Nitric oxide and prostaglandins potentiate the liver regeneration cascade

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The liver has the remarkable ability to regenerate following damage or surgical resection. Although this feature of the liver has been studied for over 100 years, the trigger of the liver regeneration cascade remains controversial. Recent experimental evidence supports the hypothesis that nitric oxide (NO) and prostaglandins (PGs), released secondary to an increase in the blood flow-to-liver mass ratio following two-thirds partial hepatectomy (PHx), work synergistically to trigger liver regeneration. To extend this research, the hypothesis that NO and PGs are potential therapeutic targets to potentiate the liver regeneration cascade is tested. The NO donor s-nitroso-n-acetylpenicillamine, the phosphodiesterase V antagonist zaprinast (ZAP) and PGI₂ each potentiated c-fos messenger RNA expression, an index of initiation of the liver regeneration cascade, following PHx. Also, the triple combination of s-nitroso-n-acetylpenicillamine, ZAP and PGI₂ potentiated c-fos messenger RNA expression. These results support the hypothesis that NO and PGs can potentiate initiation of the regeneration cascade. An additional index of liver weight restoration 48 h after PHx was also used to test the hypothesis, because this index encompasses the entire liver regeneration cascade. ZAP and 6-keto-PGF₁α, a stable metabolite of PGI₂, and the combination of ZAP and 6-keto-PGF₁α, each potentiated liver weight restoration 48 h after PHx. These results also provide support for the hypothesis that NO and PGs are possible therapeutic targets to potentiate liver regeneration following surgical resection.

Key Words: Liver regeneration; Nitric oxide; Prostaglandins; Two-thirds partial hepatectomy

Recent research has provided support for the hypothesis that an increase in the blood flow-to-liver mass ratio that ensues with partial hepatectomy (PHx) results in increased shear stress in the liver, resulting in release of nitric oxide (NO) which triggers the liver regeneration cascade (1-4). In addition, there is evidence that prostaglandins (PGs) play a role in liver regeneration (5-7), and have been suggested to trigger the liver regeneration cascade (8).

Interaction between NO and PGs has been shown to be an important part of the liver regeneration cascade. Both NO and PGs increase after two-thirds PHx (5,6,9,10), and inhibition of either NO or PG synthesis negatively affects liver regeneration (8,11,12). In situations where either NO synthase or cyclooxygenase is inhibited, an excess amount of the other may be able to compensate for the effects of the missing compound (Smith and Lautt, unpublished data).

Thus, it is hypothesized that NO and PGs interact to trigger the liver regeneration cascade and that provision of a combination of exogenous NO and PGs can potentiate liver regeneration. An index of initiation of the liver regeneration cascade, c-fos messenger (m) RNA expression after PHx, has been used to test this hypothesis. c-Fos mRNA expression has been previously shown to increase following PHx, peaking 15 min thereafter, and the expression of c-fos mRNA is dependent on the degree of PHx performed (13,14). In addition, c-fos mRNA expression increases in response to NO (15,16) and also to PGE₂ (17,18) and PGI₂ (19,20). NO signalling also involves stimulation of guanylate cyclase and...
upregulation of cyclic (c) GMP in the liver and other tissues (21). NO signalling via the cGMP pathway is regulated by breakdown of cGMP by phosphodiesterase (PDE) enzymes, specifically PDE type V (22). Inhibition of PDE V by the PDE V-specific antagonist, zaprinast (ZAP) results in an increase in cGMP signalling, and represents an additional potential compound for potentiation of the liver regeneration cascade. Thus, the NO donors, 3-morpholinosydnonimine (SIN-1) and SNAP, the PDE V antagonist ZAP, PGE2 and PGI2, and various combinations of these, were used to test the hypothesis that NO and PGs interact to potentiate the liver regeneration cascade.

Based on results obtained using the \( c-fos \) mRNA expression index, NO and PGs were administered to rats immediately following PHx, and 48 h later, liver weight restoration was measured. This is a critical test of the hypothesis that NO and PGs can potentiate the liver regeneration cascade, because liver weight restoration after PHx encompasses the entire liver regeneration cascade, while \( c-fos \) mRNA expression is only one component. The \( c-fos \) mRNA expression index is used as a screening tool to provide information regarding the stimulation of the liver regeneration cascade, and possible compounds that may be used in the potentiation thereof. Liver weight restoration 48 h after PHx was chosen as an index because it is known that liver regeneration is 50% complete within 48 h of PHx in the rat (23). Thus, administration of NO donors, PGs, ZAP; and combinations of these identified using the \( c-fos \) mRNA expression index, was used to test the hypothesis that NO and PGs can potentiate the liver regeneration cascade in vivo.

Results presented here indicated that NO and PGI2 can potentiate the liver regeneration cascade after PHx. These compounds represent possible therapeutic targets for potentiation of the liver regeneration cascade for patients undergoing hepatic resection. Further research is required to determine the optimal doses for administration following hepatic resection.

METHODS

Animals

For the acute experiments, male Sprague-Dawley rats, 250 g, were fed standard laboratory chow ad libitum until the day before the experiment, when they were made to fast for 8 h and then fed for 2 h before experimentation. For the chronic experiments, male Sprague-Dawley rats, 250 g, were fed standard laboratory chow ad libitum. Animals were treated according to the guidelines of the Canadian Council on Animal Care, and all protocols were approved by the Ethics Committee on Animal Care at the University of Manitoba, Winnipeg, Manitoba.

c-Fos mRNA expression

Surgical procedure (acute experiments): Briefly, rats were anesthetized using sodium pentobarbital. Tracheotomy was performed, and catheters were placed in the femoral artery, femoral vein and portal vein for infusion of drugs and anesthetic. Laparotomy was performed, and the animal was allowed to stabilize for 30 min. Drug or saline was then administered, and PHx or sham procedures were performed at the appropriate time. The left lateral and median lobes of the liver were removed (two-thirds PHx), or the liver lobes gently manipulated (sham procedure). The remnant liver was then removed after 15 min and immediately frozen on dry ice for RNA analysis. Experimental groups were as follows: sham, PHx, sham+SNAP, PHx+SNAP, sham+ZAP, PHx+ZAP, sham+PGE2, PHx+PGE2, sham+PGI2, PHx+PGI2, sham+ZAP (ZAP; 10 mg/kg), PHx+ZAP (10 mg/kg), sham+ZAP (30 mg/kg) and PHx+ZAP (30 mg/kg). SNAP (5 mg/kg; 0.2 mL bolus, intra-portal vein administration [IPV] over 2 min) (Sigma-Aldrich Canada Ltd), SIN-1 (5 mg/kg; 0.2 mL bolus IPV over 2 min) (Alexis Corporation, USA), PGE2 (10 µg/kg; 0.1 mL bolus over 1 min) (Sigma-Aldrich Canada Ltd), PGI2 (10 µg/kg; 0.1 mL bolus IPV over 1 min) (Sigma-Aldrich Canada Ltd) or saline (0.2 mL bolus over 2 min) were administered and PHx performed immediately thereafter. ZAP (10 mg/kg or 30 mg/kg; 0.05 mL bolus IPV over 0.5 min) was administered and PHx performed 5 min thereafter.

Based on the results from the initial potentiation experiments (Smith and Lautt, unpublished data), drug combinations included ZAP and/or SNAP and/or PGI2. Concentration, volume and route of administration for the combination drugs were the same as for the individual ones. For administration of the double drug combinations, ZAP was administered first and 3 min to 4 min thereafter, either SNAP or PGI2 was administered. In the other combination group, SNAP was administered followed immediately by PGI2. In the triple combination group, ZAP was administered followed by SNAP and then PGI2. PHx was then immediately performed, and 15 min later, the remaining liver was removed and frozen on dry ice for RNA analysis.

RNA isolation and northern blot analysis: RNA analysis has been previously described (4). Briefly, total RNA was extracted using a 3M/6M lithium chloride/urea solution, centrifuged at 25,000 rpm for 20 min at 4°C, and total RNA was extracted using phenol/chloroform. The concentration of the RNA was then determined and 20 µg of total RNA was separated by gel electrophoresis under denaturing conditions, transferred to nylon membrane and fixed by ultraviolet crosslinking. The membranes were prehybridized at 42°C for 3 h in prehybridization buffer, and \( c-fos \) mRNA was detected using a 1.8 kb complementary DNA probe labelled by the random prime method, with \( dCTP \). The membranes were hybridized overnight at 42°C, washed at 65°C and exposed to film, and the density of the \( c-fos \) mRNA band was determined by densitometry. \( c-fos \) mRNA expression was reported relative to 18S ribosomal RNA. The structure of \( c-fos \) complementary DNA used was as described by Curran et al (24). Results were analyzed using ANOVA followed by Tukey’s post hoc test, with \( P<0.05 \) deemed significant.

Liver weight restoration

Surgical procedure (chronic experiments): Male Sprague-Dawley rats were anesthetized using sodium pentobarbital (0.1 mg/100 g of 65 mg/mL solution, intraperitoneal [IP]) and the abdomen was shaved and cleaned with 70% alcohol and betadine. Laparotomy was performed and drug or saline was administered IP. The left lateral and median lobes were removed (two-thirds PHx), or the liver lobes gently manipulated (sham procedure). A microcatheter catheter (MRE-040, Braintree Scientific Inc, USA) filled with saline was placed under the skin, with one end inside the abdominal cavity and the other exiting between the shoulder blades on the back of the rat. The abdomen was sutured shut (muscle then skin layers), using Dexon II, 3-0 (Northland Healthcare Products Ltd, Canada) sutures and ensuring that the catheter was secure. The opening between the shoulder blades was sutured shut, and the catheter secured with sutures. The catheter was capped to allow further IP doses of drug or saline. Ketoprophen (an analgesic, 2.5 mg/kg) was administered subcutaneously. The rats were allowed to recover in individual cages placed on heating pads.
The animals were monitored until they were awake and able to move around the cage. They had access to food and water at all times, with some food pellets placed on the bottom of the cage for easier access. Drug or saline administration (0.5 mL bolus IP) took place at 5 h intervals, three times per day for a total of six doses during the next 48 h. Forty-eight hours after PHx, the rats were sacrificed by pentobarbital anesthesia overdose and the livers removed, slashed and blotted, and weighed. The per cent liver weight restoration after PHx was calculated as follows:

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\text{Percentage of liver weight restoration} = \frac{(48 \text{ h liver weight } - \text{ remnant liver weight}) \times 100}{\text{resected liver weight}}
\]

Where the 48 h liver weight is the weight of the liver 48 h after PHx; the remnant liver weight is the theoretical remaining liver weight following resection, as calculated above; and resected liver weight is the weight of the left lateral and median lobes removed during PHx. The standard total liver weight was calculated from a regression analysis of the total liver weight versus body weight in control rats \((n=40)\).

Results were analyzed using ANOVA followed by Tukey’s post hoc test, with \(P<0.05\) deemed significant.

**Drug preparation for liver weight restoration experiments:** For the experiments involving c-fos mRNA expression, SNAP (5 mg/kg) was dissolved in saline and a 0.2 mL bolus was infused IPV over 2 min. PGI2 (10 µg/kg) was dissolved in 95% ethanol in a stock solution, and diluted with the appropriate amount of saline to achieve the desired concentration. A 0.1 mL bolus of either SNAP was infused IPV over 1 min. ZAP was dissolved in 0.15 M sodium hydroxide, and diluted to the appropriate concentration using saline. PHx or sham procedures were performed immediately following SNAP, ZAP or PGI2 administration.

For the liver weight restoration experiments, drugs were prepared as described for the c-fos experiments. 6-keto-PGF1α, a stable metabolite of PGI2, was used in the 48 h liver weight restoration experiments rather than PGI2, due to the short half-life of PGI2. The dose of 6-keto-PGF1α was selected based on potentiation of c-fos mRNA expression using PGI2, and the finding that 6-keto-PGF1α was at least 50% less effective at vasorelaxation of smooth muscle compared with PGI2 (25). Thus, a dose of 20 µg/kg of 6-keto-PGF1α double that of PGI2 used in the c-fos experiments, was administered. The 6-keto-PGF1α was dissolved in 95% ethanol, and diluted to the appropriate concentration with saline. Doses were based on c-fos mRNA expression experiments, and a volume of 0.5 mL of drug or drug combination was administered each time. The initial dose was delivered IP at the time of surgery, and supplemental doses were administered by IP injection via the implanted catheter. For each drug or combination, the doses were given at 5 h intervals, three times per day for two days.

**RESULTS**

**c-Fos mRNA expression**

To test the hypothesis that NO and PGs are possible therapeutic targets to potentiate the liver regeneration cascade, the NO donors, SIN-1 and SNAP, or the PGs, PGE2 and PGI2, were administered immediately before PHx, and c-fos mRNA expression was evaluated. The increase in c-fos mRNA expression after PHx (1.36±0.18, \(n=7\), \(P<0.01\) versus sham [0.49±0.26]) was potentiated by PGI2 (1.45±0.21, \(n=7\), \(P=0.01\) versus sham [0.49±0.26]), and the PDE antagonist, ZAP (0.79±0.06, \(n=6\), \(P<0.01\) versus sham [0.79±0.06]). SNAP (2.12±0.21, \(n=7\), \(P<0.05\) versus PHx) (Figure 1). There was no potentiation of c-fos mRNA expression by PGE2 (1.45±0.21, \(n=7\), \(P=0.01\) versus sham [0.49±0.26]) or by the NO donor, SIN-1 (1.12±0.05, \(n=6\), \(P=0.001\) versus PHx). In the sham operated animals, neither PGE2 (0.27±0.02, \(n=4\), PGI2 (0.79±0.06, \(n=6\), SIN-1 (0.85±0.06, \(n=8\)) or SNAP (0.41±0.09, \(n=5\)) caused a significant increase in c-fos mRNA expression. Thus, PGI2 and SNAP were able to potentiate c-fos mRNA expression, an index of initiation of the liver regeneration cascade, after PHx.

**NO and PGs potentiate the liver regeneration cascade**

The PDE antagonist, ZAP, which prevents the breakdown of cGMP, was also used as an alternate means of testing the hypothesis that NO is a possible therapeutic target to potentiate the liver regeneration cascade. Compared with sham, PHx caused an increase in c-fos mRNA expression (1.60±0.09, \(n=7\), \(P<0.01\) versus sham [0.60±0.09]), and the PDE antagonist, ZAP (10 mg/kg), potentiated the increase in c-fos mRNA expression after PHx (2.07±0.14, \(n=6\), \(P<0.001\) versus PHx) (Figure 2). ZAP at a dose of 30 mg/kg tended to potentiate the increase in c-fos mRNA expression after PHx, but the increase was not significant (2.00±0.28, \(n=5\), \(P=NS\) from PHx). The 0.15 M sodium hydroxide and saline vehicle in which ZAP was dissolved had no effect on the increase in c-fos mRNA expression after PHx (1.21±0.17, \(n=6\), \(P=NS\) from PHx). In the normal, non-PHx sham operated animals, vehicle (0.44±0.06, \(n=6\), \(P=NS\) from sham), ZAP (10 mg/kg) (0.55±0.12, \(n=5\), \(P=NS\) from sham) or ZAP (30 mg/kg) (0.60±0.09, \(n=6\), \(P=NS\) from sham) did not cause an increase in c-fos mRNA expression. Thus, ZAP is also able to potentiate c-fos mRNA expression after PHx.

Based on the above results, it was hypothesized that combinations of SNAP, PGI2, and/or ZAP would further potentiate c-fos mRNA expression after PHx. The increase in c-fos mRNA expression after PHx (1.69±0.10, \(n=5\), \(P<0.01\) versus sham [0.41±0.06, \(n=5\)]) was not further potentiated by the combinations of SNAP+ZAP (5 mg/kg, 10 mg/kg) (1.65±0.20, 2.00±0.14).
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Figure 2) Potentiation of c-fos messenger (m) RNA expression after partial hepatectomy (PHx) by the phosphodiesterase antagonist zaprinast (ZAP). ZAP potentiates the increase in c-fos mRNA expression after PHx, providing further support for the hypothesis that nitric oxide is a possible therapeutic target to potentiate the liver regeneration cascade. Also, this suggests that ZAP may potentiate liver weight restoration after PHx. (PHx P<0.01 versus sham; PHx+vehicle P<0.05 versus sham; PHx+ZAP(10) and PHx+ZAP(30) P<0.001 versus sham; PHx+ZAP(10) P<0.05 versus PHx only) (data shown as mean ± SEM). rRNA Ribosomal RNA

Figure 3) Potentiation of c-fos messenger (m) RNA expression by the combination of s-nitroso-n-acetylpenicillamine (SNAP), zaprinast (ZAP) and prostaglandin (PG)I2. c-Fos mRNA expression is increased after partial hepatectomy (PHx), and this increase is further potentiated by the combination of the nitric oxide donor, SNAP, the phosphodiesterase antagonist, ZAP, and PG12. These results provide evidence that the combination of these three drugs potentiate c-fos mRNA expression, an index of initiation of the liver regeneration cascade. In addition, these results also suggest that this combination of drugs may potentiate liver regeneration in vivo (data shown as mean ± SEM). rRNA Ribosomal RNA

n=7, P=NS from PHX), SNAP+PGI2 (5 mg/kg, 10 µg/kg) (1.93±0.27, n=8, P=NS from PHX), or ZAP+PGI2 (10 mg/kg, 10 µg/kg) (2.09±0.40, n=6, P=NS from PHX) (Figure 3). However, this index was potentiated by the combination of SNAP+ZAP+PGI2 (3.35±0.17, n=6, P<0.001 versus PHX). In the normal liver, combinations of SNAP+ZAP (0.85±0.20, n=7, P=NS from sham), SNAP+PGI2 (0.84±0.08, n=8, P=NS from sham), ZAP+PGI2 (0.85±0.12, n=7, P=NS from sham) or SNAP+ZAP+PGI2 (0.66±0.10, n=5, P=NS from sham) had no effect on c-fos mRNA expression. Thus, the combination of all three drugs, SNAP, ZAP and PGI2, potentiated c-fos mRNA expression after PHx.

Liver weight restoration
To more fully test the hypothesis that NO and PGI2 are possible therapeutic targets that could potentiate the liver regeneration cascade, a more physiologically relevant index was used. This index is liver weight restoration after PHx. The drugs SNAP, ZAP and PGI2, and the triple combination of SNAP, ZAP and PGI2, were administered to rats immediately before PHx, and continued during recovery after surgery for 48 h. Forty-eight hours after PHx, liver weight was restored by 47.18±1.75% (n=4) (Figure 4). Administration of the PDE antagonist ZAP (10 mg/kg, IP), as described above, potentiated liver weight restoration to 60.85±2.46% (n=8, P<0.05) compared with PHx alone, an increase of 28.97%. Similarly, 6-keto-PGF1α (20 µg/kg, IP), a stable metabolite of PGI2, also potentiated liver weight restoration to 61.67±3.19% (n=6, P<0.05), an increase of 30.71%, after 48 h versus PHx only. There was also a trend toward an increase in liver weight restoration 48 h after PHX following administration of the NO donor, SNAP (5 mg/kg, IP, n=7) (58.59±2.73%), and following administration of a combination of ZAP (10 mg/kg, IP), 6-keto-PGF1α (20 µg/kg, IP) and SNAP (5 mg/kg, IP) (59.09±3.11%, n=7). Even though these treatments resulted in increases in liver weight restoration of 24.18% and 25.24%, respectively, they were not significantly different from PHx only.

Because both ZAP and 6-keto-PGF1α alone caused an increase in liver weight restoration after PHx, a preliminary experiment was performed in which these two compounds were administered in combination. The combination of ZAP (10 mg/kg IP) and 6-keto-PGF1α (20 µg/kg, IP) for 48 h after PHX potentiated liver weight restoration (73.10±4.50%, n=3, P<0.001 versus PHx only), resulting in a 54.94% increase compared with PHx. However, although the trend is toward further potentiation of liver weight restoration compared with either ZAP or 6-keto-PGF1α only, the increase was not significant. NO and PGI2, and the administration of ZAP+PGI2 in combination, potentiated liver weight restoration after PHx.

DISCUSSION
NO and PGI2 potentiated the liver regeneration cascade after PHx. The NO donor SNAP, the PDE V antagonist ZAP, and PGI2 potentiated c-fos mRNA expression, an index of initiation of the liver regeneration cascade, after PHx, as did the combination of SNAP, ZAP and PGI2. These results provide
support for the hypothesis that NO and PGs are possible therapeutic targets to potentiate the liver regeneration cascade.

In addition, it is interesting that SNAP, ZAP and PGI$_2$ administered individually, or as a triple combination, potentiated c-fos mRNA expression after PHx, while combinations of SNAP+ZAP, SNAP+PGI$_2$, and ZAP+PGI$_2$ did not. Although different NO donors have different characteristics (26), including mechanisms of NO release and degree of production of free radicals (SIN-1 generates superoxide), we currently have no explanation for why SNAP but not SIN-1 potentiated c-fos suppression. The implications of these results require further investigation.

c-Fos mRNA expression has proven to be a good index of initiation of the liver regeneration cascade, and has provided excellent information regarding possible therapeutic targets to potentiate liver regeneration. However, liver weight restoration after PHx was chosen as a more physiologically relevant index than c-fos mRNA expression, because liver weight restoration encompasses the entire liver regeneration cascade. The end point evaluated using this index provides information on whether NO or PGs can actually potentiate the liver regeneration cascade in vivo, rather than just one pathway involved in liver regrowth.

Liver weight restoration was evaluated 48 h after PHx, when 50% of liver weight is normally restored in the rat (23). Indeed, 47.18% of the liver weight was restored in rats that underwent PHx only. ZAP and 6-keto-PGF$_1$$\alpha$, a stable metabolite of PGI$_2$, caused an increase in liver weight restoration 48 h after PHx, while SNAP and the combination of SNAP, ZAP and 6-keto-PGF$_1$$\alpha$ also tended to cause an increase in liver weight restoration. In addition, because ZAP and 6-keto-PGF$_1$$\alpha$ caused a significant increase in liver weight restoration 48 h after PHx, the effect of the combination of ZAP and 6-keto-PGF$_1$$\alpha$ on liver weight restoration was also determined. The combination of ZAP and 6-keto-PGF$_1$$\alpha$ caused a significant increase in liver weight restoration 48 h after PHx. However, while this increase tended to be greater than either ZAP or 6-keto-PGF$_1$$\alpha$ alone, the difference was not significant. Thus, these results demonstrate that ZAP, PGI$_2$, or a combination of ZAP+PGI$_2$ can potentiate liver weight restoration after PHx.

It is also interesting that, while the triple combination of SNAP, ZAP and PGI$_2$ potentiated c-fos mRNA expression after PHx, the same combination did not cause a significant increase in liver weight restoration. The difference in results could be due to variation between the c-fos mRNA expression and liver weight restoration indices. A combination of these three compounds may affect an index involving one triggering pathway in a different manner than the effect on the entire liver regeneration cascade. While the c-fos expression provides a useful screening tool, the final validating test rests with determinations using the true end point, liver weight restoration.

Interaction between NO and PGs in disease conditions

Administration of ZAP, PGI$_2$, or a combination of ZAP+PGI$_2$ could be beneficial to patients undergoing hepatic resection. ZAP, which increases NO action through inhibition of PDE V, plays a role in dilation of capacitance and resistance vessels in the heart (27), and has been shown to be effective in the treatment of conditions such as erectile dysfunction (28). PGI$_2$ is also effective in treating chronic pulmonary hypertension by reducing pulmonary arterial pressure (29), and is being tested for use in patients with peripheral vascular conditions (30). In addition, it has been shown that the combination of NO and PGI$_2$ can be used to treat some conditions. In patients with pulmonary hypertension, Hill and Pearl (31) found that inhaled NO and PGI$_2$, administered together, had an additive effect on decreasing pulmonary arterial pressure. Also, Ziesche et al (32), demonstrated that in patients with pulmonary hypertension, who have become refractory to NO treatment, intravenous epoprostenol (PGI$_2$ analogue) administration for 13 to 29 months eliminated the refractoriness to NO treatment. The authors suggest that treatment with NO and epoprostenol may further improve O$_2$ saturation, cardiac index, mean pulmonary artery pressure and total pulmonary vascular resistance. PGI$_2$ has been administered to patients undergoing orthotopic liver transplant, and it was found that in patients who received PGI$_2$, liver oxygenation was increased and liver damage decreased (33). In addition, PGI$_2$ may play a role in preservation of liver grafts for transplantation, after removal from the donor (34). Thus, administration of NO or a PDE V antagonist (such as ZAP), or PGI$_2$ may play an important role in treating symptoms of some disease conditions. In addition, administration of NO and/or ZAP and/or PGI$_2$ in combination may further potentiate the effects of these compounds.

**Potentiation of liver regeneration by NO and PGI$_2$**

There has been no suggestion in the literature of administering a combination of ZAP and PGI$_2$ to patients undergoing liver resection to potentiate liver regeneration. The experiments described above are the first results suggesting that therapy with ZAP, which increases the effects of the NO signalling cascade, PGI$_2$, or a combination of both compounds, will potentiate liver regeneration after surgical resection, and they provide a potential option for patients whose liver must regenerate. Further research is required to determine the optimal dose and dosing schedule to potentiate liver regeneration, but results obtained in this study provide an excellent starting point and the potential for a new therapy to improve liver regeneration.

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**REFERENCES**


\[\text{NO and PGs potentiate the liver regeneration cascade}\]