th>
<th>Phase III: Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Control group</td>
<td>4.25 ± 1.79</td>
<td>4.48 ± 1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.57 ± 1.15&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group B: Allopurinol</td>
<td>4.32 ± 0.81</td>
<td>5.08 ± 1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.45 ± 1.31</td>
</tr>
<tr>
<td>Group C: Nimesulide</td>
<td>4.10 ± 0.99</td>
<td>5.23 ± 1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.72 ± 1.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; SOD, superoxide dismutase.
<sup>a</sup> P < .05 compared to the stabilization phase.
<sup>b</sup> P < .05 compared both to the stabilization phase and to the control group.
<sup>c</sup> P < .05 in this particular phase compared to the control group.

Table 4. Statistical Significance of Differences in SOD Levels: Effect of Clustering

<table>
<thead>
<tr>
<th>P&lt;sub&gt;post hoc&lt;/sub&gt;</th>
<th>A versus B</th>
<th>B versus C</th>
<th>A versus C</th>
<th>P&lt;sub&gt;ANOVA&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I: stabilization</td>
<td>-1.6% (P = .999)</td>
<td>+5.3% (P = .932)</td>
<td>+3.6% (P = .994)</td>
<td>.928</td>
</tr>
<tr>
<td>Phase II: ischemia</td>
<td>-13.4% (P = .701)</td>
<td>-2.9% (P = .992)</td>
<td>-16.7% (P = .650)</td>
<td>.479</td>
</tr>
<tr>
<td>Phase III: reperfusion</td>
<td>+25.0% (P = .617)</td>
<td>+19.6% (P = .168)</td>
<td>+49.7% (P = .008)</td>
<td>.010</td>
</tr>
<tr>
<td>Total</td>
<td>P = .923</td>
<td>P = .963</td>
<td>P = .865</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: SOD, superoxide dismutase.

Measurement of Neopterin

Neopterin was measured using enzyme-linked immunosorbent assay. We used 6 standards of known neopterin concentrations from the World Health Organization in the standard curve: 0, 1, 2, 4, 12, 37, and 111 ng/mL. Concentrations between 0.96 and 2.48 ng/mL were considered normal. Values equal to or greater than 2.48 were considered abnormal.

Measurement of SOD Activity

Superoxide dismutase activity (in U/mg Hb) in red blood cells was spectrophotometrically determined with the BIOXYTECH SOD-525 kit (OXIS Health Products Inc, Portland, Oregon). The blood was taken from the femoral vein in each phase of the experiment, as for neopterin. Superoxide dismutase are metalloenzymes that catalyze the dismutation of superoxide ion into oxygen and hydrogen peroxide, according to the following reaction:

\[ 2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \]

Different types of SOD have been described. The 3 most studied classes are distinguished by the catalytic metal at the active site: Cu/Zn, Mn, or Fe. Cu/Zn enzymes are found primarily in eukaryotes, Fe-SOD is found mainly in prokaryotes, and Mn-SOD is found in prokaryotes and eukaryotes.
Cu/Zn-SOD is localized in the cytosol and nucleus, while Mn-SOD is localized in the mitochondrial matrix. Superoxide dismutase enzymes provide a defense system that is essential for the survival of aerobic organisms.

Principles of the Procedure

The BIOXYTECH SOD-525 method is based on the SOD-mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene R1 in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. The chromophore has not been isolated or characterized.

Interference due to mercaptans (RSH), such as reduced glutathione, is controlled by pretreating samples with 1,4,6-trimethyl-2-vinylpyridinium R2, which directly eliminates mercaptans by means of a fast alkylation reaction.

The kinetic measurement of the 525 nm absorbance change is performed after the addition of R1. The SOD activity is determined from the ratio of the autoxidation rates in the presence (Vs) and in the absence (Vc) of SOD. The Vs/Vc ratio as a function of SOD activity is independent of the type of SOD (Cu/Zn-SOD, Mn-SOD, and Fe-SOD) being measured. One SOD-525 activity unit is defined as the activity that doubles the autoxidation rate of the control blank (Vs/Vc = 2). The relationship between the Vs/Vc ratio and SOD activity is described by Equation (1).

\[
Vs/Vc = 1 + [SOD]/a[SOD] + b
\]

where Vs is the rate of sample containing SOD, Vc is the average rate of blank (SOD = 0) samples, SOD is the SOD...
activity of the sample in SOD-525 units, $a$ is the dimensionless coefficient, and $b$ is the coefficient in SOD-525 units.

One SOD-525 activity unit has a $V_s/V_c$ ratio of 2; therefore, $b = 1 - a$. The determination of $a$ and $b$ by experimentally measuring a range of SOD samples results in $a = 0.073$ and $b = 0.93$ SOD-525 units.

>Conversion of SOD activity. If the original sample contains 5 mg protein/mL, the SOD activity can be expressed as SOD activity per milligram protein by dividing the units/mL by the protein concentration (12 700 U/mL)/(5 mg/mL) = 2540 U/mg.

**Histopathology**

Pancreatic tissue samples were prepared in 10% formaldehyde. Thin sections (3 mm) were obtained with an electronic rotary microtome (Microm HM 340 E, Thermo Scientific Microm, Walldorf, Germany). Histopathologic evaluations were performed by light microscopy after staining the specimens with hematoxylin–eosin. The following histological parameters were studied:

1. edema (extravascular and intralobular),
2. acinar cell necrosis,
3. fat necrosis,
4. polymorphonuclear infiltration,
5. inflammatory infiltrates with lymphocytes and plasma cells, and
6. extravasation of red blood cells (hemorrhagic infiltrations).

The evaluation was made blindly using 3 grades of lesion severity: mild, moderate, and severe.

**Results**

**Neopterin Levels**

The mean neopterin level increased significantly in group B (allopurinol) during reperfusion (10.64 ± 7.47 vs 8.94 ± 2.46 ng/mL) compared to group A. Neopterin concentration in group C (nimesulide) decreased during ischemia (5.67 ± 2.76 vs 7.74 ± 3.59) and during reperfusion compared to group A (4.34 ± 2.87 vs 8.94 ± 2.86). Group C also exhibited a stronger decrease in neopterin compared to group B during ischemia (5.67 ± 2.76 vs 7.45 ± 8.89) and during reperfusion (4.34 ± 2.87 vs 10.64 ± 7.47; Table 1). Statistical significance, whenever observed, is depicted in Table 2.

**The SOD Activity**

Group A had mean SOD values of 4.25 ± 1.79, 4.48 ± 1.60, and 5.57 ± 1.15 ng/mL at phase I (stabilization), phase II (ischemia), and phase III (reperfusion), respectively. Group B (allopurinol) had SOD levels of 4.32 ± 0.81, 5.08 ± 1.10, and 4.45 ± 1.31 ng/mL at the end of the 3 respective phases. In group C, the mean SOD values were 4.10 ± 0.99, 5.23 ± 1.60, and 3.72 ± 1.30 ng/mL, respectively, at the same time points (Table 3). During reperfusion, the SOD level in group A was 25% higher than in group B and 49.7% higher than in group C. In the allopurinol group, the SOD level was 19.6% higher than in the nimesulide group. The difference between the nimesulide group and control group was statistically significant ($P < .05$).

During ischemia, the SOD level in group C was 16.7% higher than in group A and 2.9% higher than in group B, while the SOD level in group B was higher by 13.4% compared with group A. There were statistically significant differences between the 3 groups in the ischemic period. Statistical significance, whenever observed, is depicted in Table 4.

**Light Microscopy**

Group A showed moderate-to-severe acute pancreatitis lesions (Figures 1–3). Group A had focal pancreatic parenchymal necrosis with destruction of acinar cells and abundant inflammatory cell infiltrates, mainly neutrophils, lymphocytes, and plasma cells. There were also foci of steatonecrosis and extensive bleeding perfusion.

In group B (Figures 4–7), there were slight-to-moderate changes indicative of acute pancreatitis, such as limited acinar cell necrosis, interstitial tissue swelling, inflammatory cell infiltration (mainly with lymphocytes, plasma cells and a few neutrophils), foci of fat necrosis, and hemorrhagic infiltration. These results show that, compared with group A, the preparations of group B had fewer foci of parenchymal necrosis, fewer foci of steatonecrosis, less extensive inflammatory phenomena, and less severe bleeding perfusion.

Group C animals (Figures 8–11) showed a limited degree of deterioration indicative of acute pancreatitis. The
changes consisted mainly of a small degree of inter- and intralobular edema, few foci of hemorrhagic infiltrates and mild inflammatory cell infiltrates. In this group no foci of pancreatic parenchymal necrosis were observed. In contrast with groups A and B, group C had no foci of steatonecrosis. Inflammatory phenomena and bleeding perfusion in group C were significantly less extensive than in groups A and B.

The islets of Langerhans in the 3 groups appeared normal (Figures 12 and 13). Additionally, the vessels showed no evidence of vasculitis (Figure 14). In some preparations of group A, the formation of inflammatory granulation tissue, tumor capillaries, inflammatory infiltrates, and foreign body reactions in the presence of polymorphonuclear giant cells were observed.

Discussion
The pancreas is highly susceptible to ischemic damage. Ischemia/reperfusion of the pancreas induces acute pancreatitis in animal models. Ischemia/reperfusion causes an inflammatory reaction similar to acute pancreatitis and is considered a major determinant of the progression of pancreatitis produced by other causes.

Microcirculatory derangements caused by I/R play a pivotal role in acute pancreatitis. Microvascular perfusion failure of the
pancreas is the primary cause of clinical and experimental pancreatitis. Reduction of blood flow in the pancreatic microcirculation results in the formation of thrombi in the capillaries, activation of leukocytes, intrapancreatic release, and activation of digestive enzymes as well as in the formation of oxygen-derived free radicals and proinflammatory cytokines.\(^{18,19}\)

As in other organs, ROS, which are mainly derived from the xanthine dehydrogenase/xanthine oxidase system, are important etiological agents of I/R pancreatic injury.\(^7\) Reperfusion significantly exacerbates ischemia-induced injury via the formation of ROS, such as superoxide anion, hydroxyl radical, hydrogen peroxide, and peroxynitrite. The ROS cause disintegration of cell membranes by stimulating the free chain reaction known as lipid peroxidation and trigger the extravasation of granulocytes in the pancreatic tissue. In experimental pancreatitis during reperfusion, the venules can become occluded by a variety of causes, such as the expression of adhesion molecules on leukocytes and/or endothelial cells and superoxide and nitric oxide activation. The adherence of leukocytes plays an important role in I/R tissue damage due to their release of proteinases and generation of superoxide radicals (respiratory burst).\(^{17,18}\)

Several pharmacological agents that might attenuate reperfusion injury of the pancreas have been tested. Allopurinol, an effective inhibitor of the enzyme XO, has been used for several decades for the treatment of gout and hyperuricemia. Because the inhibition of XO limits the formation of ROS as well as uric acid production, allopurinol has been used experimentally for the treatment of conditions associated with I/R injury in the intestine, liver, and pancreas.\(^{11,19,20}\)

The SODs are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As
such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The level of SOD was depleted by 12.4% during reperfusion compared with ischemia in group B (allopurinol), while in the control group SOD increased by 24.3% in reperfusion compared with ischemia. Moreover, the histopathological findings in group B were milder, with no focal necroses and less edema, in comparison with group A.

Neopterin is a messenger of the immune system. It is synthesized and released by macrophages and monocytes after activation by the cytokine interferon-gamma and is indicative of a proinflammatory immune status. This activation occurs in ischemia, inflammation, cancer, transplantation, and autoimmunization.21,22

Neopterin level in pancreatic I/R seemed not to be influenced by the administration of allopurinol compared with control. Therefore, the production of neopterin might not be as strongly influenced by the XO system or by ROS as by the general activation of the immune system and inflammatory mechanisms that characterize acute pancreatitis. In addition, the pancreas expresses lower levels of XO compared with intestine and liver.23,24,25

Of particular interest are our findings regarding nimesulide. To our knowledge, this is the first study to investigate nimesulide in I/R of the pancreas as an anti-inflammatory and antioxidant drug. Nimesulide is a preferential cyclooxygenase 2 inhibitor and nonsteroidal anti-inflammatory drug (NSAID).12,13 Its action is primarily through inhibition of arachidonic acid. Because of its chemical nature (weak acid), nimesulide selectively inhibits the biosynthesis of prostaglandins (PGs), increasing the synthesis of prostaglandin PGH2 under certain conditions. This effect seems to significantly reduce the side effects of the drug compared with other NSAIDs.10,26,27 Therefore, this study selected nimesulide as a possible antioxidant with potential applications in clinical practice. This choice was based on the following properties:

1. It blocks the “respiratory burst,” reducing superoxide anion production.28 This effect is likely due to repressive action on phosphodiesterase or protein kinase C (PKC), which are involved in the “respiratory burst” of PMN.29 There are, however, other aspects of the relationship between nimesulide and PKC.30 It is possible that the intracellular pH is not altered by the action of nimesulide, as with other NSAIDs that definitely inhibit PKC.31,24 Furthermore, it appears that nimesulide does not affect the mobility or phagocytic–bactericidal properties of PMN.27 Therefore, its antioxidant action can most likely be exercised safely without the risk of intracellular acidosis.10,11
2. It acts as a scavenger of hypochlorite ion by reducing the level of the toxin chloramine.28
3. Its antihistaminic and general anti-inflammatory action seems to affect the mediators of inflammation, such as the

Figure 12. Islets of Langerhans without lesions (group A: control; hematoxylin and eosin [H&E], ×100).

Figure 13. Islets of Langerhans without lesions (group A: control; hematoxylin and eosin [H&E], ×160).

Figure 14. Vessels and nerves without alterations (group A: control; hematoxylin and eosin [H&E], ×100).
kallikreins and nitric oxide, which have been implicated in the pathogenesis of experimental pancreatitis.\(^2\)\(^{12}\)\(^{13}\)\(^{18}\)\(^{21}\)\(^{28}\)\(^{32}\)\(^{33}\)

In this study, SOD decreased during reperfusion compared with ischemia to a significantly greater degree in the nimesulide group (28.2\%\( P = 0.008\)) than in the allopurinol group (12.4\%). In contrast, in the control group, SOD increased by 24.3\%. These results show that the SOD levels in phase III (reperfusion) and in the groups receiving antioxidants were lower in comparison with the control group. Because SOD is a key antioxidant system of the body that neutralizes ROS, an increase in its activity reflects the level of exposure to oxidative stress. Our results are in accordance with the findings reviewed by Zimmerman et al in the level of exposure to oxidative stress.

Even more interesting, however, is the fact that only in the nimesulide group was a decrease in neopterin observed during reperfusion (23.4\%). In the control group, neopterin remained high (15.5\% increase), while in the allopurinol group, it continued to increase (42.8\%) but did not reach a level significantly higher than the control (3.9\% difference). During reperfusion, the neopterin level in group C was significantly lower than group A.

The above findings suggest that nimesulide suppresses the level of neopterin, an important messenger of the immune system, through its pleiotropic anti-inflammatory and antioxidant actions.\(^2\)\(^{12}\)\(^{13}\) In contrast, allopurinol acts locally on the XO system, whose involvement in experimental pancreatitis appears to be limited.\(^3\)\(^{12}\) Thus, administration of allopurinol reduces oxidative stress (as evidenced by SOD levels) to a lesser extent than nimesulide and does not alter the concentration of a less specialized indicator of inflammatory processes, neopterin. This phenomenon can be explained by the relatively lower concentrations of XO observed in pancreas compared with small intestine and liver.\(^2\)\(^{23}\)\(^{24}\)

Light microscopy revealed a significant beneficial effect of nimesulide. Group C showed low-grade lesions of acute pancreatitis but normal acinar cells and mild intercellular edema. In contrast, the control group showed moderate-to-severe lesions of acute pancreatitis (necrosis, acute inflammatory cell infiltrates, and extensive bleeding), while the allopurinol group displayed an intermediate phenotype. It should, however, be noted that the lower ROS production (as reflected in the levels of SOD and neopterin) during reperfusion in the nimesulide group compared with the allopurinol group may have been related to the low concentration of XO in the pancreas and to the different routes of drug administration (allopurinol: iv; nimesulide: intraperitoneal).

In conclusion, it is not clear whether XO is causatively involved in I/R injury of the pancreas, but it seems likely that allopurinol administration can be beneficial. Moreover, the present study has shown—to the best of our knowledge, for the first time in an in vivo model—that nimesulide is more potent as an antioxidant compared to allopurinol and can significantly attenuate the damage caused by ischemia and reperfusion.

Although prophylactic therapy with nimesulide is possible, further studies are needed to confirm our findings before definitive conclusions can be drawn.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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