# *N*-Acetyl-Cysteine: Protective Agent or Promoter of Gastric Damage? (43255)

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*Abstract.* **N-acetyl-cysteine (NAC), when given orally, has been shown to prevent gastric damage induced by ethanol, but when administered intraperitoneally, it appears to potentiate such damage. In an effort to resolve these seemingly discordant findings, fasted rats (six per group) received 1 ml of saline or 20% NAC orally or intraperitoneally (ip). Two hours or 15 min later, they received 1 ml of 100% ethanol orally. At sacrifice 5 min later, rats receiving oral pretreatment with 20% NAC at both 15 and 120 min prior to ethanol exposure demonstrated a significant reduction in the magnitude of gastric injury when compared with saline controls. In contrast, actual promotion of ethanol damage was noted when NAC was given intraperitoneally, but was more pronounced when NAC was administered 15 min prior to exposing the mucosa to 100% ethanol. In all animals receiving intraperitoneal NAC, large amounts of peritoneal fluid (4–6 ml/rat) were recovered at the time of sacrifice, most of which occurred within 15 min of NAC administration; these more pronounced peritoneal effects at 15 min after NAC correlated with the more severe injury from ethanol at this time period compared to 120 min after intraperitoneal NAC. Saline controls had no peritoneal fluid. Mucosal glutathione (GSH) levels generally paralleled these results in that a significant decrease in tissue GSH occurred at 15 min following intraperitoneal NAC when compared with controls; at 120 min after intraperitoneal NAC, GSH levels were similar to control values. Additional experiments demonstrated that within 15 min following NAC administration, systemic blood pressure dropped by approximately 20% and basically remained unchanged over the next 2 hr; intraperitoneal saline had no sustained adverse effects on blood pressure. It was concluded that the inability of NAC to prevent ethanol injury when given intraperitoneally in contrast to orally is related to the drop in blood pressure secondary to NAC's peritoneal irritant effects, which presumably altered gastric mucosal blood flow, thus obivating its ability to prevent ethanol damage under these conditions. Furthermore, the decreased levels in mucosal GSH following the hypotension induced by intraperitoneal NAC suggest that perturbations in GSH metabolism may also have contributed to the decreased resistance to ethanol injury.** [P.S.E.B.M. 1991, Vol 197]

A number of sulfhydryl compounds have been shown to protect the gastric mucosa from injury induced by various noxious substances, including alcohol (1–3). One such agent is *N*-acetyl-cysteine (NAC), a source of cysteine, a key substrate for glutathione (GSH) synthesis (4). When administered orally, this compound has been noted to possess potent protective properties against ethanol-induced damage in the rat stomach comparable to those of prostaglandin (3). Recently, it was noted that NAC, when administered intraperitoneally in the rat, actually exacerbated gastric injury induced by ethanol (5). The mechanism underlying this adverse effect was not apparent in this study, but appeared to be independent of perturbations in GSH metabolism. In an effort to resolve these seemingly discordant findings, namely, the protective effect of NAC when administered orally and the promotion of damage when given intraperitoneally, the present study was undertaken. An abstract of our findings has been published (6).

## Materials and Methods

All experiments were performed on Sprague-Dawley rats of either sex weighing approximately 200 g and housed in wide mesh-bottom cages to prevent ingestion

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of hair and feces. Following a 24-hr fast in which water but not food was allowed *ad libitum*, animals were randomly assigned to one of a number of experimental groups as shown in Table I. Regardless of the pretreatment protocol, all animals received 1 ml of 100% ethanol orally. At sacrifice 5 min later, stomachs were quickly removed and the magnitude of macroscopic damage involving the glandular gastric epithelium was quantitated using computerized planimetry; the magnitude of injury was reported as the percentage of glandular mucosa damaged.

The concentration of NAC chosen for these experiments was 20%, which was prepared by dissolving 1200 mg/kg of NAC in saline; this concentration was shown previously in our studies to effectively prevent lesion formation induced by absolute alcohol (3). It is also the concentration of NAC used by Lu *et al.* (5) in which intraperitoneal administration of this agent was shown to promote gastric damage by ethanol. The 20% NAC solution was buffered to pH 7.0 by the addition of NaOH; titration to neutrality was carried out to maintain NAC in solution. For control experiments, saline was also titrated to pH 7.0. The osmolarity of the resultant NAC solution was approximately 2 *M*. The stability of this solution was verified by adding 0.3 ml of 1.0 *N* HCl to 1 ml of neutralized NAC, which reduced the pH to only 3.75. This amount of HCl is exceedingly high compared with the quantity of HCl that is normally secreted by the rat stomach (no more than 150 mEq/liter) and that would be resident in the gastric lumen when NAC was given orally. Thus, the NAC would never encounter enough acidity to hydrolyze the amide link between cysteine and acetic acid.

In a separate series of experiments, rats receiving intraperitoneal saline or NAC were subjected to a similar protocol (see Table I), except that they were sacrificed at the time they would have received ethanol. At the time of sacrifice, samples of glandular gastric mucosa were obtained for measurement of GSH levels. The GSH assay employed followed the method of Anderson (7). Levels of GSH were reported as micromoles per gram of tissue wet weight.

Because of the results obtained from the aforementioned experiments, in which NAC was noted to have irritant effects on the peritoneal cavity when administered intraperitoneally (refer to Results), an additional series of studies was performed to see what effect this NAC-induced peritoneal irritation had on systemic blood pressure (BP). For these experiments, rats were anesthetized with methoxyflurane, and the effects of intraperitoneal saline or NAC on BP were measured via a previously placed femoral artery catheter. After a 30-min equilibration period to allow the BP to stabilize, experiments were commenced, and the effect of intraperitoneal saline or NAC on BP was evaluated for a total of 120 min.

All results were reported as mean ± SE. Differences among experimental groups were statistically analyzed using the Mann-Whitney *U* test for nonparametric data. A $P$ value of 0.05 or less was considered statistically significant.

## Results

In animals receiving oral saline of intraperitoneal saline prior to exposing the gastric mucosa to 100% ethanol, regardless of the time interval between these two treatments, damage was consistently observed. This damage was limited to the glandular portion of the stomach, sparing the forestomach. The majority of this injury involved the acid-secreting portion, with only occasional involvement of the antrum. Lesions were typically longitudinal, paralleling the long axis of the stomach, and measured 4–10 mm in length and 1–3 mm in width. Although the amount of glandular stomach injured varied considerably among the groups receiving saline pretreatment, no statistical differences were evident. These findings are summarized in Figure 1.

Oral pretreatment with 20% NAC 15 min prior to exposing the gastric mucosa to 100% ethanol significantly reduced the magnitude of gastric injury as compared with pretreatment of animals receiving only saline. Similar observations were noted when NAC was given orally 120 min prior to ethanol exposure (see Fig. 1). In contrast to the protective capabilities of 20% NAC administered orally, actual promotion of ethanol-induced damage was noted when NAC was given intraperitoneally when compared with animals receiving saline pretreatment. These promoting effects of NAC on gastric damage were especially prominent when NAC was given 15 min prior to exposing the gastric mucosa to 100% ethanol (see Fig. 1).

**Table I.** Experimental Protocol

| | Pretreatment | Damaging agent | Sacrifice |
|---|---|---|---|
| Mucosal injury studies | Oral or intraperitoneal saline or 20% NAC | Oral 100% ethanol 15 or 120 min after pretreatment regimen | 5 min after 100% ethanol |
| Tissue glutathione studies | Intraperitoneal saline or 20% NAC | | 15 or 120 min after pretreatment regimen |

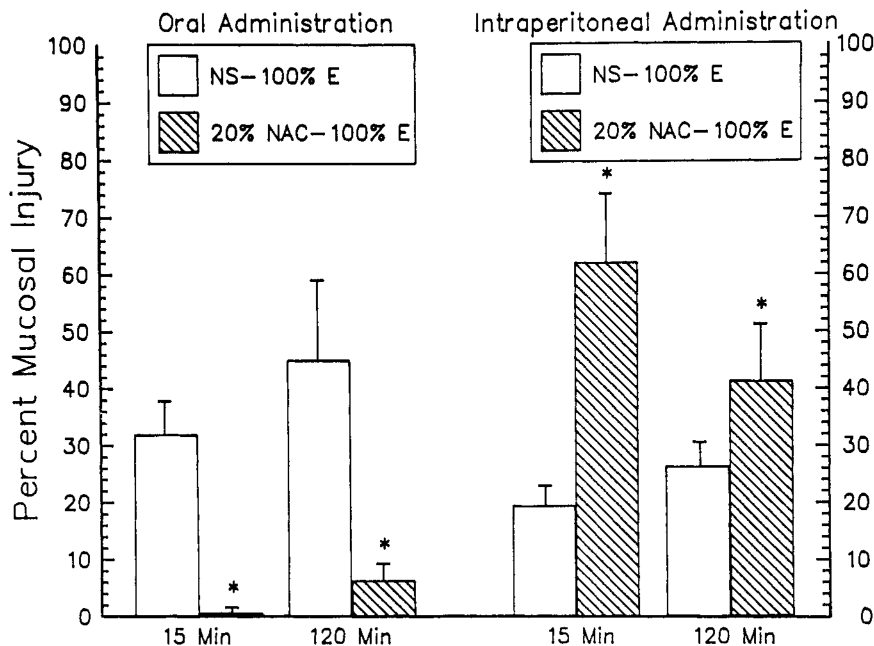


**Figure 1.** Effects of oral or intraperitoneal saline (NS) or 20% NAC on gastric damage induced by 100% ethanol (E). Fifteen or 120 min refers to time of 100% E administration after NS or NAC. $n = 6$ per group. $^{*}P < 0.01$ versus corresponding control group.

Coincident with the promotion of gastric damage by NAC given intraperitoneally was the clear evidence of obvious peritoneal irritation by this substance at the time of animal sacrifice. This was characterized by large amounts of peritoneal fluid recovered in animals receiving intraperitoneal NAC in contrast to those receiving saline, in which no peritoneal fluid was recovered. The majority of this peritoneal irritation was observed in animals receiving ethanol 15 min after NAC administration. These findings are summarized in Table II.

Mucosal GSH levels averaged about 2.0 $\mu$mol/g wet weight in animals receiving intraperitoneal saline and sacrificed 15 to 120 min later (see Fig. 2). Animals given NAC intraperitoneally 120 min prior to sacrifice also demonstrated GSH levels comparable to those receiving intraperitoneal saline in the same time period. In contrast, GSH levels were significantly lower than control counterparts in animals receiving intraperitoneal NAC prior to sacrifice 15 min later (see Fig. 2).

In experiments in which the effects of NAC administered intraperitoneally on systemic BP were performed, BP was noted to drop by almost 20% of basal within 15 min of giving NAC. This diminution in BP

**Table II.** Residual Peritoneal Volume after Intraperitoneal Saline or 20% NAC[a]

| | Saline | 20% NAC |
|---|---|---|
| After 15 min | 0 | $4.1 \pm 0.3^{b}$ |
| After 120 min | 0 | $5.8 \pm 0.4^{b}$ |

[a] Residual peritoneal volume was calculated in milliliters.
[b] $p < 0.001$ versus saline.

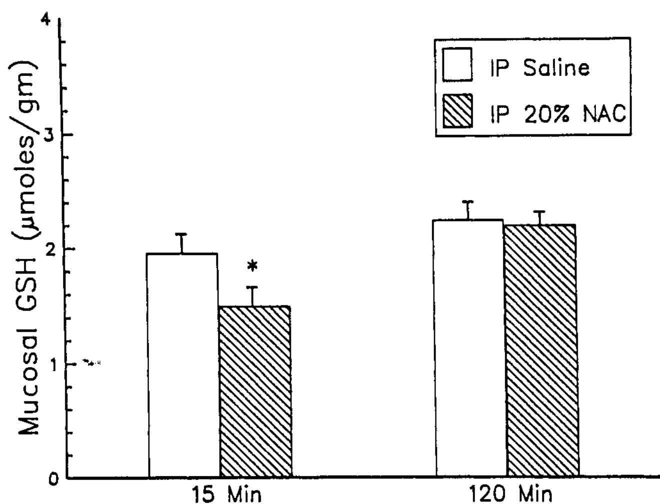


**Figure 2.** Effects of intraperitoneal saline or 20% NAC on gastric mucosal GSH levels. Fifteen or 120 min refers to time of sacrifice after intraperitoneal administration of saline or 20% NAC. $n = 6$ per group. $^{*}P < 0.01$ versus corresponding control group.

remained basically unchanged over the subsequent 2 hr of observation. In animals receiving intraperitoneal saline, a transient drop in BP was noted within the first 15 min of its administration, but the magnitude of this drop was significantly less than that observed with NAC. This transient BP drop quickly returned to baseline levels and remained there for the duration of the study. These BP findings are tabulated in Figure 3.

## Discussion

Several studies have clearly shown that NAC, when administered orally, can effectively prevent or markedly

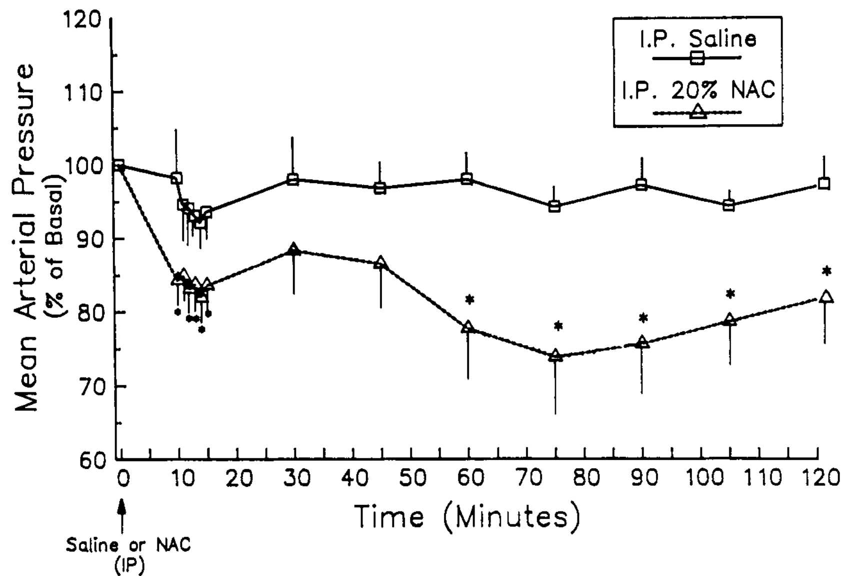


**Figure 3.** Effects of intraperitoneal saline or 20% NAC on mean arterial blood pressure. $n = 6$ per group. $^*P < 0.05$ versus corresponding value with intraperitoneal saline.

attenuate the magnitude of gastric injury induced by various noxious substances when applied to the gastric mucosa (3, 8, 9). Although the mechanism underlying this protective action has remained undefined, a likely possibility is that it is mediated through enhancement of GSH synthesis by NAC's provision of cysteine, as this latter substance forms a major component of the GSH molecule (10, 11). In fact, studies involving the protective action of NAC against liver injury convincingly demonstrated that this is probably the mechanism involved (4). Such a mechanism would also make sense in the stomach since evidence exists linking GSH with gastric defense. This evidence is based on the observations that perturbations in GSH metabolism appear to correlate with enhancement of gastric injury, and stimulation of GSH synthesis obviates such injury (1, 2, 12–15).

Despite the clear protective action of NAC when administered orally (3, 8, 9), a recent study by Lu and associates (5) noted that this capability was missing when NAC was administered intraperitoneally. The experimental model used in that study was a well-established rat alcohol-injury preparation in which the damaging agent was 100% ethanol. The reason intraperitoneal pretreatment with NAC did not prevent injury by absolute alcohol was not apparent in that study. One possible explanation for such a result was that NAC was not effectively absorbed via the intraperitoneal route, as many agents are, and thus the amount of this agent reaching the stomach was not sufficient to provide a protective effect. While this possibility may have played a role, it does not explain the fact that alcohol injury was actually exacerbated by the intraperitoneal NAC. This latter finding suggested that NAC, when administered by the intraperitoneal route, was inducing some other event that was deleterious to the stomach and making it more likely for alcohol injury to occur.

To explore further the possible explanation for these seemingly paradoxical effects of NAC on alcohol injury when administered orally versus intraperitoneally, the present study was undertaken. It was our belief that if NAC was in fact doing something to the peritoneal cavity that altered its protective action, this effect may be a function of the amount of time that NAC was in contact with the peritoneum. Since in the earlier study Lu's group (5) administered NAC over 2 hr prior to giving alcohol orally, it seemed appropriate to see whether the NAC effects observed by these investigators would be present only if NAC was in prolonged contact with the peritoneum or whether such effects were also evident with shorter exposure times. Thus, NAC was given intraperitoneally and allowed to remain in contact with the abdominal cavity for both a short period, such as 15 min, and a longer period, such as 2 hr, prior to exposing the gastric epithelium to 100% ethanol. To allow appropriate comparisons with its protective effects orally, a similar pretreatment scheme was followed when NAC was administered by this route prior to ethanol exposure.

Our results clearly show that oral administration of NAC is protective against alcohol injury, confirming our earlier observations (3). Interestingly, this protective effect was almost as efficient with a 120-min pretreatment period as it was with a shorter period. These findings lend strong credence to the contention that

NAC mediated this protective action through some biochemical process, rather than through a topical barrier effect, for it to be sustained for such a prolonged duration. In view of what is known about this agent and its participation in the synthesis of GSH (4), the most likely mechanism for its protective action is through enhancement of GSH synthesis. Exactly how GSH itself promotes this protection remains to be determined. One possibility is through the antioxidant activity of this substance and its ability to scavenge hydrogen peroxide and other hydroperoxides (10, 11). The recent findings that ethanol injury may involve oxygen radical formation makes this notion a tenable one (16–18).

Our findings also confirm the earlier observations of Lu and associates (5) by showing that intraperitoneal NAC does not possess protective activity against alcohol injury, regardless of the length of the pretreatment period. Of equal note, our results provide an explanation for the absence of this protective action, in that intraperitoneal NAC was shown to be a potent irritant to the peritoneal lining. In fact, such irritation was so potent that the peritoneal fluid recovered indicated that as much as 25–40% of the rat's circulating blood volume may be lost to the abdominal cavity through such irritation. This conclusion is based on the observation that a 200-g rat has approximately 12 ml of circulating blood volume (19). Subtracting the 1 ml of NAC solution that was introduced into the abdomen leaves an average loss of approximately 3 ml in the first 15 min following its administration and almost 5 ml 120 min after administration. It was not surprising, therefore, to see the effects of this peritoneal irritation on mean arterial blood pressure and to understand why the drop in blood pressure in response to intraperitoneal NAC never recovered its baseline values. Our results, therefore, are consistent with the hypothesis that the shock-like state induced by intraperitoneal NAC resulted in the redistribution of splanchnic blood flow away from the gastrointestinal tract to more vital organ systems, thus making the gastric mucosa less resistant to topical exposure to 100% ethanol. Our findings are also consistent with the observations of other investigators, in which shock has been noted to enhance the damaging effects of acid (20, 21), aspirin (22), taurocholate (21, 23), and ethanol (24), when topically applied to the gastric mucosa, and to prevent the protective action of a prostaglandin against aspirin damage (22).

How intraperitoneal NAC induced its irritant effects is unclear. Because of the hyperosmolarity of the NAC solution (approximately 2 *M*), it is tempting to speculate that the hypertonicity evoked the large outpouring of peritoneal fluid. That osmolarity alone may not be the only explanation for our results is supported by other studies (unpublished) in our laboratory in which intraperitoneal dimethylthiourea, an oxygen radical scavenger, administered in a solution of similar osmolarity, was noted to prevent 100% ethanol-induced injury in the rat stomach and to have no peritoneal irritant effects (i.e., no peritoneal fluid formation). Thus, a component of NAC's effect when administered intraperitoneally may be related to direct chemical irritation of the peritoneal lining.

The explanation for our tissue GSH results is also uncertain. The finding that GSH levels were significantly decreased after NAC administration when compared with saline controls at the 15-mm sacrifice time is consistent with the effects of NAC on blood pressure. It is known, for example, that shock can depress tissue GSH levels apparently by disturbing GSH synthesis (25). Thus, not only the shock, but possibly the depressed GSH also, may have contributed to the exacerbation in gastric injury observed in animals treated with intraperitoneal NAC and then exposed to 100% ethanol 15 min later, as compared with their saline-treated counterparts. What is less certain is why GSH levels returned to control values at the 120-min sacrifice time even though NAC still continued to have adverse effects on blood pressure. One possibility is that sufficient NAC may have been absorbed by this time, despite its peritoneal irritant effects, and was directly synthesized into GSH by the gastric mucosa. The fact that most of the peritoneal irritant effects were observed within the first 15 min after NAC's administration and the aggravation in ethanol injury was most pronounced at this time period would be consistent with this notion. Another possibility is interorgan transport of GSH from an organ highly supplied with GSH, such as the liver, to another organ that needs GSH, as Griffith and Meister (26) have shown can occur. Whether either or both of these considerations are tenable explanations for our data will require further study. What is clearly not apparent from our studies is why Lu's group (5) found increased levels of GSH over control values when evaluated 2 hr after intraperitoneal NAC in the face of findings similar to ours with respect to an inability of intraperitoneal NAC to prevent ethanol-induced gastric damage.

Our results confirm previous observations that NAC is protective against alcohol injury when administered orally, but this protective action is lost when it is administered intraperitoneally. This promotion of damage via the intraperitoneal route appears to be secondary to the peritoneal irritant effects of NAC, since intraperitoneal administration of NAC induced the production of large amounts of peritoneal fluid with a corresponding drop in mean arterial blood pressure. These findings are consistent with the hypothesis that intraperitoneal NAC induces a shock-like state and thereby alters gastric mucosal blood flow, thus obviating the ability of this agent to prevent ethanol damage under these conditions.

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