Treatment of Acute Focal Cerebral Ischemia with Propranolol

JOHN R. LITTLE, M.D., JOHN P. LATCHAW, JR., M.D., ROBERT M. SLUGG, A.B., RONALD P. LESSER, M.D.,* AND NICHOLAS T. STOWE, PH.D.†

SUMMARY Propranolol has been found to have a protective effect in experimental myocardial ischemia. Protection of ischemic kidneys was subsequently demonstrated following treatment with propranolol and its weaker beta blocking isomer, d-propranolol. The objective of the present investigation was to study the effects of propranolol (i.e., racemic d,l mixture) and d-propranolol upon regional cerebral blood flow (rCBF) and early ischemic changes following experimental middle cerebral artery (MCA) occlusion. Thirty adult cats, lightly anesthetized with ketamine hydrochloride, underwent 3 hours of right MCA occlusion. Ten cats were untreated. Ten cats were given a continuous infusion of propranolol (1 mg/kg/hr) for 4 hours beginning 1 hour before MCA occlusion and a 4 mg/kg bolus immediately before occlusion. Ten cats were given a continuous infusion of d-propranolol (0.5 mg/kg/hr) for 4 hours beginning 1 hour before MCA occlusion and a 2 mg/kg bolus immediately before occlusion. The therapeutic agents were injected directed into the right carotid artery. The rCBF in the right Sylvian region was not significantly different in the 3 groups. EEG changes also were similar. Carbon filling defects were found to be smallest in the d-propranolol-treated group. Light microscopic studies demonstrated a reduction in infarct size in the propranolol and d-propranolol groups. The findings of the investigation indicated that propranolol and d-propranolol do not have a deleterious effect on rCBF after MCA occlusion and suggested that these agents have a protective effect upon ischemic cerebral tissue.

Propranolol has been found to have a protective effect in experimental myocardial ischemia.1-3 Protection of ischemic kidneys was subsequently demonstrated following treatment with propranolol (i.e., Inderal) and its weaker beta blocking isomer, d-propranolol.4-6 The actions of these agents upon cerebral blood flow and cerebral tissue during ischemia, however, have not been evaluated.

A recent experimental study by David and Sundt6 suggested that propranolol might reduce cardiac output and regional cerebral blood flow (rCBF) in the nonischemic cerebral hemisphere despite the maintenance of arterial blood pressure. This could have serious implications in cerebral ischemia as many stroke-prone patients receive this medication.

The objective of the present investigation was to study the effects of propranolol (i.e., racemic d,l-propranolol) and its weaker beta blocking isomer, d-propranolol, upon rCBF and early ischemic changes following experimental middle cerebral artery (MCA) occlusion.

Methods

Right Middle Cerebral Artery Exposure

Thirty adult cats (mean weight 3.7 kgs) were anesthetized with ketamine hydrochloride (40 mg/kg intraperitoneally). Administration of ketamine hydrochloride took place two hours or longer before right MCA occlusion. Additional doses of ketamine hydrochloride were not given.

Catheters were inserted into the right femoral artery and vein through a groin incision. A tracheostomy was performed through a longitudinal midline incision and mechanical ventilation instituted. Skeletal muscle paralysis was achieved with d-tubocurare (1 mg/kg IV). Additional d-tubocurare was administered during an experiment if voluntary movement was observed. Needle electrodes were placed subcutaneously in the right and left thoracic areas for EKG recording. A reference electrode was placed in the tracheostomy wound. A small catheter was inserted into the right carotid artery through the lingual artery for subsequent injection of Xenon-133 (133Xe) and for the direct administration of the therapeutic agents.

Arterial blood gases (femoral artery) were determined when necessary to maintain the PaCO2 in the 30–35 mm Torr range (i.e., normal range for conscious adult cats10) and the PaO2 above 100 mm Hg. Arterial blood pressure (femoral artery) and EKG were monitored continuously. Blood hematocrit was determined with the blood gas samples prior to each 133Xe rCBF measurement. A heating pad was placed over the trunk to maintain the core temperature at 37°C.

The head of each cat was shaved and placed in a headholder which provided unobstructed access to the right orbit. The orbital contents were evacuated on the right side and a small craniectomy, continuous with the superolateral margin of the optic foramen, was performed. Using microsurgical techniques, the dura and arachnoid membranes were opened. The proximal segment of the right MCA was dissected from the adjacent structures in preparation for application of a miniature aneurysm clip. Prior to the 133Xe clearance studies and treatment, the scalp and temporalis muscles were separated from the skull and retracted.

Regional Cerebral Blood Flow Measurement

Regional cerebral blood flow (rCBF) was measured by the 133Xe clearance technique. The 133Xe window (centered at 81 keV) was determined with a multi-
channel analyzer. A colimated 1.5 cm sodium iodide crystal, recessed 5.0 cms, was applied to the skull overlying the right Sylvian cortex. The $^{133}$Xe (200$\mu$ Ci in 0.5 ml normal saline) was rapidly injected into the right carotid artery through the lingual artery catheter. Measurements were recorded on a multi-channel analyzer for a 10-minute period. Kinetic analysis was used to calculate the rCBF.\textsuperscript{11, 12} The rCBF measurements were performed immediately before and after occlusion, and at 90 minutes and 3 hours after occlusion.

**Electroencephalography**

During the preocclusion treatment period, small holes (i.e., 1.5 mm) were drilled bilaterally 1 cm from the midline on the previously exposed skull. Holes were placed bilaterally in the midfrontal, posterior frontal, and parietal regions. Small stainless steel bolt electrodes were screwed into these holes to a depth contacting but not penetrating the dura. The location of the electrodes was in the border zone between the anterior cerebral artery and MCA territories and not in the core area of ischemia (i.e., Sylvian region). Another hole was drilled in the midline over the frontal air sinus and a screw was inserted for use as a reference electrode. The left temporals muscle was used for a ground. Tracings were recorded on a Grass model 6 electroencephalograph with recorded amplitudes 20% down at 1 and 70 Hz. The EEG was recorded for 2 minute periods before treatment, 30 minutes before right MCA occlusion, immediately before and after occlusion, and then every 30 minutes for the duration of each experiment.

**Treatment Groups**

The 30 cats were alternately assigned to the untreated, racemic propranolol (d and l stereoisomers), and d-propranolol groups (10 animals/group). Treatment was started 1 hour before right MCA occlusion and continued until the completion of each experiment. The agents were injected into the right carotid artery through the lingual artery catheter. The 10 untreated cats received 40 ml of 0.9% saline by slow infusion (10 ml/hour) starting 1 hour before right MCA occlusion and 1 ml of 0.9% saline was rapidly injected immediately before occlusion. Ten cats received 4 mg/kg of propranolol dissolved in 40 ml of 0.9% saline by slow infusion (10 ml/hour) starting 1 hour before right MCA occlusion and 4 mg/kg of propranolol dissolved in 1 ml of 0.9% saline immediately before occlusion. Ten cats received 2 mg/kg of d-propranolol dissolved in 40 ml of 0.9% saline by slow infusion (10 ml/hour) starting 1 hour before right MCA occlusion and 2 mg/kg dissolved in 1 ml of 0.9% saline immediately before occlusion. All cats received atropine (0.6 mg/kg subcutaneously) one hour before the administration of saline or the therapeutic agents.

**Right Middle Cerebral Artery Occlusion**

Following completion of the 1 hour treatment and preocclusion studies, the exposed right MCA was occluded with a miniature aneurysm clip. The clip remained in place for 3 hours.

**Perfusion**

Thirty minutes before perfusion, Evans blue (i.e., 0.5 ml of a 10% solution) was given intravenously. Intra-arterial carbon-fixative perfusion was carried out at the end of the 3 hour ischemic period after completion of the $^{133}$Xe clearance studies. A midline thoracotomy was performed. The right MCA was reopened by removing the aneurysm clip in order to improve delivery of the carbon-fixative solution to the ischemic tissue. A large cannula was passed through a left ventriculostomy incision into the ascending aorta and secured with a ligature. The descending aorta was clamped and the right atrium incised. The animals were perfused with 50 ml of isotonic saline followed by a mixture of colloidal carbon (125 ml) and phosphate-buffered 4% formaldehyde (125 ml) at a constant pressure of 120 mm Hg. The brain of each cat was removed, sliced coronally, and placed in fixative solution for 48 hours.

**Examination of the Brains**

The coronal brain slices were photographed. The presence or absence of Evans blue staining and shift of midline structures, if any, were recorded. The distribution of carbon staining was graded according to a previously described system.\textsuperscript{13} Grade “0” indicated normal vascular filling. Grade “1” referred to a few circumscribed foci of poor filling, not more than 3 mm in diameter; Grade “2” indicated a large area of improper subcortical filling; and, Grade “3” referred to an extensive cortical and subcortical region of impaired filling.

Thin (i.e., 10$\mu$m) semi-serial coronal sections were prepared from paraffin-embedded slices of both hemispheres, sustained with hematoxylin and eosin and periodic acid Schiff stains, and examined with a light microscope. The cross-sectional area of gray matter where moderate and severe neuronal alterations (i.e. Grades II and III\textsuperscript{14, 15}) predominated were determined with a Keuffel and Esser planimeter (Keuffel and Esser Company, New York, New York) in coronal sections of the right cerebral hemispheres 3 mms posterior to the temporal lobe tip. The percentage of gray matter surface area, that is (ischemic gray area/total gray area $\times$ 100) where severe ischemic neuronal alterations predominated was determined. The means and standard deviations of the three groups were calculated and comparisons made using the Kruskal-Wallis H test.

**Results**

**Vital Signs**

Systemic stability was maintained in the 30 cats undergoing right MCA occlusion. Arterial blood pressure was similar in the three groups however, heart rate was reduced in the cats treated with propranolol and d-propranolol (table 1). Hematocrit remained stable throughout each experiment (untreated 40 ± 3%; propranolol 37 ± 3%; d-propranolol 39 ± 2%).
Regional Cerebral Blood Flow

The results of the $^{133}$Xe clearance studies are displayed in figure 1. The rCBF was similar in the 3 groups immediately before right MCA occlusion, that is, 1 hour after initiation of treatment with propranolol or d-propranolol. No significant difference in rCBF was seen between the three groups throughout the 3 hour occlusion period. Reduction of rCBF < 18 ml/100 gm/min was recorded one or more times after MCA occlusion in 6 untreated cats, 4 propranolol-treated cats, and 5 d-propranolol-treated cats.

EEG Studies

The EEG background consisted of 5–30 hertz activity of up to 100$\mu$V. In addition, 1–3 hertz activity of up to 30$\mu$V was frequently seen. The record of 1 propranolol cat could not be analyzed due to technical factors. In order to correct for bilateral EEG voltage changes during the course of the procedure, the right and left amplitudes were compared. In 2 untreated cats preocclusion right-sided amplitude was more than 33% greater than that on the left and in 2 untreated cats, 1 propranolol-treated cat, and 1 d-propranolol treated cat, it was more than 33% lower initially. In all other animals activity was initially symmetrical.

There was greater than a 33% reduction in the right-to-left amplitude ratios immediately after occlusion in 4 untreated cats, 3 propranolol-treated cats and 3 d-propranolol-treated cats. At 3 hours, there was at least a 33% reduction in 3 untreated cats, 4 propranolol-treated cats, and 3 d-propranolol-treated cats. Taking each group as a whole, the right-to-left ratio showed 17% and 25% decrease at 90 minutes and 3 hours in the untreated group; 14% and 25% decrease in the propranolol group; and 24% and 18% decrease in the d-propranolol group. There were no significant differences between the groups for each analysis time. Moreover, intragroup variabilities and standard deviations were large.

Morphological Studies

1. Macroscopic findings. The right MCA and its major branches were well filled with carbon-fixative solution. This confirmed reopening of the right MCA after removal of the aneurysm clip. Little or no right to left shift of midline structures was observed in the 3

---

**Table 1 Systemic Arterial Blood Pressure and Heart Rate in 30 Cats Undergoing Right MCA Occlusion**

<table>
<thead>
<tr>
<th></th>
<th>Untreated cats</th>
<th>Propranolol treated cats</th>
<th>d-propranolol treated cats</th>
<th>Untreated cats</th>
<th>Propranolol treated cats</th>
<th>d-propranolol treated cats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour preocclusion</td>
<td>127 ± 5</td>
<td>109 ± 7</td>
<td>103 ± 7</td>
<td>168 ± 10</td>
<td>152 ± 9</td>
</tr>
<tr>
<td></td>
<td>Immediate preocclusion</td>
<td>133 ± 4</td>
<td>109 ± 10</td>
<td>122 ± 6</td>
<td>170 ± 9</td>
<td>131 ± 9</td>
</tr>
<tr>
<td></td>
<td>Immediate postocclusion</td>
<td>130 ± 3</td>
<td>110 ± 10</td>
<td>121 ± 7</td>
<td>167 ± 10</td>
<td>106 ± 5</td>
</tr>
<tr>
<td></td>
<td>90 minutes postocclusion</td>
<td>132 ± 5</td>
<td>120 ± 11</td>
<td>134 ± 7</td>
<td>164 ± 10</td>
<td>107 ± 3</td>
</tr>
<tr>
<td></td>
<td>3 hours postocclusion</td>
<td>136 ± 3</td>
<td>122 ± 7</td>
<td>128 ± 6</td>
<td>163 ± 10</td>
<td>108 ± 4</td>
</tr>
</tbody>
</table>

---

**REGIONAL CEREBRAL BLOOD FLOW IN RIGHT SYLVIAN REGION OF CATS UNDERGOING RIGHT MCA OCCLUSION**

**Figure 1. Regional cerebral blood flow in right Sylvian region of 30 cats undergoing right MCA occlusion.**
groups. Three untreated cats had small gray matter foci of Evans blue staining, whereas, none of the treated cats had gross evidence of increased permeability to this vital dye. Impaired carbon-filling in the right MCA territory was observed in 10 untreated cats, 9 propranolol-treated cats, and 8 d-propranolol-treated cats (table 2). None of the d-propranolol treated cats were found to have a large cortical and subcortical area of impaired carbon filling (i.e., Grade 3).

2. Microscopic findings. Severe ischemic neuronal alterations were present in the caudate nucleus and/or cortex supplied by the right MCA of 10 untreated, 9 propranolol-treated, and 8 d-propranolol-treated cats. Astrocytic swelling and capillary narrowing were seen in the same areas as the ischemic neurons. Severe ischemic changes were consistently observed in the cortex underlying the detector probe in those cats with rCBF reduction to < 18 ml/100 gm/min. The percentage of gray matter cross-sectional area where severe ischemic neuronal alterations predominated was 23 ± 17% in the untreated group, 8 ± 10% in the propranolol-treated group, and 8 ± 10% in the d-propranolol treated group. There was evidence of an overall significance level of α = 0.10. The data indicated that the infarct area in the untreated group was larger than that in both the propranolol and d-propranolol groups (i.e., p < 0.02 and p < 0.02 respectively). There was no evidence that there was a significant difference between the two treatment groups (p > 0.80).

Discussion

Pharmacological Properties of Propranolol

A number of actions of propranolol and/or d-propranolol could potentially play a role in protecting ischemic tissue. Pendleton and associates16 found a shift to the right of the hemoglobin-oxygen dissociation curve of erythrocytes with propranolol and d-propranolol. In their study, the weaker beta blocking d isomer produced a greater shift than racemic propranolol. This action could increase the availability of oxygen to the ischemic tissue. Because the d isomer was equally or more effective in shifting the curve than the racemic mixture, this action was not attributed to the beta-blocking properties of the 1 isomer.

Racemic propranolol and d-propranolol appear to have "membrane stabilizing" effects. Langslet17 has shown both that racemic propranolol and d-propranolol inhibit osmotically-induced erythrocyte lysis. This membrane stabilizing effect was confirmed by Pendleton and associates.16 As well, these agents have been shown to have local anesthetic effects which are comparable to lidocaine.18

<table>
<thead>
<tr>
<th>Grade of carbon perfusion</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated cats</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Propranolol-treated cats (10)</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>d-propranolol-treated cats (10)</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 2 Carbon Perfusion in the Right Cerebral Hemisphere of 30 Cats Undergoing Right MCA Occlusion

Blocking of beta adrenergic receptors in the brain could modify the effects of ischemia. Meyer and associates19, 20 produced a reduction in cerebral oxygen consumption, glucose metabolism, and rCBF in patients with brain ischemia and infarction by injecting propranolol (1.45 μg gm/kg/min) into the carotid artery. Increased cerebrovascular resistance and reduction in rCBF were attributed partly to the depressant effects of propranolol on cerebral metabolism and reduced CO₂ production. However, propranolol was also thought to act directly on microvascular receptors with consequent blocking of the beta-adrenergic dilator tonus.

Oleson and associates31 studied the ability of propranolol to cross the blood-brain barrier and its effects on the cerebral circulation in man. Significant amounts of ¹⁴C-propranolol were found in the brain within a few minutes of intravenous injection. Approximately 66% of propranolol in the blood crossed the blood-brain barrier in a single passage, that is, an extraction rate similar to water. They found only slight reductions of rCBF following the administration of propranolol (0.15 mg/kg). This was interpreted as a response to the inhibition of parenchymal beta-adrenergic receptors rather than to a direct vascular effect.

Effects of Propranolol in Heart and Kidney Ischemia

Propranolol appears to have a protective effect in experimental myocardial ischemia.1-5 Sommers and Jennings5 demonstrated a significant reduction in myocardial necrosis after transient ischemic episodes of 20–25 minutes duration in dogs treated with propranolol. The incidence of ventricular fibrillation, however, was not altered. Protection was attributed partly to the inotropic-sparing action of the drug. Prolongation of the ischemic period to 40 minutes in a subsequent study showed a similar protective effect.3

A protective effect of propranolol was demonstrated by Stowe and associates6 in the treatment of ischemically damaged canine kidneys prior to transplantation. The dog kidneys were subjected to 30 minutes of warm ischemia followed by hypothermic pulsatile perfusion for 24 hours. The kidneys were autotransplanted with immediate contralateral nephrectomy. In this model only 50% of the untreated control group survived whereas survival was 100% in the propranolol-treated group. Protection was thought to be related to the blockade of beta-mediated renin release and/or to a membrane-stabilizing effect.

In the same experimental model, Stowe and associates6 subsequently studied the effects of treatment with racemic propranolol and d-propranolol in kidneys subjected to 60–120 minutes of warm ischemia. The protective effect of these two agents was similar. Because the d isomer has less than 1% of the beta blocking activity of the 1 isomer, the findings of the study indicated that the protective action did not reside in the beta-adrenergic blocking properties of the 1 isomer.

Effects of Propranolol in Cerebral Ischemia

The events that follow experimental occlusion of the MCA in cats appear to resemble the neurological and
pathological changes in acute major cerebral artery occlusion in humans. Although substantial neurological, EEG, and CBF changes occur within minutes of MCA occlusion, irreversible injury to cerebral tissue in the ischemic zone usually is not present with occlusion periods less than one to two hours. Previous studies have demonstrated that occlusion of the MCA in conscious or ketamine-anesthetized cats invariably results in a large cortical and subcortical infarct. Occlusion of the MCA resulted in EEG amplitude depression rather than the development of slow activity. These findings were consistent with previous reports. Sundt and Michenfelder demonstrated a decrease in amplitude in association with minor degrees of ischemia and the development of lower voltage with more irregular components when ischemia was more severe. Hossmann and Schuier found that EEG power gradually decreased over the entire range of flow rates and suggested that with increasing ischemia an increasing proportion of the neuropil was inhibited. Because of the position of the rCBF detector probe, we placed the electrodes in a more sagittal plane (i.e., border zone between the anterior cerebral artery and the MCA) than those in the study of Hossmann and Schuier. This probably accounts for the less severe EEG changes seen in our study.

Progressive impairment of microcirculatory filling with carbon perfusion has been demonstrated previously in experimental models of acute focal cerebral ischemia. A large cortical and subcortical area of severely impaired filling was invariably seen with MCA occlusion of 6 hours and longer. This was initially thought to represent a state of so-called "no reflow", however, subsequent studies of albumin and erythrocyte transit indicated that flow persisted despite an apparent increase in microcirculatory resistance. Although impairment of carbon perfusion in the present investigation was relatively mild in the 3 groups of cats following 3 hours of MCA occlusion, carbon filling defects were found to be smallest in the d-propranolol-treated group.

Light and electron microscopic studies in fixative perfused animals have clearly demonstrated the morphological changes in ischemic neurons. The changes are distinct from the so-called "dark neurons" which are thought to result from inadequate fixation. In the present investigation, the tissue studied was well fixed by perfusion and immersion. Also, the ischemic changes in the glia and microvasculature in the areas containing ischemic neurons were consistent with previous descriptions.

Severe ischemic changes in the right MCA territory were seen in 10 untreated cats, 9 propranolol-treated cats, and 8 d-propranolol-treated cats. The mean size of the infarcts, however, were smaller in the propranolol-treated and d-propranolol-treated groups compared with the untreated groups. These findings suggested that propranolol and d-propranolol had a protective effect in ischemic cerebral tissue.

Effects of Propranolol on Cardiac Function and Cerebral Blood Flow

The d isomer of propranolol appears to have minimal chronotropic and inotropic effects on the heart. Barrett has compared the effects of racemic propranolol and d-propranolol in anesthetized dogs. Doses of 0.25 mg/kg of racemic propranolol and d-propranolol (equivalent to a total dose of 12.5 mg intravenously in the human) were studied. Racemic propranolol produced a 23% reduction in heart rate, an 18% reduction in cardiac contractile force, and a 15% reduction in tension/time index. Conversely, d-propranolol, which is weaker in beta-adrenergic blocking properties, produced only a 2% reduction in heart rate, a 6% reduction in contractile force, and a 4% rise in tension/time index.

A previous report suggested that the beta-adrenergic blocking properties of racemic propranolol on the heart may have a deleterious effect upon CBF in ischemia. Davis and Sundt recently studied the effects of racemic propranolol upon rCBF, cardiac output, and mean arterial blood pressure, at varying levels of PaCO₂ in cats. They found that propranolol, in a dosage insufficient to change mean arterial blood pressure, decreased both cardiac output and rCBF. The agent abolished the cardiac output response to elevations in PaCO₂ but not the rCBF response. These findings also were thought to indicate that the rCBF reduction was not the result of impaired cerebral autoregulation.

The findings of our study did not confirm those of Davis and Sundt. Despite the use of higher doses of propranolol in the present investigation, rCBF before and after MCA occlusion was not significantly different from the untreated cats. The different methods of propranolol administration, that is, intracarotid in our study and intravenous in the study of Davis and Sundt could be a factor in explaining the disparity in the results of the two investigations. The findings of our study, however, suggest that the use of propranolol does not increase the risk of cerebral infarction and may, in fact, reduce ischemic injury.

Acknowledgments

This investigation was partly supported by a grant from the American Heart Association (Northeast Ohio Chapter) and the Cleveland Foundation. The assistance of George W. Williams Ph.D. and Sarah B. Forsythe, M.S., Department of Biostatistics in the statistical analysis of the data was appreciated. The authors appreciated the donation of the racemic propranolol and d-propranolol from Ayerst Corporation.

References

4. Eliahou HE, Iaina A, Solomon S and Gavendo S: Alleviation of
Treatment of acute focal cerebral ischemia with propranolol.
J R Little, J P Latchaw, Jr, R M Slugg, R P Lesser and N T Stowe

doi: 10.1161/01.STR.13.3.302

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/13/3/302

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/