A NOVEL, POTENT AND SELECTIVE INHIBITOR OF THE ACTIVITY OF INDUCIBLE NITRIC OXIDE SYNTHASE (GW274150) REDUCES THE ORGAN INJURY IN HEMORRHAGIC SHOCK

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An enhanced formation of nitric oxide (NO) by the inducible NO synthase (iNOS) may contribute to the pathophysiology of hemorrhagic shock. This study investigates the effect of a novel, potent and selective inhibitor of iNOS activity (GW274150) on the circulatory failure and the organ injury and dysfunction associated with hemorrhagic shock in the anesthetised rat. Hemorrhage (sufficient to lower mean arterial blood pressure to 45 mmHg for 90 min) and subsequent resuscitation with shed blood resulted (within 4 h after resuscitation) in a delayed fall in blood pressure, renal and liver injury and dysfunction as well as the pancreatic injury. Pre-treatment of rats with GW274150 (5 mg/kg at 30 min prior to the onset of hemorrhage) attenuated the renal dysfunction as well as the liver and pancreatic injury caused by hemorrhage and resuscitation. Interestingly, GW274150 did not reduce the delayed fall in blood pressure associated with hemorrhagic shock. We propose that an enhanced formation of NO from iNOS contributes to the organ injury and dysfunction in hemorrhagic shock, and that highly selective inhibitors of iNOS activity, such as GW274150, may represent a novel therapeutic approach for the therapy of hemorrhagic shock.

Keywords: oxygen radicals, superoxide dismutase, catalase, superoxide anions, multiple organ failure, GW274150
INTRODUCTION

Following the discovery that inhibitors of nitric oxide (NO) synthase (NOS) attenuate the hypotension (1) and the vascular hyporeactivity to pressor agents (2,3) caused by endotoxin in rats and dogs (4), numerous studies have documented beneficial hemodynamic effects of NOS inhibitors in animal models of shock (of different aetiology) (see 5) as well as in humans with septic shock (6). An enhanced formation of NO by iNOS also contributes to the delayed circulatory failure associated with hemorrhagic shock (7,8). As there is evidence that NOS inhibitors, which are more potent inhibitors of the activity of endothelial NOS (eNOS, NOS III), may cause excessive vasoconstriction and augmentation of the adhesion of platelets and neutrophils to the endothelium (see 5,9 for review), many investigators and pharmaceutical companies have strived to develop selective inhibitors of inducible NOS (iNOS or NOS II). Since 1992, several compounds have been reported to be relatively selective inhibitors of iNOS activity (i.e. agents which are more potent inhibitors of iNOS than eNOS activity). These include aminoguanidine (10), l-amino-2-hydroxyguanidine (II), aminoethyl-isothiourea (AE-ITU) (12,13) and N-iminoethyl-L-ornithine (14), to name but a few. It should, however, be stressed that these compounds are at best 30-fold more potent inhibitors of iNOS activity than eNOS activity and, hence, should be classified as partially selective inhibitors of iNOS activity (15). It should be stressed that many of the above agents are also fairly potent inhibitors of eNOS activity and compounds such as aminoguanidine and AE-ITU exert many non-specific effects, which make any data obtained with such compounds in animal models of disease difficult to interpret (15,16).

In 1995, we discovered that certain amidines are relatively selective inhibitors of iNOS activity (17). Garvey and colleagues have recently reported that one analogue of acetamidine, named 1400W is a highly selective inhibitor of human (greater than 5000x) and murine (1000x) iNOS activity (18). GW274150 is a novel inhibitor of iNOS activity that has been identified from a series of acetamidine amino acids, which like 1400W, have a very high degree of selectivity for iNOS versus both eNOS (>250-fold) and nNOS (>80-fold). GW274150 is a sulphur-substituted acetamine amino acid, which causes a NADPH-dependent inhibition of iNOS activity, which is slow in onset, long-lasting and reversible with L-arginine (15,19). The half-life of GW274150 in the rat is 5h and a single injection of this iNOS inhibitor (3 mg/kg) abolishes the increase in nitrite and nitrate caused by an injection of endotoxin, even if endotoxin is injected 14 hours after administration of GW274150 (15).

The effects of GW274150 (or of 1400W) in animal models of hemorrhagic shock have not yet been investigated. Here we investigate the effects of GW274150 on the circulatory failure and MODS (multiple organ injury/dysfunction, e.g. renal dysfunction, liver injury and dysfunction and the pancreatic injury) caused by severe hemorrhage resuscitation in the anesthetised rat.
MATERIALS AND METHODS

The experiments described in this article were performed in adherence to National Institutes of Health guidelines on the use of experimental animals. All experiments were performed in adherence with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London.

This study was carried out on 35 male Wistar rats (Tuck, Rayleigh, Essex, UK) weighing 250-320 g receiving a standard diet and water ad libitum. All animals were anesthetised with thiopentone sodium (120 mg/kg i.p.) and anesthesia was maintained by supplementary injections of thiopentone sodium as required. The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37 °C with a homeothermic blanket. The right femoral artery was catheterised and connected to a pressure transducer (Senso-Nor 840, Senso-Nor, Horten, Norway) for the measurement of phasic and mean arterial blood pressure (MAP) and heart rate (HR), which were displayed on a data acquisition system (MacLab 8e, ADI Instruments, Hastings, UK) installed on an Apple Macintosh computer. The right carotid artery was cannulated to allow blood withdrawal (see below). The jugular vein was cannulated for the administration of drugs. The bladder was also cannulated to facilitate urine flow and to prevent the possibility of the development of post-renal failure. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilise for 15 min. Then, blood was withdrawn from the catheter placed in the carotid artery in order to achieve a fall in MAP to 45 mmHg within 10 min. Thereafter, MAP was maintained at 45 mmHg for a total period of 90 min by either withdrawal (during the compensation period) or re-injection of blood. It should be noted that in none of these experiments, the amount of shed blood re-injected during the 90 min period of hemorrhage exceeded 10% of the total volume of the blood withdrawn. The amount of blood withdrawn in rats subjected to hemorrhage and treated with vehicle (control-group) was 8.0±0.3 ml; the amount of blood withdrawn in rats subjected to hemorrhage and treated with GW274150 was 8.4±0.3 ml; (P>0.05). At 90 min after initiation of hemorrhage, the shed blood was re-injected into the animal.

Evaluation of the effects of GW274150 on circulatory failure and MODS:

Experimental design

Four experimental groups were used for the experiments:
1) Hemorrhage-Saline: At 30 min prior to the onset of hemorrhage, control rats were treated with the vehicle (saline, 1 ml/kg i.v. bolus) for GW274150 (HS + Saline, n=12).
2) Hemorrhage-GW274150: At 30 min prior to the onset of hemorrhage, all animals received GW274150 (5 mg/kg i.v. bolus) (HS + GW274150, n=12).
3) Sham-Saline: Rats were subjected to the same surgical procedure without causing a hemorrhage and were treated with the vehicle (saline, 1 ml/kg i.v. bolus) for GW274150 (Sham + Saline, n=7).
4) Sham-GW274150: Rats were subjected to the same surgical procedure as in group 3, but received GW274150 (5 mg/kg i.v. bolus) (Sham + GW274150, n=4).

Quantification of organ function and injury

Four hours after resuscitation (end of the experiment), 1.5 ml of blood was collected into a serum gel S/I .3 tube (Sarstedt, Germany) from the catheter placed in the right carotid artery. The blood sample was centrifuged (1610 x g for 3 min at room temperature) to separate serum. All serum samples were analysed within 24 h by a contract laboratory for veterinary clinical chemistry (Vetlab Services, Sussex, UK)
The following marker enzymes were measured in the plasma as biochemical indicators of multiple organ injury/dysfunction: (1) Liver injury was assessed by measuring the rise in serum levels of alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury), bilirubin and \( \gamma \)-glutamyltransferase (\( \gamma \)-GT) (20). (2) Renal dysfunction was assessed by measuring the rises in serum levels of creatinine (an indicator of reduced glomerular filtration rate, and hence, renal failure) and urea (an indicator of impaired excretory function of the kidney and/or increased catabolism) (20). Pancreatic injury was evaluated by measuring the serum levels of lipase and amylase (20).

**Immunohistochemical localisation of nitrotyrosine**

Tyrosine nitration, an index of the nitrosylation of proteins by peroxynitrite and/or oxygen-derived free radicals, was determined by immunohistochemistry. Briefly, at the end of the experiment, the relevant organs (lung and liver) were fixed in 10% buffered formaldehyde and 8 mm sections were prepared from paraffin embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% \( \mathrm{H}_2\mathrm{O}_2 \) in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in phosphate buffered saline (PBS) for 20 min. Nonspecific adsorption was minimised by incubating the section in 2% normal goat serum in phosphate buffered saline for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin. The sections were then incubated overnight with 1:1000 dilution of primary anti-nitrotyrosine antibody or with control solutions. Controls included buffer alone or non specific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin-biotin peroxidase complex.

**Myeloperoxidase activity**

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation, was determined in the following manner: At the end of the experiment, sections of lung and liver were obtained and weighed. The tissue was homogenised in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 x g at 4° C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM \( \mathrm{H}_2\mathrm{O}_2 \). The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 mol of peroxide per min at 37° C and was expressed in milliunits per gram weight of wet tissue.

**Materials**

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK). Thiopentone sodium (Intraval Sodium®) was obtained from Rhone Merieux Ltd. (Harlow, Essex, UK). GW274150 was from Alexis Corporation (Nottingham, U.K.). All stock solutions were prepared in non-pyrogenic saline (0.9% NaCl; Baxter Healthcare Ltd., Thetford, Norfolk, UK).

**Statistical evaluation**

All data are presented as means ± s.e.mean of \( n \) observations, where \( n \) represents the number of animals or blood samples studied. For repeated measurements (hemodynamics) a 2-factorial analysis of variance (ANOVA) was performed. Data without repeated measurements (multiple
organ injury/failure) was analysed by 1-factorial ANOVA, followed by a Dunnett’s test. A $P$-value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of GW274150 on the delayed vascular decompensation (circulatory failure) caused hemorrhage

Baseline values (at 30 min prior to the onset of hemorrhage) of MAP in all groups of animals ranged from 117±5 to 124±6 mmHg, and were not significantly different between groups (Table 1). In sham-operated rats (no hemorrhage), administration of GW274150 did not affect MAP (Table 1). In rats subjected to hemorrhage, resuscitation with shed blood led to an immediate increase in blood pressure from ~ 45 mmHg to 91 ±5 mmHg. Thereafter, there was a progressive decline in MAP to approximately 60 mmHg at the end of the experiment (Table 1). Interestingly, GW274150 had no significant effect on the delayed fall in MAP associated with hemorrhage and resuscitation (Table 1).

Baseline values of heart rate (HR) in all groups of animals ranged from 354±10 to 404±17 beats per minute (bpm), and were not significantly different between groups (Table 2). In sham-operated animals, administration of GW274150 did not result in any significant alterations in heart rate (Table 2). Hemorrhagic shock did also not cause a significant alteration in heart rate (Table 2, $P$>0.05). Similarly, GW274150 had no significant effect on heart rate (Table 2).

Effects of GW274150 on the multiple organ dysfunction syndrome caused by hemorrhage in the rat

Effects on the renal injury/dysfunction: In sham-operated rats, administration of GW274150 did not result in any significant alterations in the

<table>
<thead>
<tr>
<th>Group</th>
<th>-2h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sham + Saline</td>
<td>124±4</td>
<td>100±3</td>
<td>98±2*</td>
<td>93±5*</td>
<td>93±5*</td>
</tr>
<tr>
<td>2. Sham + GW274150</td>
<td>117±5</td>
<td>119±3*</td>
<td>114±2*</td>
<td>113±3*</td>
<td>111±3*</td>
</tr>
<tr>
<td>3. HS + Saline</td>
<td>117±6</td>
<td>86±4</td>
<td>79±3</td>
<td>69±5</td>
<td>62±4</td>
</tr>
<tr>
<td>4. HS + GW274150</td>
<td>122±3</td>
<td>91±4</td>
<td>84±3</td>
<td>79±2</td>
<td>74±3</td>
</tr>
</tbody>
</table>

Group 1: Rats were subjected to the surgical procedure without causing a hemorrhage and treated with vehicle (saline, 1 ml/kg i.v. bolus; n=7); Group 2: Rats were subjected to the surgical procedure but treated with GW274150 (5 mg/kg i.v. bolus, 30 min prior to hemorrhage; n=4); Group 3: Rats were subjected to a hemorrhage for 1.5 h and resuscitation (4 h). Prior to the onset of hemorrhage, rats were treated with the vehicle (saline, 1 ml/kg i.v. bolus; n=12); Group 4: Rats were subjected to the same procedure as group 3, but treated with GW274150 (5 mg/kg i.v. bolus, 30 min prior to hemorrhage, n=12). *$P$<0.05 when compared to HS + Saline.
serum levels of creatinine (Fig. 1). When compared with sham-operated rats, hemorrhage/resuscitation resulted in significant rises in the serum levels of creatinine, demonstrating the development of renal dysfunction (Fig. 1). Treatment of rats subjected to hemorrhage and resuscitation with GW274150 resulted in a significant attenuation of the renal dysfunction caused by severe hemorrhage and resuscitation (Fig. 1).

**Table 2:** Heart rate (bpm) in all experimental groups studied before the hemorrhage (-2 h) and 1, 2, 3 and 4 h after resuscitation.

<table>
<thead>
<tr>
<th>Group</th>
<th>-2h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sham + Saline</td>
<td>404±17</td>
<td>383±18</td>
<td>374±21</td>
<td>384±19</td>
<td>373±23</td>
</tr>
<tr>
<td>2. Sham + GW274150</td>
<td>360±15</td>
<td>390±6</td>
<td>357±10*</td>
<td>372±4</td>
<td>356±8</td>
</tr>
<tr>
<td>3. HS + Saline</td>
<td>354±10</td>
<td>422±10</td>
<td>421±12</td>
<td>398±9</td>
<td>383±10</td>
</tr>
<tr>
<td>4. HS + GW274150</td>
<td>354±8</td>
<td>386±6*</td>
<td>382±8</td>
<td>378±10</td>
<td>377±7</td>
</tr>
</tbody>
</table>

Group 1: Rats were subjected to the surgical procedure without causing a hemorrhage and treated with vehicle (saline, 1 ml/kg i.v. bolus; n=7); Group 2: Rats were subjected to the surgical procedure but treated with GW274150 (5 mg/kg i.v. bolus, 30 min prior to hemorrhage; n=4); Group 3: Rats were subjected to a hemorrhage for 1.5 h and resuscitation (4 h). Prior to the onset of hemorrhage, rats were treated with the vehicle (saline, 1 ml/kg i.v. bolus; n=12); Group 4: Rats were subjected to the same procedure as group 3, but treated with GW274150 (5 mg/kg i.v. bolus, 30 min prior to hemorrhage, n=12). *P<0.05 when compared to HS + Saline.

**Figure 1:** Semm levels of creatinine in rats subjected to (i) the surgical procedure without causing a hemorrhage and treated with either vehicle (open column, Sham+Saline, saline, 1 ml/kg i.v. bolus; n=7) or GW274150 (striped column, 5 mg/kg i.v. at 30 min prior to the proposed hemorrhagic period, n=4) or (ii) to hemorrhage for 1.5 h and resuscitation with the shed blood, and were treated with either vehicle (black column, HS+Saline, saline, 1 ml/kg i.v. bolus; n=12) or GW274150 (grey column, 5 mg/kg i.v. bolus; n=12). Interventions were administered 30 min prior to hemorrhagic period.*P<0.05 when compared with HS+Saline.
Figure 2A: Serum levels of (A) ALT, (B) bilirubin and (C) \(\gamma\)-GT in rats subjected to (i) the surgical procedure without causing a hemorrhage and treated with either vehicle (open column, Sham+Saline, saline, 1 ml/kg i.v. bolus; n=7) or GW274150 (striped column, 5 mg/kg i.v. at 30 min prior to the proposed hemorrhagic period, n=4) or (ii) to hemorrhage for 1.5 h and resuscitation with the shed blood, and were treated with either vehicle (black column, HS+Saline, saline, 1 ml/kg i.v. bolus; n=12) or GW274150 (grey column, 5 mg/kg i.v.bolus; n=12). Interventions were administered 30 min prior to hemorrhagic period. *\(P<0.05\) when compared with HS+Saline.
Effects on the liver injury/dysfunction: In sham-operated rats, administration of GW274150 did not result in any significant alterations in the serum levels of ALT, bilirubin or γ-GT (Fig. 2). When compared with sham-operated rats, hemorrhage/resuscitation resulted in significant rises in the serum levels of ALT, bilirubin and γ-GT (Fig. 2), demonstrating the development of liver injury/dysfunction. Treatment of rats subjected to hemorrhage and resuscitation with GW274150 attenuated the liver injury and dysfunction caused by severe hemorrhage and resuscitation (Fig. 2).

Effects on the pancreatic injury: In sham-operated rats, administration of GW274150 did not result in any significant alterations in the serum levels of amylase (Fig. 3). When compared with sham-operated rats, hemorrhage/resuscitation resulted in significant rises in the serum levels of amylase (Fig. 3), demonstrating the development of pancreatic injury. Treatment of rats subjected to hemorrhage and resuscitation with GW274150 attenuated the rise in the serum levels of amylase caused by hemorrhage and resuscitation (Fig. 3).

Effects of GW274150 on the formation of nitrotyrosine in the lung and liver of rats subjected to hemorrhage and resuscitation.

When compared to organs obtained from sham-operated rats, which had not been subjected to hemorrhage and resuscitation, the lungs and the liver of rats subjected to hemorrhage and resuscitation showed substantial histological

![Figure 3: Serum levels of amylase in rats subjected to (i) the surgical procedure without causing a hemorrhage and treated with either vehicle (open column, Sham+Saline, saline, 1 ml/kg i.v. bolus; n=7) or GW274150 (striped column, 5 mg/kg i.v. at 30 min prior to the proposed hemorrhagic period, n=4 or (ii) to hemorrhage for 1.5 h and resuscitation with the shed blood, and were treated with either vehicle (black column, HS+Saline, saline, 1 ml/kg i.v. bolus; n=12) or GW274150 (grey column, 5 mg/kg i.v. bolus; n=12). Interventions were administered 30 min prior to hemorrhagic period.*P<0.05 when compared with HS+Saline.](image)
alterations consistent with shock-induced organ injury. Most notably, the lungs
(Fig. 4b) and liver (Fig. 5b) of rats subjected to hemorrhage and resuscitation
exhibited a marked staining for nitrotyrosine when compared to organs obtained
from sham-operated rats (Fig. 4a and Fig. 5a). In contrast, the degree of
nitrotyrosine staining in these organs (Fig. 4c and Fig. 5c) was markedly reduced
in rats pre-treated with GW274150 prior to the onset of hemorrhage. It should be
noted that the administration of GW274150 in sham-operated rats did neither
cause any histological alterations nor staining for nitrotyrosine (data not shown).

Effects of GW274150 on the polymorphonuclear leukocyte accumulation in the
lung and liver of rats subjected to hemorrhage and resuscitation.

In sham-operated rats, administration of GW274150 did not result in any
significant alterations in myeloperoxide activity (neutrophil infiltration) within the
lung (Fig. 6a) and the liver (Fig. 6b). When compared with sham-operated rats,
hemorrhage/resuscitation resulted in significant rises in myeloperoxidase activity
within the lung (Fig. 6a) and the liver (Fig. 6b), demonstrating an increase in
neutrophil accumulation within the aforementioned organs. Treatment of rats
subjected to hemorrhage and resuscitation with GW274150 attenuated the rise in
organ neutrophil infiltration caused by severe hemorrhage and resuscitation (Fig. 6).

DISCUSSION

We report here for the first time that that pre-treatment of rats with
GW274150, a novel and selective inhibitor of iNOS activity (15,19) attenuates (i)
the renal dysfunction, (ii) the liver injury, and (iii) the pancreatic injury caused by
hemorrhage and resuscitation. What then, is the mechanism by which GW274150
protect the kidney, liver and pancreas of the rat against injury and dysfunction?
GW274150 is a novel inhibitor of iNOS activity that has been identified from a
series of acetamidine amino acids, which like 1400W, have a very high degree of
selectivity for iNOS versus both eNOS (>250-fold) and nNOS (>80-fold). (15,19)
GW274150 is a sulphur-substituted acetamine amino acid, which causes a
NADPH-dependent inhibition of iNOS activity, which is slow in onset, long-
lasting and reversible with L-arginine (15,19). The half-life of GW274150 in the
rat is 5 h and a single injection of this iNOS inhibitor (3 mg/kg) abolishes the
increase in nitrite and nitrate caused by an injection of endotoxin, even if
endotoxin is injected 14 hours after administration of GW274150 (15). At doses
up to 100 mg/kg i.v., GW274150 does not affect the blood pressure in mice,
suggesting that even high doses of this compound do not inhibit eNOS activity in
rodents in vivo. Similarly, doses of 20 mg/kg of GW274150 do not inhibit nNOS
activity in the rat. There is limited evidence that doses of 1-5 mg/kg of
GW274150 reverse the iNOS-dependent delayed GI transit in a rat model of post-
operative ileus (15). We, therefore, propose that the beneficial effects of
Figure 4: The lungs of rats subjected to hemorrhage and resuscitation. Staining for nitrotyrosine.
(A) sham-operated rats
(B) after hemorrhage and resuscitation
(C) after pretreatment with GW 274150
Figure 5: The livers of rats subjected to hemorrhage and resuscitation. Staining for nitrotyrosine.
(A) sham-operated
(B) after hemorrhage and resuscitation
(C) after pretreatment with GW 274150
GW274150 (5 mg/kg) reported here are due to inhibition of iNOS activity in the kidney, liver and pancreas. This hypothesis is supported by the finding that the NO scavenger NOX also reduces the degree of liver injury and improves survival in a rodent model of severe hemorrhagic shock (21).

We also report in this study that GW274150 reduces the degree on neutrophil accumulation (measured as an increase in myeloperoxidase activity) in the lungs and liver of rats subjected to hemorrhagic shock. In addition, pre-treatment of rats with GW274150 also reduced the nitration of proteins (nitrotyrosine formation) caused by severe hemorrhage and resuscitation.

This formation of nitrotyrosine moieties is most likely due to the formation of peroxynitrite (22,23), but may also be generated when nitrite interacts with...
myeloperoxidase (24). We have previously reported that other interventions, which reduce the nitration of tyrosine residues in proteins also reduce the organ injury and dysfunction associated with hemorrhagic shock (25).

Using the iNOS inhibitor N^6-(iminoethyl)-L-lysine or iNOS knockout mice, Hierholzer and colleagues have recently reported that the activation of the transcriptional factors nuclear factor kappa B and signal transducer and activator of transcription 3 and increases in IL-6 and G-CSF messenger RNA levels in the lungs and livers measured 4 h after resuscitation from hemorrhagic shock were iNOS dependent (26). Furthermore, they documented that iNOS inhibition resulted in a marked reduction of lung and liver injury produced by hemorrhagic shock. These findings support the view that an enhanced formation of NO from iNOS is essential for the up-regulation of the inflammatory response in resuscitated hemorrhagic shock and participates in end organ damage under these conditions (26).

There is good evidence that prolonged periods of hemorrhage (e.g. > than 5 h) result in the induction of iNOS protein and activity in many tissues (7, 27) including the vasculature (7). The induction of iNOS in the vessel wall then contributes to the delayed vascular decompensation. In contrast, there is also evidence that an enhanced formation of NO from iNOS does not contribute to the vasodilatation in rodent (27) and porcine models of hemorrhagic shock, in which shorter periods of hemorrhage (e.g. Wigger’s type models) have been employed. In the model used in our study, 90 min of hemorrhage (to 45 mmHg) followed by 4 h of resuscitation lead to a moderate fall in blood pressure, which was not significantly attenuated by GW274150. This finding supports the view that the hypotension observed in models of hemorrhagic shock, which employ a relatively short period of hemorrhage, are not - or at least not exclusively -mediated by an enhanced formation of NO by iNOS.

In conclusion, this study demonstrates for the first time that GW274150, a novel and potent inhibitor of iNOS activity (15,19) attenuates the renal, liver, and pancreatic injury/dysfunction caused by severe hemorrhage and resuscitation in the anesthetised rat. These results support the view that the overproduction of NO from iNOS contributes to the organ injury associated with hemorrhagic shock. We propose that highly selective inhibitors of iNOS activity, such as GW274150, may be useful in the therapy of conditions associated with hemorrhagic shock.

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