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# Pharmacokinetics of Naloxone: An Insight into the Locus of Effect on Stress-Ulceration[1]

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**ABSTRACT**

The purpose of this study was to determine if the beneficial effect of naloxone on formation of acute gastric mucosal lesions was brought about *via* central or peripheral mechanisms by measuring blood concentrations of naloxone in rats during a 4-hr period of restraint stress. The study involved administration of naloxone to rats at doses of 5, 20 and 40 $mg \cdot kg^{-1} \cdot hr$ by either the intravenous or enteral routes. Blood samples were collected throughout the period of restraint and gastric stress-lesions were counted at the end of the experiments. Both routes of administration were equally effective in preventing stress-ulceration, with only rats receiving drug intravenously showing the presence of naloxone in blood samples. Inverse linear relationships existed between mean trough blood concentrations and lesions ($P = .0003$), as well as a linear correlation between area under the time-concentration curve and mean trough concentrations ($P = .0001$). Although our results show tight correlation between blood levels and effect on lesions in the group given drug intravenously, the effect must be on peripheral rather than central opiate receptors as no detectable blood levels were found when naloxone was given enterally.

Naloxone has the ability to bring about changes in the hemodynamic status which are beneficial to patients and animals in septic and hemorrhagic shock (Albert *et al.*, 1982; Demaria *et al.*, 1985; Lechner *et al.*, 1985; Weissglas *et al.*, 1982). Gastric mucosal injury related to stress is thought to be related to gastric hemodynamics (Cheung, 1984). Therefore, the possibility that naloxone may be useful in the prophylaxis of stress ulceration has recently been investigated. However, early reports concerning the efficacy of naloxone in preventing stress-ulceration were conflicting primarily due to a lack of information regarding naloxone's site of action, mechanism of action and optimal dose (Arrigo-Reina and Ferris, 1980; Moran *et al.*, 1984; Nafradi *et al.*, 1983; Waisman *et al.*, 1985). We have recently shown that naloxone is effective in preventing stress-induced gastric ulceration in the rat (Kleiman *et al.*, 1989). The effect was dose-related and was apparent with i.v. and i.g. administration. However, i.v. administration was slightly more effective.

Opiate receptors known to affect gastric functions are present both in the central nervous system (Rozé *et al.*, 1980; Taché *et al.*, 1980) and the gastric wall (Ekblad *et al.*, 1985), so either or both sites might mediate naloxone's protective effect in this model. The fact that i.v. administration was more efficacious suggests that receptors remote from the gastric wall might have importance in naloxone's beneficial effects. However, our knowledge of human naloxone kinetics suggests (Fishman *et al.*, 1973) that naloxone has minimal bioavailability when given p.o. The aim of this study was to expand on our previous findings by providing pharmacokinetic data for naloxone in the rat restraint model. We planned to determine if naloxone blood concentrations correlated with the gastric mucosal protection observed in order to determine which receptors contribute to the effect in this model.

## Materials and Methods

**Animal model.** The protocol was approved by the Animal Care Committee at the University of Iowa, Iowa City, Iowa.

After a 24-hr fast with water allowed *ad libitum*, stress-ulceration was induced in 80 lightly anesthetized (chloral hydrate 0.3 $mg \cdot kg^{-1}$) male Sprague-Dawley rats (250–300 g) by restraining them on their backs for 2 hr at room temperature followed by an additional 2 hr at 4°C. Animals were divided into 7 different treatment groups: control (20 rats) and 6 groups of 10 rats/group which received naloxone every hour over the 4-hr study period at doses of 5, 20 or 40 $mg \cdot kg^{-1}$ every hour given by the i.v. ($N = 10$) or i.g. ($N = 10$) routes. Each animal had received both an i.v. injection and an i.g. volume to assure equal manipulation, as well as equal fluid balance for data analysis. All animals had a placement of a jugular venous catheter on the day of experiments; however, they were fully recovered before initiation of stress; as well as an hourly orogastric tube insertion during stress to allow i.g. administration of naloxone or vehicle. Each dose of i.v.

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**ABBREVIATIONS:** i.v., intravenous; i.g., intragastric; AUC, area under concentration curve.

naloxone was administered in 0.5 ml of saline with 2.0 ml of vehicle given i.g., whereas animals received naloxone by the i.g. route in 2.0 ml of saline per dose with 0.5 ml of vehicle given i.v. Blood samples (0.5 ml) were taken *via* an indwelling jugular venous catheter before drug administration at 30, 60, 90, 120, 180 and 240 min after initiation of stress. Blood samples were stored immediately at −70°C until naloxone analysis.

After experimentation animals were sacrificed by an overdose of pentobarbital (150 $mg \cdot kg^{-1}$). The stomachs were removed, incised along the greater curvature and the pH of the luminal contents was determined by means of a pH meter. The stomachs were gently cleaned and examined under a dissecting microscope (×4 magnification) by investigators blind to the treatment group. Each individual lesion was separated according to size and was scored as 1.0 if ≥2 mm and 0.5 if <2 mm and >1 mm. A total lesion score for each rat was then calculated as the sum of the individual scores. The total lesion score was used for statistical analysis.

**Naloxone analysis.** Analysis of whole blood samples for naloxone was performed using a reversed-phase ion-pair high-performance liquid chromatography method (Asali *et al.*, 1983). The mobile phase was a mixture of acetonitrile (85%) and triethylamine (0.06% v/v) in an aqueous phosphoric acid solution at a pH of 5.0. The flow rate was 1.5 $ml \cdot min^{-1}$ and the column was Bio-Rad C-18 5 μm (150 × 4.0 mm inside diameter). Detection of naloxone was performed by UV absorption at 214 nm with a limit of detection of 0.2 μM. Assay variation was less than 10% at all concentrations tested.

Preparation of blood samples for the measurement of naloxone involved dilution of whole blood (0.25 ml) with an equal volume of double-distilled water. Naltrexone (5 μg) was added to each diluted sample as the internal standard. The mixture was adjusted to a pH of 10 using bicarbonate buffer (1 M, 0.25 ml) followed by addition of 5 ml of diethyl ether. After vortex mixing (30 sec) and centrifugation (2000 rpm for 2 min) the ether layer was removed and transferred to a tube containing 100 μl of phosphoric acid (pH 2.5). After further vortex mixing and centrifugation 20 μl of the aqueous layer was injected onto the column. The retention times for naloxone and naltrexone were 2.3 and 4.5 min, respectively.

**Pharmacokinetic and statistical analysis.** Pharmacokinetic analysis involved calculation of the AUC (0–240 min) by the log trapezoidal method. In addition, the mean and median trough blood concentrations over the study period (60, 120, 180 and 240 min after drug administration) were determined for each animal and the half-life of elimination of calculated from the median values using times of 30 to 60 and 90 to 120 min. Statistical analysis was performed for the stress ulcer scores for pooled i.v. and i.g. naloxone for each dose using the one-way analysis of variance. Pearson's correlation coefficient was computed for relationships between plasma concentrations, AUC and gastric lesions.

## Results

A summary of the blood concentrations (mean ± S.E.M.) at each time point after i.v. administration of naloxone is illustrated in figure 1 for each dosing group. With the exception of several animals absorbing naloxone in the i.g. group at 240 min, no blood concentrations were observed for p.o. administered naloxone. Pharmacokinetic parameters (half-life of elimination, AUC and median trough concentrations) are summarized in table 1. Trough concentrations of naloxone and AUC values were dependent upon the dose of naloxone administered, whereas naloxone's half-life of elimination was equal for all doses. A test of normality for our data showed lesion scores to be normally distributed for each dose level administered ($P > .05$). Table 2 summarizes the total stress-lesion scores for both i.v. and i.g. routes for a given naloxone dose. Analysis of variance was performed while eliminating the control group in order to determine if the route of administration influenced the effect. This was found not to be of statistical significance ($F = 0.42$, $P = .52$), which then allowed us to pool the i.v. and i.g. group by dose, including the control group. The naloxone dose effect was significant ($F = 29.20$; $P = .0001$), with doses 5, 20 and 40 $mg \cdot kg^{-1} \cdot hr$ being different from the control group.

Inverse linear correlations were shown to exist between the mean trough blood concentration of naloxone and reduction in lesion score formed for the i.v. route of administration ($r = -0.53$; $P = .0003$). In addition, AUC values correlated with mean trough concentrations ($r = 0.90$; $P = .0001$) and correlated inversely with the lesion score formed ($r = -0.57$; $P = .0001$). However, although the efficacy of i.g. administered naloxone was not statistically different from that of i.v. naloxone ($P > .05$), it was not related to blood concentrations.

## Discussion

Our data show that although i.g. naloxone diminishes gastric lesions in the rat restraint model of stress ulceration, no detectable blood levels were achieved when the drug was given through this route of administration. This is in agreement with previous reports suggesting that the oral bioavailability of naloxone is on the order of 0.1% in both rats (Weinstein *et al.*, 1973, 1974) and humans (Fishman *et al.*, 1973). For animals given naloxone i.v., attenuation of gastric damage was related to both measured blood concentrations and the AUC. No such relationship could be demonstrated in rats given naloxone i.g., as blood levels were not detectable. These findings demonstrate that central opiate receptors are unlikely to play a major role in the gastric cytoprotective effect of naloxone during cold restraint.

The short half-life of elimination for naloxone in the present work (16–31 min) is in agreement with previously published pharmacokinetic data and shows that naloxone is quickly cleared from systemic circulation (Garrett *et al.*, 1986; Ngai *et al.*, 1976; Stile *et al.*, 1987). This in part may explain why previous studies concerning the efficacy of naloxone in a number of experimental models were contradictory in their conclusions. These reports utilized single doses (5–10 $mg \cdot kg^{-1}$) of naloxone given by the i.m. or i.v. routes (Arrigo-Reina and Ferris, 1980; Moran *et al.*, 1984; Nafradi *et al.*, 1983; Waisman *et al.*, 1985). The short half-life of elimination (with resultant short duration of action) suggests that by administering naloxone as a single low dose bolus, very little drug will remain in systemic circulation at the completion of the period of stress. Therefore, the animals used in these studies were probably not receiving prophylaxis for the entire period of experimentation. In contrast, our study administered naloxone on an hourly basis in order to maintain steady-state concentrations throughout the period of stress.

It is still unclear how naloxone protects the gastric mucosa. When administered by the i.v. route, it is known that naloxone can bring about hemodynamic changes, thereby maintaining blood flow to vital organs during septic and hemorrhagic shock (Albert *et al.*, 1982; Demaria *et al.*, 1985; Lechner *et al.*, 1985; Weissglas *et al.*, 1982). It is now thought that stress leads to gastric mucosal ischemia in both experimental and clinical stress-ulceration (Miller, 1983; Silen, 1985). Other investigators have suggested that the long acting opiate antagonist naltrexone protects the gastric mucosa by enhancing perfusion during cold restraint (Morley *et al.*, 1982). However, our data indicate that enhancement of systemic hemodynamics must not be

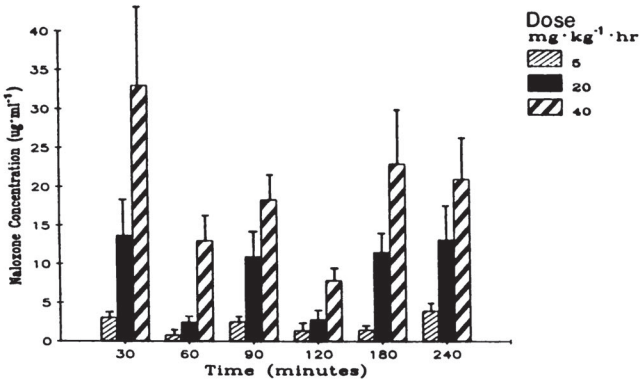


**Fig. 1.** Mean blood concentration (± S.E.M.) data for each dose group after i.v. administration of naloxone.

TABLE 1

**Pharmacokinetic data for naloxone after i.v. administration (median values)**

Numbers in parentheses represent range of blood concentration data.

| Dose | Half-Life | AUC 0–240 min | Trough Concentration |
|---|---|---|---|
| *mg·kg⁻¹·hr* | *min* | *μM·min·ml⁻¹* | *μM* |
| 5 | 16.4 (10.0–75.0) | 473.4 (338.7–621.4) | 5.3 (2.2–6.8) |
| 20 | 16.2 (8.5–38.3) | 1368.2 (1059.0–4229.0) | 19.4 (6.9–29.1) |
| 40 | 15.3 (7.0–48.1) | 3761.0 (2183.0–6099.2) | 41.9 (22.9–69.1) |

TABLE 2

**Total stress-lesion scores for both i.v. and i.g. naloxone (mean ± S.D.)**

| Dose | Total Lesion Score | |
|---|---|---|
| | i.v. group | p.o. group |
| *mg·kg⁻¹·hr* | | |
| 0 | 12.47 ± 1.30 | |
| 5 | 7.10 ± 1.40*† | 9.95 ± 1.91* |
| 20 | 3.22 ± 0.60* | 5.09 ± 1.31* |
| 40 | 1.38 ± 1.69* | 1.75 ± 1.28* |

* Different from 0 mg·kg⁻¹·hr at $P < .05$.
† Different from 20 and 40 mg·kg⁻¹·hr at $P < .05$.

necessary for naloxone's effect, as i.g. naloxone does not reach the systemic circulation in significant amounts.

Although current stress-ulcer prophylaxis using H-2 blockers or antacids has reduced the incidence of clinically important bleeding to 2 to 3% among seriously ill patients (Lucas, 1981; Marrone and Silen, 1984), several studies have suggested that routine suppression of normal gastric acidity may result in overgrowth of gram negative organisms within the gastric lumen (Hillman *et al.*, 1982). This intragastric growth of pathogens in patients receiving stress-ulcer prophylaxis has been linked to increased rates of nosocomial pneumonias in intensive care units (Du Moulin *et al.*, 1982; Driks *et al.*, 1987). Our previous work with naloxone (Kleiman *et al.*, 1989) and other studies (Morley *et al.*, 1982) show that peripherally administered naloxone does not alter gastric pH at cytoprotective doses.

The patients at greatest risk of stress-ulceration are often traumatized or postsurgical and are in need of opiate agonists for pain control. Intravenous naloxone for stress-ulcer prophylaxis would certainly interfere with the opiates' analgesic effect. However, our data indicate that i.g. naloxone could be clinically useful in stress-ulcer prophylaxis as it will not interfere with concurrent pain medications as well as not alter gastric pH.

In conclusion, our study shows that i.g. naloxone prevents gastric lesions from cold restraint without affecting systemic blood levels. Hourly i.v. administration resulted in steady-state blood levels that correlated with the cytoprotective effect. The cold restraint model did not affect naloxone's half-life (16–31 min) when given i.v. These findings suggest that gastric, rather than central, opiate receptors are responsible for naloxone's cytoprotective effects. As i.g. naloxone affects neither gastric pH or the analgesic effects of systemic opiates, it may prove useful for prevention of stress-ulceration in clinical situations.

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