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Citation for published version (APA):
Mundin, G., McDonald, R., Smith, K., Harris, S., & Strang, J. (2017). Pharmacokinetics of concentrated naloxone nasal spray over first 30 minutes post-dosing: analysis of suitability for opioid overdose reversal. *Addiction*. Advance online publication. https://doi.org/10.1111/add.13849

Pharmacokinetics of concentrated naloxone nasal spray over first 30 minutes post-dosing: analysis of suitability for opioid overdose reversal

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Running head: Pharmacokinetics of naloxone nasal spray

Word count: *2,110 words (excl. references, instructions, etc.)*

Clinical trial registration details: This manuscript reports on data from a Phase I study conducted in the U.S. in 2004. The study was not registered on ClinicalTrials.gov and therefore does not have an NCT identifier number. Since the study was conducted in 2004, i.e. prior to the U.S. Food and Drug Administration Amendments Act of 2007 (FDAAA, signed September 27, 2007), registration was/is not required.

ABSTRACT (293 words)

Background and Aims: Lack of non-injectable naloxone formulations has impeded widespread take-home provision for the prevention of heroin/opioid overdose deaths. For non-injectable formulations that are finally being investigated, rapid onset of action and sufficient bioavailability will be vital. We present analysis of data from a study of concentrated naloxone nasal spray formulations. Our aims are: to assess 1) pharmacokinetic properties and 2) suitability for overdose reversal in terms of naloxone absorption within 30 minutes post-dosing.

Design and interventions/comparator: Open-label, randomized, 4-way crossover Latin-square pharmacokinetic study of naloxone administration by three routes: intranasal at two doses (8mg/0.4mL, 16mg/0.4mL) versus sublingual (16mg/mL) versus intravenous reference (1mg/mL).

Setting: Clinical Pharmacology Unit at The Ohio State University (Columbus, Ohio, USA).

Participants: 12 healthy volunteers (age 20-41; 7 female).

Measurements: From blood plasma naloxone concentrations, 1) standard pharmacokinetic parameters, including maximum plasma concentration (Cmax) and mean absolute bioavailability (F%, relative to intravenous injection), were determined; as well as 2) partial area under the curve (AUC) values, tmax (time to maximum plasma concentration), and T50% (time to 50% of maximum plasma concentration) as measures of early absorption.

Findings: 1) Bioavailability was F%=25-28% for intranasal naloxone. Sublingual had low bioavailability (F%=2%) and was not considered further. Mean Cmax values for 8mg (12.83ng/mL) and 16mg (18.25ng/mL) intranasal exceeded 1mg intravenous (9.64ng/mL) naloxone. 2) Following intranasal administration, T50% was reached within 8 minutes and tmax within 20 minutes. Mean naloxone absorption from dosing to 30 minutes (AUC30) was greater following 8mg (4.17h*ng/mL) and 16mg (5.91h*ng/mL) intranasal than following 1mg intravenous (1.70h*ng/mL) administration.

Conclusions:
Concentrated naloxone nasal spray has a promising pharmacokinetic profile, with substantial bioavailability. Its early absorption time-course suggests that concentrated nasal naloxone is suitable for emergency administration in the community, where rapid restoration of respiratory function is essential for opioid overdose reversal.

1. Introduction:

Opioid overdose constitutes a major international public health problem (1). Overdose deaths from heroin and other opioids can be prevented through timely administration of the antagonist naloxone.

Naloxone was, until recently, only licensed as injection. Regulatory criteria for non-injectable naloxone have been proposed (2) and, in 2015/16, a first naloxone nasal spray (4mg/0.1mL) was approved in the US (3) and Canada (4), with 44-47% mean bioavailability relative to intramuscular injection (5).

Some opioid overdoses have insidious onset, while others occur rapidly. Darke and Duflou (6) recently analysed the time course of opiate metabolites post-mortem and concluded that heroin overdose death occurred within 20-30 minutes of injecting in 43% of cases, suggesting the time window for naloxone administration may be very narrow (7). Hence, analysis of naloxone pharmacokinetics in the first 20-30 minutes is particularly important.

In this new analysis of previously unpublished data from a 2004 pharmacokinetic study of naloxone nasal spray (which investigated abuse liability of an oral oxycodone/naloxone formulation), we consider the potential of the studied high-concentration intranasal (IN) naloxone formulations from the different perspective of overdose reversal, with two aims: to assess 1) their pharmacokinetic properties and 2) naloxone absorption in the clinically-relevant period of the first 30 minutes post-administration.

2. Methods:

2.1. Study design:

We report data from a pharmacokinetic study with healthy volunteers conducted in 2004 by Purdue Pharma LP (US). Ethics approval was granted by the Western Institutional Review Board (Olympia, WA, US). Its key features (eligibility criteria, etc.) are summarised in the web-appendix. Participants received naloxone in four dose/route combinations (one per session) in a 4-way crossover Latin square design. The four naloxone sessions compared 1mg/mL intravenous (IV) reference with 16mg/mL sublingual (SL) administration and two IN doses: 8mg/0.4mL from 20mg/mL and 16mg/0.4mL from 40mg/mL solution.

Naloxone hydrochloride 10mg/10mL vials for 1mg/mL IV bolus injection were obtained from Bristol-Meyers Squibb (USA). The SL dose (16mg/mL; prepared from naloxone-hydrochloride powder (Mallinckrodt Pharmaceuticals, USA) in 0.9% sodium-chloride solution) was

administered by having subjects retain the solution under the tongue for 5 minutes. IN solution was prepared by dissolving naloxone-hydrochloride powder (see above; 11.0g for 20mg/mL; 22.0g for 40mg/mL solution) in sodium-citrate stock solution (9.35g for 20mg/mL; 20.9g for 40mg/mL) and brought up to 500mL volume using 0.9% sodium-chloride solution. IN solution was atomized using metered dose nasal spray devices (comprising a pump spray assembly threaded onto small amber glass bottle), with two 0.1mL aerosol actuations delivered per nostril, for a 0.2mL total volume per nostril. Subjects were required to remain upright (seated or standing) with the head tilted slightly forward from dosing until 4 hours post-dosing. Pharmacokinetic blood samples were drawn into tubes containing the anticoagulant K_2EDTA. Blood was collected pre-dosing and at minutes 1, 2, 4, 10, 30, 40; and hours 1, 2, 4, 6, 8, 12, 16, 24.

Bioanalysis was conducted by Purdue Pharma L.P. (Ardsley, NY, USA). Naloxone plasma concentration was determined by a validated liquid extraction method using liquid chromatography–mass spectrometry (LC-MS/MS). The range of quantification was 0.01-1.0ng/mL. Concentrations below the limit of quantification were set to zero for pharmacokinetic calculations.

2.2. Outcome measures for this new analysis

Our interest was the potential of IN naloxone for opioid overdose reversal, and consequently we focused on the pharmacokinetics within the first half-hour, examining plasma naloxone sample concentrations from dosing to 30 minutes.

Partial area-under-the-curve (AUC) values were determined for these sampling points using Phoenix WinNonlin 6.4. AUC values are expressed as h*ng/mL, i.e. hour(s) times nanograms per millilitre, representing naloxone exposure over time.

We also introduced the exploratory parameter T50%, defined as time from dosing to concentration equal to 50% of maximum plasma concentration (Cmax) (8).

2.3. Statistical analysis:

Inferential statistics were calculated using SPSS Statistics 23. Analysis of variance (ANOVA) was conducted to determine differences in naloxone absorption by treatment arm. Following WHO guidance (9), dose-dependent AUC data were log-transformed to allow for normal distribution in the ANOVA. Tukey's HSD test was used for post-hoc comparisons, with significance level at p<.05.

3. Results:

3.1. Study participants and sensitivity analysis

Twelve eligible healthy subjects were entered into the study, which is within the FDA recommendation of 6-36 subjects (10); 5 were males (age 20-41 years, height 165-193cm, weight 74-106kg) and 7 females (19-48 years, 157-168cm, 51-83kg). Subject 12 did not attend the final 8mg IN session, and Subject 7 failed to attend the 16mg SL and 1mg IV sessions. These three sessions were handled as missing data. The plasma naloxone concentration from Subject 3 was clearly anomalous at 20 minutes following IV administration, being 5-9 times greater than adjacent time points (10, 30 minutes) with an AUCt-value (26.85h*ng/mL) four times greater than the group median (6.64h*ng/mL). We have excluded all IV data for this individual. Consequently, values reported below refer to sample sizes of n=10 (1mg IV), n=11 (8mg IN, 16mg SL), and n=12 (16mg IN), unless otherwise specified.

3.2. Pharmacokinetics:

Plasma naloxone concentrations over the first 6 hours are displayed in Figure 1 (left-hand graph) and with expanded depiction of the first 30 minutes (right-hand graph). IV administration (1mg) was characterized by rapid uptake and subsequent decline; whereas SL administration (16mg) showed minimal absorption. Both IN administrations (8mg, 16mg) had similar time profiles, reaching peak concentrations in less than 30 minutes post-dosing. (The 12 subjects' individual plasma-concentration curves are provided as web-appendix).

*** insert Figure 1 about here ***

Pharmacokinetic parameters are shown in Table 1. The two IN administrations (8mg, 16mg) displayed similar uptake, with rapid median tmax of 20 minutes (0.33 h) for both doses. T50% was 7-8 minutes for both IN doses (8mg IN: x̄=0.12h; 16mg IN: x̄=0.13h), and hence slower than from IV administration (4 minutes; x̄=.06h,). Cmax values following 8mg IN (x̄=12.83ng/mL) and 16mg IN (x̄=18.25ng/mL) were greater than those following 1mg IV (x̄=9.64ng/mL). Cmax values following 16mg SL were extremely low (x̄=0.90ng/mL).

*** insert Table 1 about here ***

3.3. Bioavailability

Dose-adjusted AUC data (per mg) from IN and SL administrations were compared against the 1mg IV reference. Since comparisons were not possible for missing and excluded sessions

(see Section 3.1), absolute bioavailability was determined for sample sizes of n=9 (8mg IN) and n=10 (16mg IN, SL).

The mean absolute bioavailability (F%) from dosing to last measureable concentration (AUCt) was 2.0% for SL naloxone; hence it was not considered further. IN administration had F% of 27.7% (8mg) and 24.6% (16mg; see Table 2).

Mean bioavailability values for partial AUC at 1, 2, 4, 10, 20, and 30 minutes post-dosing are reported in Table 2, with similar increase over time for both IN doses (8mg, 16mg): >5% at 4 minutes, ≥13% at 10 minutes, ≥20% at 20 minutes.

*** insert Table 2 about here ***

3.4. AUC30 and nasal dose equivalent to 1mg IV bolus

Observed AUC30 values following 8mg IN (x̄=4.17h*ng/mL) and 16mg IN (x̄=5.91h*ng/mL) were greater than following 1mg IV (x̄=1.70h*ng/mL; see Table 1).

These AUC30 values were dose-adjusted, log-transformed and compared in a one-way, between-subjects ANOVA. AUC30 values differed significantly as a function of naloxone treatment [$F_{(3,40)}=255.11$, $p<0.001$]. Post-hoc tests showed that dose-adjusted, log-transformed AUC30 was significantly higher with IV (x̄=3.21, SD=0.15) versus both IN concentrations (8mg IN: x̄=2.68, SD=0.19; 16mg IN: x̄=2.53, SD=0.18). However, there was no significant difference between both IN concentrations (p=0.230), suggesting naloxone absorption was proportional to IN dose administered.

Hence, with dose-adjusted AUC30 values for 8mg (x̄=0.52 h*ng/mL per mg) and 16mg IN (x̄=0.37 h*ng/mL per mg) and 1mg IV (x̄=1.70 h*ng/mL) (from above observed values), we calculate, for AUC30, the IN-dose equivalent to 1mg IV would be 3.3mg IN (20mg/mL formulation) and 4.6mg IN (40mg/mL).

3.5. Safety

No serious adverse events occurred. Side effects reported after naloxone administration included fainting (3 cases; one each after 8mg IN, 16mg IN, 1mg IV), headache (2 cases) and gastrointestinal symptoms (5 cases). These 10 cases were distributed by treatment as follows: 8mg IN (3 cases); 16mg IN (5 cases); 16mg IN (0 cases); 1mg IV (2 cases).

4. Discussion:

Recent WHO guidelines (11) recommend that, similar to adrenaline/epinephrine for the treatment of allergic shock (12), naloxone should be offered to anyone in the community likely to suffer or witness an opioid overdose ('take-home naloxone', THN). However, the lack of

licensed non-injectable naloxone formulations until late 2015 (which continues outside North America) has hindered widespread THN (13-17). Once non-injectable solutions exist, naloxone may be provided more widely.

Our analysis identifies a promising pharmacokinetic profile for concentrated naloxone nasal spray. In 2008, Dowling et al. (18) reported only 4% absolute bioavailability with a nasal spray adaptation of a commercially-available concentration of naloxone (2mg/5mL), although the authors suggested the extremely low bioavailability may be a result of excessive volume at the nasal membrane. In sharp contrast, we now report that, at much higher concentrations (8mg/0.4mL, 16mg/0.4mL), there is a mean absolute bioavailability between 25-28%. Even though originally studied for different reasons, we conclude that concentrated solutions of naloxone administered as nasal spray have bioavailability adequate for overdose reversal.

We also report that, crucially, half of the maximum observed concentration (T50%) was reached within 8 minutes and maximum concentration (tmax) within 20 minutes of IN administration. This time profile suggests that concentrated naloxone nasal spray may be suitable for the reversal of overdoses from heroin and other short-acting opioids (e.g. fentanyl), where rapid restoration of respiratory function within 30 minutes of opioid use may be essential (6).

These results are broadly consistent with the recent paper by Krieter et al. (19) who reported a Cmax of 10.3ng/mL for a 8mg/0.2mL IN dose as well as tmax values of 18-30 minutes and bioavailability of 44-54% (relative to intramuscular reference) for 0.1-0.2mL of 20mg/mL and 40mg/mL IN formulations. However, absence of an intramuscular reference in this study means that a direct bioavailability comparison between the studies is not possible.

We did not find a significant difference between the two nasal formulations in their dose-adjusted naloxone absorption (AUC30). This allowed us to estimate an IN dose-equivalent that would deliver the same naloxone exposure within 30 minutes as the reference (1mg/mL IV bolus injection). We calculate that a nasal dose of 3.3mg (at 20mg/mL) and 4.6mg (40mg/mL) will provide, over the clinically-critical initial 30-minute period, the same AUC over 30 minutes as 1mg/mL IV.

Algorithms exist for injectable naloxone to guide correct initial and repeat dosing (20) but have yet to be developed for IN naloxone. The T50% data suggest that initial IN absorption is delayed compared to the IV bolus, with IN administration taking 7-8 minutes to attain half of the peak concentration (versus 4 minutes for IV), and IN absolute bioavailability only surpassing 10% between 4-10 minutes (see Table 2). If this finding is robust, then lay

responders may need to be advised to wait some minutes before administering a second IN dose to avoid risk of precipitating over-antagonism.

Several limitations need to be borne in mind. Some averages were based on low subject numbers (see Table 1). There was also variability in the tmax values for IV administration (median: 4 minutes), due to two outliers at 4 hours. It is unclear if the low SL bioavailability resulted from subjects possibly swallowing the solution. For the nasal route, only a 0.2mL-volume per nostril was tested in this study, meaning that a volume-absorption relationship cannot be determined. Finally, while it is generally assumed that atomization at a droplet size greater than 10μm increases nasal absorption (21), the droplet size distribution was not characterized in this study, and its potential impact on nasal deposition cannot be determined.

We should also give consideration to how quickly the nasal spray versus injectable naloxone can be administered, which then needs to be considered alongside pharmacokinetics-derived speed of onset. For example, in a Vancouver ambulance study, differences in time-to-recovery comparing IV versus subcutaneous naloxone, disappeared when the greater time to establish IV access was accounted for (22).

This data analysis focuses on the clinically relevant first 30 minutes, and it also introduces the measure of T50%. Also, while our findings support good bioavailability in healthy subjects, concentrated naloxone nasal spray has yet to be formally tested in the target population of opioid users.

The emergence of supportive pharmacokinetic data for concentrated IN naloxone, along with approval of a first nasal naloxone spray in North America (3)(4), constitutes a significant advancement for the field, after concerns over off-label use of injectable naloxone-hydrochloride solution as nasal spray sparked a lively debate in early 2016 (8).

The time-lag between the original study conducted thirteen years ago (with its results subsequently archived) and this new analysis warrants concern. This new analysis identifies the potential of concentrated naloxone nasal spray for overdose reversal (hence authorship of this research report is across academia and industry). There has recently been considerable public investment to conduct healthy volunteer studies of nasal naloxone (19): the field could have progressed faster if there had been awareness of the above data. In future, a mechanism is needed to ensure awareness of relevant data by industry and academia."

5. Conclusion:

Concentrated naloxone nasal spray appears to be a feasible formulation with adequate speed of onset and acceptable bioavailability in the concentrated form. This appears directly relevant to prevention of opioid overdoses in medical settings and in the community (THN). The above data find concentrated nasal spray solutions (at 20mg/mL and 40mg/mL) to have acceptable bioavailability and plasma levels over the clinically-critical first 30 minutes, with moderate uptake from 4-10 minutes onwards. Further examination is required (and is in progress) and dose-titration protocols and repeat-dosing guidance will need development, especially for wider distribution to non-medical persons (family members, peers, drug users themselves). We conclude that concentrated naloxone nasal sprays hold real promise, may enable wider THN provision, and can thereby contribute to the prevention of fatalities from heroin/opioid overdose.

Table 1: Pharmacokinetic parameters (mean, SD)

Parameter	n	Unit	1mg IV	8mg IN	16mg IN	16mg SL
AUC20	10-12	h*ng/mL	1.24 (0.62)	2.50 (1.35)	3.58 (2.25)	0.11 (0.09)
AUC30	10-12	h*ng/mL	1.70 (0.62)	4.17 (1.68)	5.91 (0.30)	0.22 (0.11)
AUCt	10-12	h*ng/mL	8.83 (4.90)	20.07 (4.93)	32.81 (10.22)	2.67 (1.78)
Cmax	10-12	ng/mL	9.64 (12.66)	12.83 (4.47)	18.25 (7.50)	0.90 (0.37)
T50%	10-12	h	0.06 (0.05)	0.12 (0.06)	0.13 (0.07)	0.24 (0.10)
Tmax^	10-12	h	0.07 (0.03, 4.00)	0.33 (0.07, 0.50)	0.33 (0.07, 0.67)	0.67 (0.50, 36.00)

Annotations: AUC20 = partial area under the curve (AUC) from dosing to 20 minutes; AUC30 = partial AUC from dosing to 30 minutes; AUCt = AUC from dosing to last measurable time point; Cmax = maximum observed plasma concentration; Tmax = time to Cmax; ^median (min, max).

Table 2: Absolute bioavailability (F%) based on partial AUCs (1-30 min. post-dosing) & AUCt

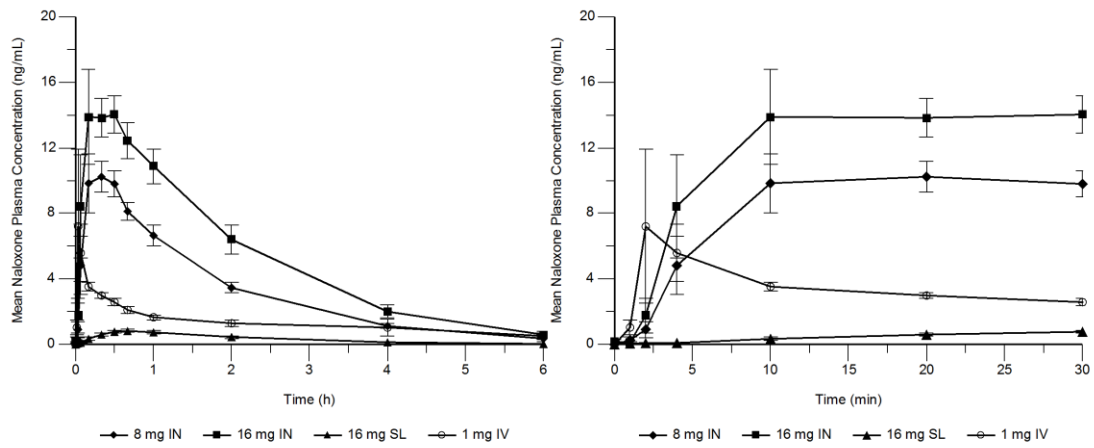|	AUC1	AUC2	AUC4	AUC10	AUC20	AUC30	AUCt
8 mg IN	3.4%	2.4%	6.2%	17.5%	27.6%	33.1%	27.7%
16 mg IN	1.2%	1.7%	5.0%	13.0%	19.5%	23.2%	24.6%

Figure 1 | Mean naloxone plasma profiles within 6 hours (left) and expanded depiction of first 30 minutes (right) post-dosing (excl. Subject 3 IV outlier)

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