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INTESTINAL ABSORPTION OF IPAZILIDE AND ITS N-DESETHYL METABOLITE

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ABSTRACT

Rat in-situ intestinal perfusion experiments were conducted to determine the absorption potential of ipazilide and its metabolite, N-desethyl ipazilide (NDI). Ipazilide (1.54, 3.85 and 15.4 mg/mL) and NDI (1.50 and 3.60 mg/mL) were perfused, through the rat jejunum and duodenum at the rate of 0.1 mL/min for 60 min in separate experiments. The concentrations of each were determined in systemic and hepatic portal plasma samples. Each compound was detectable in portal plasma following its

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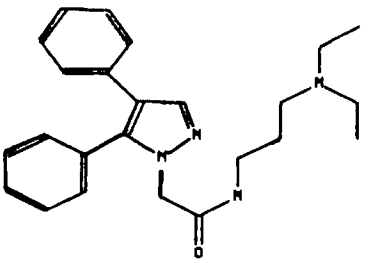
duodenal administration at all dose levels. Ipazilide was detected in systemic plasma only after duodenal perfusion of the high concentration (15.4 mg/mL) solution. NDI was undetected in the ipazilide perfusates. Conversely, systemic concentrations of NDI were detected as a result of perfusing either 1.50 or 3.60 mg/mL solutions. Intestinal metabolism of ipazilide to NDI was not seen.

Ipazilide was well absorbed from the duodenum following lumenal perfusion at all levels. Jejunal absorption of ipazilide following administration of 1.54 and 15.4 mg/mL solutions, did not occur. NDI, in contrast, was equally well absorbed from either intestinal segment. The limited number of perfusion studies conducted did not permit determination of absorptive dose proportionality.

INTRODUCTION

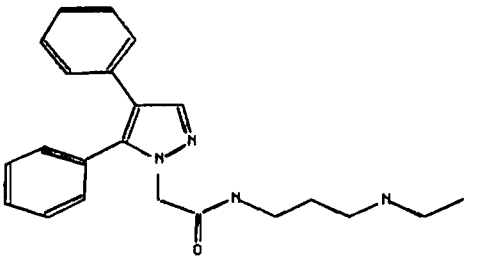
The in-situ lumenal perfusion of the rat intestine has been extensively used to study absorption and metabolism of xenobiotics (1,2,3). Water soluble compounds have been perfused through the intestine and quantifiable drug levels have been assayed in hepatic portal blood samples. Expansion of this approach to include assays of metabolites and collection of systemic blood samples provides an indication of sustained drug levels after hepatic elimination.

Two diphenylpyrazolacetamide derivatives, that have demonstrated antiarrhythmic activity, were investigated for their absorption potential. The N-desethyl derivative (NDI) is an active metabolite of ipazilide. Each compound was perfused through the rat intestinal lumen at different dose levels.



Ipazilide

N-[3-(Diethylamino) propyl]-4,5-diphenyl-1H-pyrazole-1-acetamide (E)-2-butenedioate (1:1)



N-desethyl ipazilide

N-[3-(Ethylamino)propyl]-4,5-diphenyl-1H-pyrazole-1-acetamide (E)-2-butenedioate (1:1) monohydrate

MATERIALS AND METHODS

All animal care and use procedures have been approved by Sterling Research Group's institutional animal care and use committee.

Male albino, Sprague Dawley rats (Charles River) ranging in weight from 346 to 395 grams were fasted 24 hours prior to surgery, and given water ad libitum. Anesthesia was effected with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The animals were maintained under anesthesia, at 37°C on a slide warmer (Precision Scientific, Chicago, IL) throughout surgery and the subsequent perfusion experiment.

A midline abdominal incision was made to permit access to the intestine (duodenum or jejunum), hepatic portal vein and vena cava inferior. To introduce the infusion solution into the intestine, the proximal end of a 10 cm segment was incised, a 2 cm glass cannula inserted isoperistaltically into either the duodenum at the pyloris, or the jejunum, 3 cm posterior to the Ligament of Treitz, and secured with a 4-0 silk suture. The segment's distal end was similarly cannulated, antiperistaltically with a 3 cm teflon cannula (4 mm O.D., HPLC Mobil Phase Tubing, Waters Associates, Milford, MA) to allow perfusate collection into a glass vial. A 20 cm PE-50 cannula (Clay Adams, Parsippany, NJ) fitted with a 5 mm, 23 gauge needle tip was filled with heparinized normal saline (200 IU/mL), inserted into the hepatic portal vein and secured with instant adhesive (Super Glue Corp., Hollis, NY). The vena cava inferior was cannulated in a manner identical to that for the portal vein to allow collection of systemic blood.

Plastic wrap (Reynolds Manufacturing, Richmond, VA) was placed over the opened abdomen to prevent dehydration during the experiment.

PERFUSION METHODS

Solutions of ipazilide at 1.54 mg/mL and 3.85 mg/mL, and of NDI at 1.50 and 3.60 mg/mL, were prepared in Krebb's buffer immediately prior to perfusion and maintained at room temperature during use. A 15.4 mg/mL solution of ipazilide also was tested. Each solution contained approximately 0.5 μCi of nonabsorbable C^{14} PEG-4000 (Amersham Corporation, 2636 South Clearbrook Drive, Arlington Heights, IL).

The inlet cannula was attached, via teflon tubing, to a 30 cc glass syringe containing the perfusion solution. Each perfusion was carried out for 60 minutes, at a rate of 0.1 ml/min using a syringe pump (Model 431A, Sage Instruments, Memorial Drive, Cambridge, MA). Perfusate fractions were collected at 10, 20, 30, and 45 and 60 minutes while systemic blood was sampled at 30, 45 and 60 minutes. Collected blood samples were replaced with equivalent volumes of heparinized saline. All blood samples were centrifuged to separate the plasma fraction for HPLC analysis.

At the end of each perfusion, the cannulations were examined visually to verify integrity and the animals

were sacrificed with a sodium pentobarbital overdose (125 mg/kg).

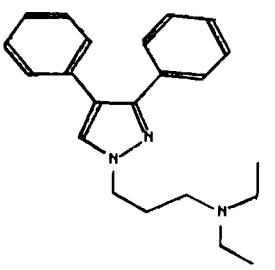
Standard Preparation

All solvents were HPLC grade (J.T. Baker Inc., Phillipsburg, NJ). Solutions of ipazilide and NDI were similarly prepared by dissolving 100.0 mg of compound in purified water, to achieve a 2 mg/mL concentration. Stock solutions of each drug were made by adding 50 μL of the aqueous solution to 950 μL of unmedicated rat plasma. Appropriate dilutions were made in rat plasma from the stock solutions, yielding concentrations of 6, 4, 2, 1, and 0.5 mcg/mL of each standard. Ipazilide and NDI standards were prepared in blank Krebb's buffer for perfusate assays.

Sample Preparation

Samples were prepared for analysis by solid phase extraction (Bond Elut CN cartridge, 1 mL, Analytichem International, Harbor City, CA). The cartridge sorbent was conditioned by washing with 1 mL portions of methanol, water and sodium borate buffer (0.05 M, pH 11.2).

To 100 μL of each plasma sample and standard in plasma, 100 μL of internal standard (4 μg/mL) was added. The preparation was vortex mixed for 30 seconds and transferred to the prepared Bond Elut column. Following evacuation of the plasma, the sorbent was washed with two



Internal Standard

4 mcg/mL in water N,N-Diethyl-3,4-diphenyl-1H-pyrazole-1-propanamine

1 mL portions of the sodium borate buffer (0.05 M, pH 11.2) and these eluents discarded. Two 1 mL methanol aliquots were added and the eluents were evaporated at 40°C under continuous nitrogen flow and then reconstituted with 300 μL of mobile phase.

Analysis

The reconstituted samples were assayed by HPLC using the following conditions:

Mobile Phase: Acetonitrile - 0.05 M ammonium acetate (aqueous) - glacial acetic acid (500:500:2).

Flow Rate: 1.5 mL/min (Model 510 Pump, Waters Associates, Milford, MA).

Injection: 50 μL for plasma samples and 5 μL for perfusates (WISP 710B, Waters Associates, Milford, MA).

Guard Column: New Guard RP-18; 15 x 3.2 mm, 7 μm, (Brownlee Labs, Santa Clara, CA).

Analytical Column: Partisil 10, ODS-3, 240 x 4.6 mm (Whatman Chemical Separation Inc., Clifton, NJ).

Detection: 254 nm, 0.005 aufs for plasma samples and 2 aufs for perfusates (Spectroflow 757 Variable Wavelength detector, Kratos Analytical, Ramsey, NJ).

The perfusates were assayed by direct injection using the same HPLC conditions. Standards were freshly prepared prior to use at 80, 100 and 120% of the target assay values for the unperfused solutions. Drug concentrations were calculated by linear regression analysis of the peak heights. The interpolated values of the perfusate were corrected for intestinal water flux by using scintillation readings (Tri-Carb 1500, Packard Instrument Company, Warrensville Road, Donner Grove, IL) of the unperfused and perfused samples in accordance with equation 1.

$$\frac{\text{DPM Unperfused}}{\text{DPM Perfusate}} \times \text{Interpolated Value} = \text{Corrected Concentration} \quad \text{Eq. 1}$$

Calculations

Linear regression analysis of the peak height ratios of drug/internal standards was used to interpolate concentrations in the sample plasma.

RESULTS AND DISCUSSION

HPLC Assays

A typical chromatogram for ipazilide, NDI, and the internal standard extracted from rat plasma is shown with a chromatogram of a blank plasma extract in Fig. 1.

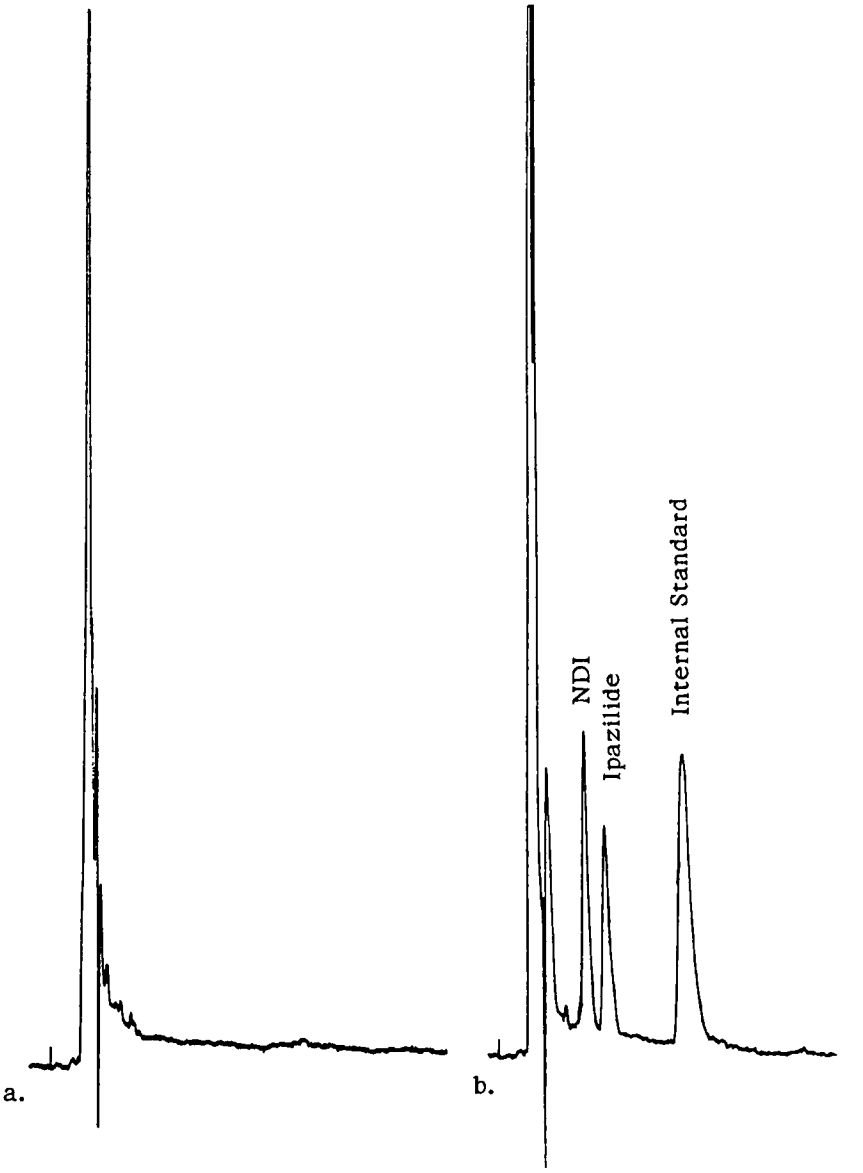


FIGURE 1

Typical HPLC chromatogram of blank rat plasma (a) and of NDI (3 μg/mL), ipazilide (3 μg/mL) and the internal standard (4 μg/mL) extracted from rat plasma (b).

TABLE 1

Typical Statistics of Plasma Calibration Curves for Ipazilide and NDI Calibration Data in Rat Plasma

Range	$S^a_{y,x}$	m^b	S^c_m	b^d	$S_b{}^e$	n^f	r^g
Ipazilide	0.30	3.34	0.157	-0.10	0.21	5	0.997
0.5-6 μg/mL	0.30	3.45	0.02	-0.17	0.02	5	0.999
	0.31	3.28	0.12	0.02	0.16	5	0.998
NDI	0.36	2.81	0.17	0.01	0.27	5	0.995
0.5-6 μg/mL	0.38	2.67	0.19	0.31	0.32	5	0.993
	0.34	3.00	0.17	0.07	0.26	5	0.995

[a]Standard error of estimate about regression of concentration (μg/mL) on peak height ratio. [b]Slope. [c]Standard error of slope. [d]Intercept. [e]Standard error of intercept. [f]Number of calibration points. [g]Correlation coefficient.

Typical standard calibration data are shown in Table 1 for ipazilide and NDI references in rat plasma. Correlation coefficients were 0.997 for ipazilide and 0.995 for NDI (Table 1). The minimum quantifiable level was 0.50 μg/mL for both compounds.

Ipazilide Intestinal Perfusion

Following jejunal perfusion with 1.54 mg/mL or 15.4 mg/mL solutions, the perfusate concentrations remained unchanged (Fig. 2). NDI was not detected in the perfusate and neither ipazilide or NDI were detected in systemic or portal plasma samples. Therefore, ipazilide was not absorbed from the rat jejunum and there was no

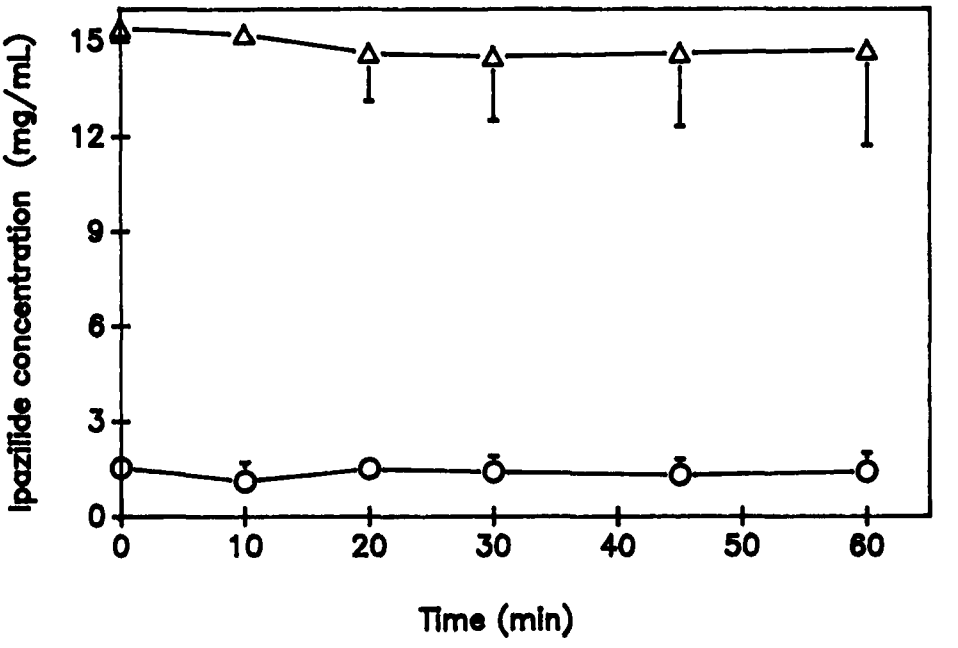


FIGURE 2
Plots of jejunal perfusate concentrations of ipazilide versus time (doses: 1.54 mg/mL, O; 15.4 mg/mL, Δ; n=4).

jejunal metabolism of ipazilide. Jejunal peristalsis was visually unaffected and no discoloration of the organ was seen.

Ipazilide concentrations in the duodenal perfusate decreased during the perfusion (Fig. 3). NDI was undetected in the perfusates. Perfusion of the duodenum with 15.4 mg/mL ipazilide resulted in loss of peristalsis within 30 minutes. The duodenum became discolored within 45 minutes of perfusion with the 15.4 mg/mL solution. At this point, the perfusion was concluded due to compromised duodenal integrity. Perfusate concentrations

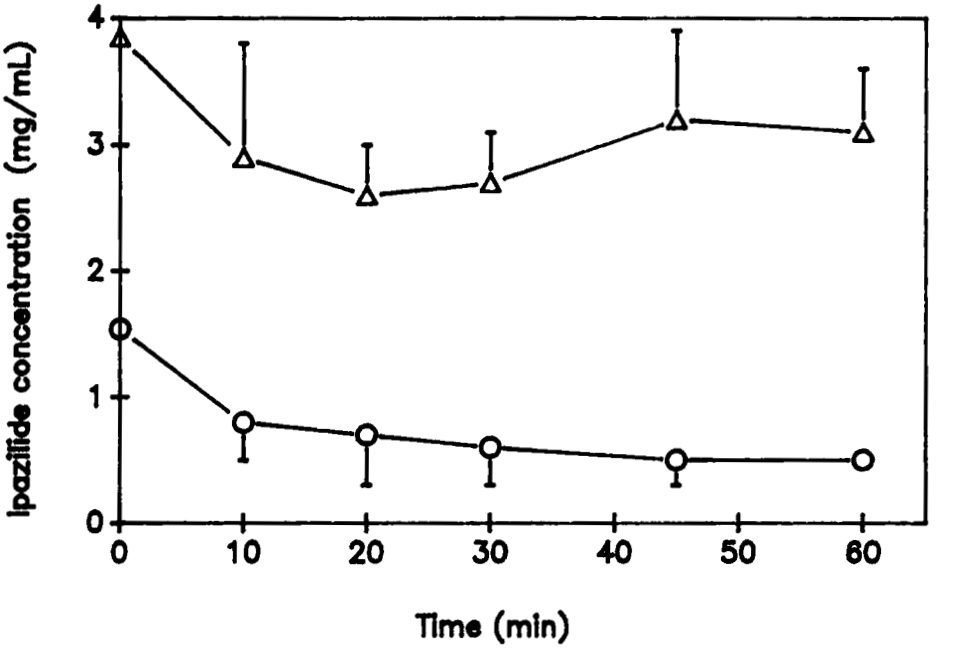


FIGURE 3
Plots of duodenal perfusate concentrations of ipazilide versus time (doses: 1.54 mg/mL, O; 3.85 mg/mL, Δ; n=4).

of the 1.54 and 3.85 mg/mL ipazilide solutions reached steady state within 10 minutes of the perfusion. Duodenal perfusion with ipazilide concentrations of 1.54 and 3.85 mg/mL caused no discoloration or apparent loss of peristalsis.

Portal blood plasma levels of ipazilide reached steady state at 30 minutes during duodenal perfusion of 1.54 mg/mL, and at 45 minutes with the 3.85 mg/mL solution (Fig. 4).

Steady state portal plasma levels were not observed with the duodenal perfusion of 15.4 mg/mL ipazilide.

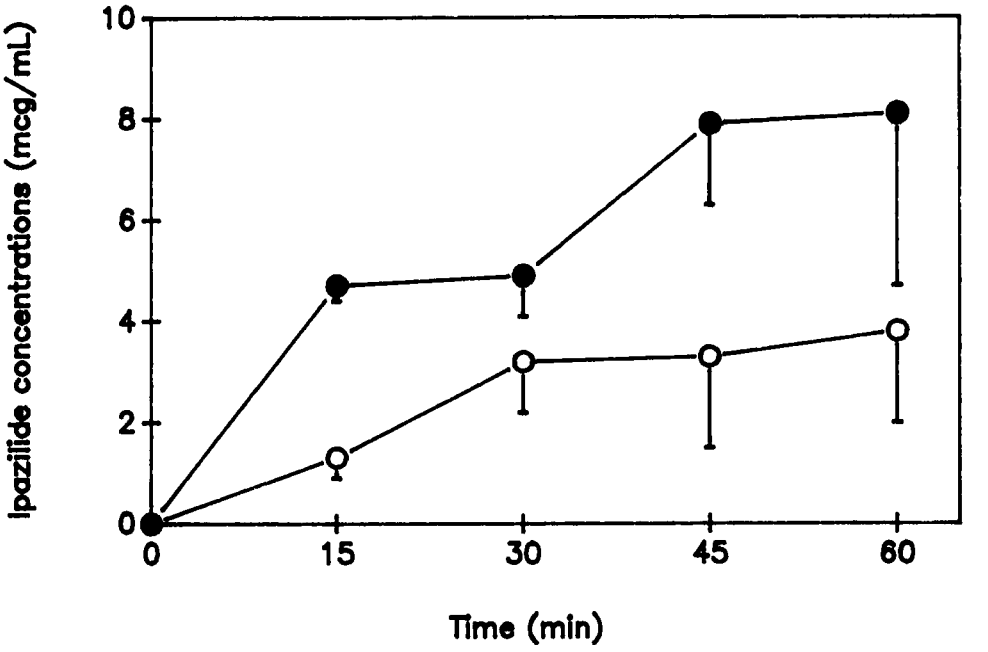


FIGURE 4
Plots of hepatic portal plasma concentrations of ipazilide following duodenal perfusions (doses: 1.54 mg/mL, O; 3.85 mg/mL, ●; n=4).

However, with the 15.4 mg/mL solution, NDI was detected in systemic plasma samples.

NDI Intestinal Perfusion

The duodenal and jejunal perfusions of NDI at 3.6 mg/mL did not result in significant differences for drug absorption, hepatic portal plasma levels, or systemic plasma concentrations (Fig. 5, 6 and 7). Steady state hepatic portal levels were reached within 30 min for the duodenal perfusion whereas the jejunal perfusion resulted in declining portal blood levels after 30 min. The

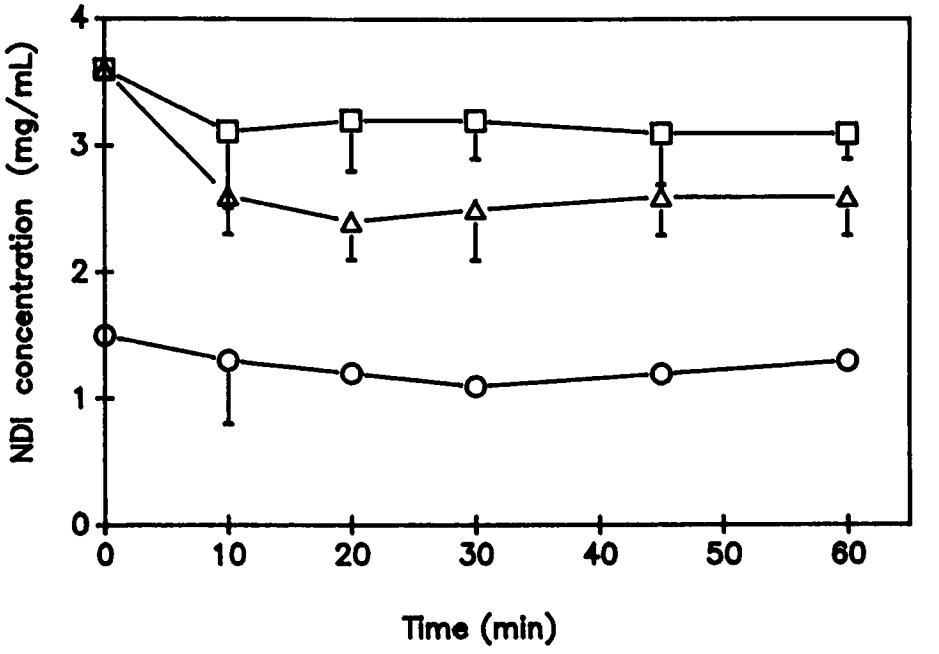


FIGURE 5

Plots of NDI perfusate concentrations following jejunal administration (3.60 mg/mL dose, □) and duodenal (3.60 mg/mL dose, Δ; 1.50 mg/mL dose, O; n=4).

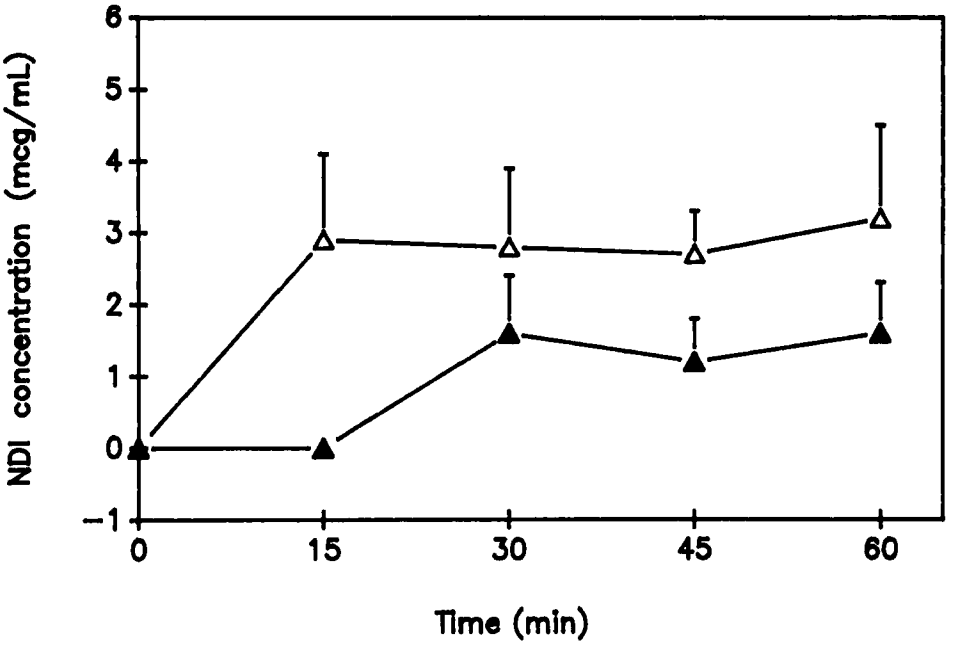


FIGURE 6

Plots of hepatic portal plasma (Δ) and systemic plasma (▲) concentrations following duodenal perfusion with 3.6 mg/mL NDI, n=4.

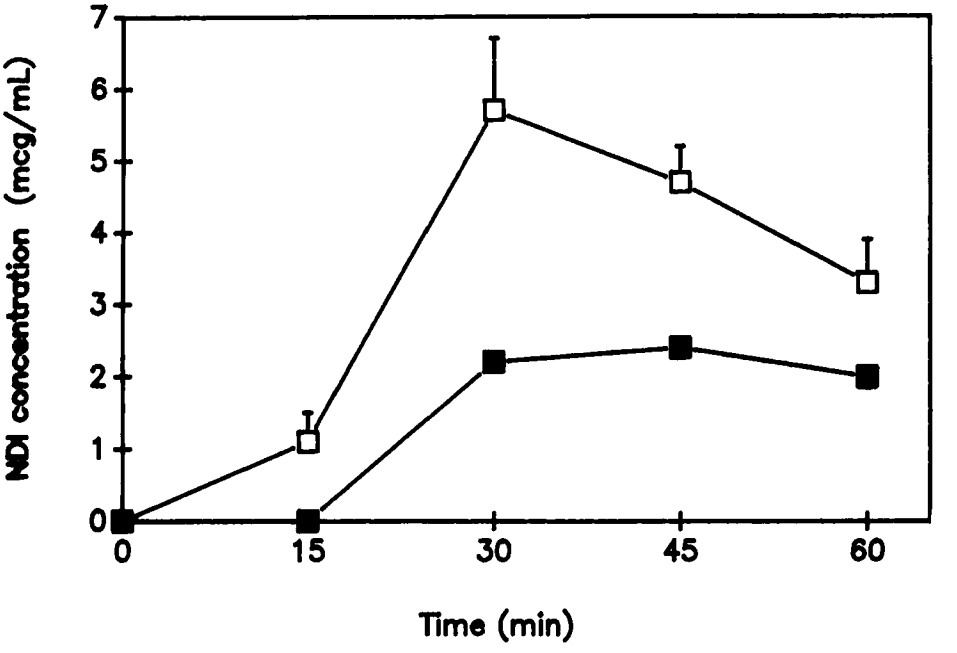


FIGURE 7
Plots of hepatic portal plasma (□) and systemic plasma (■) concentrations following jejunal perfusion with 3.6 mg/mL NDI, n=4.

duodenal perfusion at 1.5 mg/mL showed continued increases in portal plasma NDI concentrations (Fig. 8). Steady state was not achieved, but significant systemic plasma concentrations were detected within 45 min.

Detection of systemic levels of perfused NDI is in contrast to the absence of any systemic ipazilide, using similar duodenal perfusion concentrations. This could be a result of less extensive liver metabolism of NDI and/or smaller volume of distribution. No overt effects of NDI on the intestine were seen. NDI was equally well absorbed from the duodenum and the jejunum, in contrast

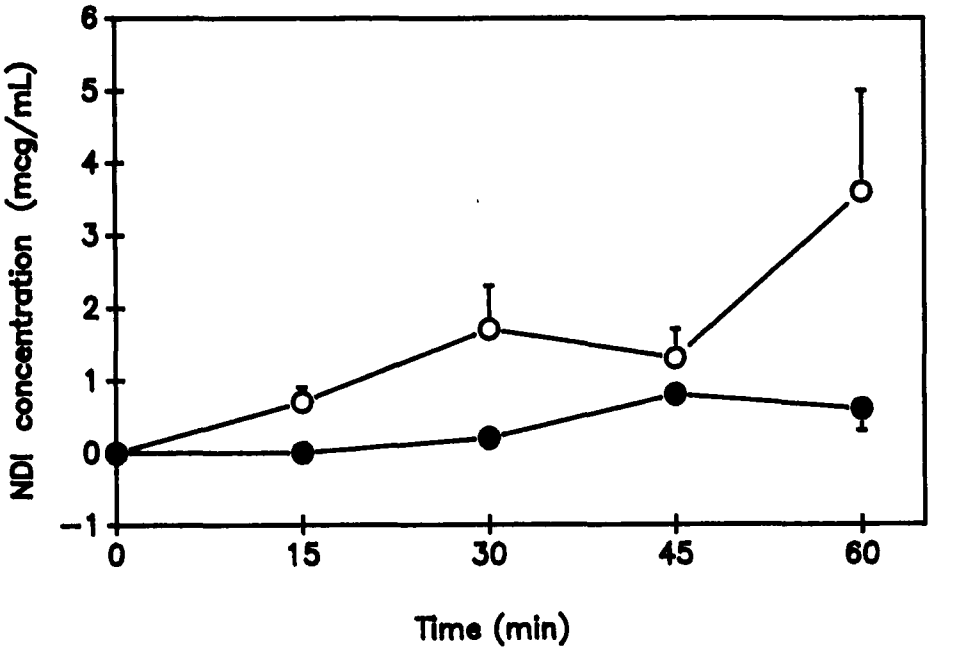


FIGURE 8
Plots of hepatic portal plasma (O) and systemic plasma (●) concentrations of NDI following 1.5 mg/mL duodenal perfusion, n=4.

to ipazilide which was only absorbed from the duodenum. The lack of absorption of ipazilide from the rat jejunum was surprising especially in consideration of the small changes expected in any physical-chemical properties between ipazilide and its des-ethyl derivative. While differential absorption of compounds occur along the different sections of the GI tract and carrier-mediated processes are site specific, the total lack of absorption for ipazilide from the jejunum was unexpected.

CONCLUSION

The in-situ intestinal perfusion studies have shown that a small chemical change (desethylation) can profoundly alter absorption characteristics, and physiologic function. Ipazilide is well absorbed from the rat duodenum but not the jejunum, whereas the desethyl derivative was equally well absorbed from both intestinal segments. Systemic levels of NDI were readily achieved but only traces of ipazilide were detected in the systemic circulation. NDI was shown to be a metabolite of ipazilide, but the metabolism was not due to intestinal lumen activities.

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