Biological Disposition of Noscapine I

Kinetics of Metabolism, Urinary Excretion, and Organ Distribution

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Biologic disposition studies of noscapine in the rabbit, rat, and mouse indicate a rapid rate of biotransformation of this compound. When noscapine was administered intravenously to rabbits, it disappeared from the blood by first-order kinetics with a half-life of about 9 minutes. In mice, the drug was found to have a half-life of 8.8 minutes and a first-order rate constant of 7.9×10^{-2} minutes^{-1}. Tissue distribution of noscapine in rats indicates that it is taken up by various organs within 1 minute after intravenous administration. High concentrations were found in the brain and the kidneys. However, the drug disappeared rapidly from most of the organs studied, especially from the brain which, at the end of 30 minutes, was virtually free of noscapine. Less than 1 per cent of the injected dose can be recovered unchanged from the urine of rabbits.

NOSCAPINE IS one of the principal alkaloids of opium where it occurs in concentrations ranging from 4 to 10%. It was isolated in 1817 by Robiquet, who named it narcotine. Actually, the narcotic properties of noscapine are rather insignificant. Its most important pharmacological activity is its antitussive effect, which was pointed out first by Chopra *et al.* in 1930 (1). In recent years, extensive pharmacological and clinical investigations have confirmed its value as a potent nonaddictive cough suppressant (2, 3).

Little is known about the fate and biological disposition of noscapine. Cooper and Hatcher (4) found that the drug disappeared rapidly from the blood after it was administered intravenously to cats. Only traces of noscapine could be detected in the urine over a period of several days following administration. These findings were confirmed by Vedsö (5), who used humans. He observed that noscapine was excreted in the urine partly free and partly as a metabolite which, on acid hydrolysis, produced a noscapine-like substance. The total excretion of noscapine and metabolite was less than 1% during the first 6 hours after administration. Vedsö concluded that the rapid disappearance of noscapine from the circulation was not due to renal excretion but was caused by the drug being deposited in the tissues. However, no conclusive evidence was presented to support this. The present investigation was undertaken to clarify many of the problems associated with the biological disposition of noscapine.

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A primary requirement for such a study is a sensitive method of analysis capable of quantitative determination of submicrogram amounts of noscapine. A number of analytical procedures have been described for noscapine, but the first method to possess satisfactory sensitivity and specificity was developed by Vedsö (6). It is based on production of an intensely fluorescent compound when noscapine is autoclaved in an alkaline buffer solution.

EXPERIMENTAL

The alkaloid used in our experiments was noscapine hydrochloride N. F. XI.

Estimation of Noscapine.—The method described by Vedsö was modified to increase its specificity and to reduce blank values. Vedsö measured the fluorescence of an ether extract of noscapine in the range of 480 to 580 mμ, using the 365 mμ line of mercury for excitation. The authors found, however, that the excitation spectrum has three maxima; the most intense is at 260 mμ. The fluorescence spectrum has a single maximum at 470 mμ (Fig. 1). The specificity of the Vedsö method was improved greatly when the excitation was carried out at 260 mμ and the intensity of the fluorescence measured at 470 mμ. The linear relationship between the concentration of noscapine and the fluorescence is illustrated in Fig. 2.

For extraction of noscapine from biological fluids, it was important to select a solvent system which would permit a high recovery of the alkaloid with single extraction techniques and at the same time keep the extraction of extraneous materials to a minimum. In a series of experiments, aqueous solutions of noscapine hydrochloride and rabbit urine containing known quantities of the drug were made alkaline and extracted with an organic solvent. The organic layer was separated and extracted with 0.1 *N* hydrochloric acid, as described under *Procedure*. Blank values were determined from extracted urine samples without added noscapine. The recoveries of noscapine and the blank readings obtained with the different solvent systems are given in Table I. A solvent mixture containing 4 parts of cyclohexane and 1 part of benzene was chosen as most satisfactory.

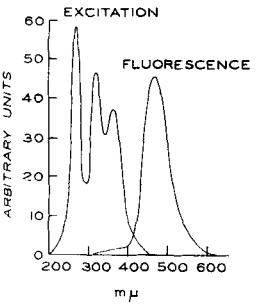

Fig. 1.—Excitation and fluoroescence spectra of noscapine after autoclaving at pH 9.2.

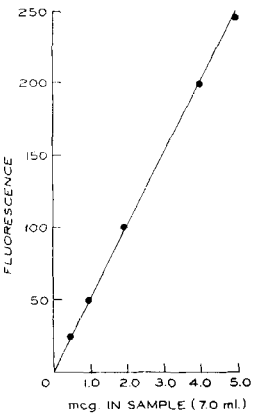

Fig. 2.—Standard curve for noscapine.

TABLE I.—RECOVERIES OF NOSCAPINE AND URINE BLANK READINGS OBTAINED AFTER A SINGLE EXTRACTION WITH VARIOUS SOLVENTS

Organic Solvent	Recovery of Noscapine[a] Aqueous Soln., %	Rabbit Urine, %	Urine Blank[a] Arbitrary Units
Ether	65.8	54.5	16
Chloroform	3.3	2.6	11
Benzene	75.7	63.8	9
Cyclohexane–benzene (4:1)	99.4	89.4	3

[a] Average values based on at least two determinations.

Procedure.—Acidified urine or tissue homogenate was diluted with 1 *N* hydrochloric acid to a concentration preferably in the range of 0.5 to 5 mcg. of noscapine per milliliter. One milliliter was pipeted into a 15-ml. glass-stoppered centrifuge tube, made alkaline with solid sodium bicarbonate, and extracted with 5.0 ml. of a cyclohexane–benzene mixture (4:1). The layers were separated by centrifugation, and 4.0 ml. of the organic phase was transferred to another centrifuge tube and shaken with 6.0 ml. of 0.1 *N* hydrochloric acid. After centrifugation, 5.0 ml. of the acid layer was placed in a Pyrex test tube, mixed with 0.5 ml. of 1 *N* sodium hydroxide and 1.5 ml. of borate buffer of pH 9.2. The test tube was covered with aluminum foil and heated in an autoclave at 120° for 30 minutes. After cooling to room temperature, the fluorescence was determined at 470 mμ in a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 260 mμ.

For the analysis of blood samples, 1.0 ml. of blood was precipitated with 1.0 ml. of 10% trichloroacetic acid. The mixture was centrifuged, and 1.0 ml. of the supernatant liquid was analyzed as described.

Blank readings were obtained for the various fluids and tissues and subtracted from the sample readings. Since the blank values were very low (equivalent to less than 0.05 mcg. of noscapine) and remained reasonably constant for a particular fluid or tissue, average blank values were used for the routine analyses.

The recoveries of authentic noscapine added to biological fluids and tissue homogenates are shown in Table II.

Specificity.—The specificity of the analytical procedure was studied by Vedsö (6), who observed no fluorescence from codeine, narceine, and papaverine when treated in the same way and in the same concentration range as noscapine. However, morphine gave an increase in fluorescence after autoclaving of about one-half that produced by noscapine. This is probably due to formation of pseudomorphine. When the method is used for the drug in biological media, a more satisfactory test for specificity is to compare the partition behavior of the authentic drug with that recovered from biological materials following administration to the intact animal. For this purpose, noscapine hydrochloride was injected intravenously in mice. The animals were homogenized in 1 *N* hydrochloric acid 9 minutes after administration. The homogenate was alkalinized with sodium bicarbonate and extracted with cyclohexane–benzene (4:1). The amount of noscapine present in a 5.0-ml. aliquot of the organic extract was determined. Separate 5.0-ml. portions were equilibrated with buffer

TABLE II.—RECOVERIES OF NOSCAPINE AFTER ADDITION TO AQUEOUS SOLUTIONS, RABBIT URINE, RABBIT FECES, AND RAT TISSUES

Material	Recovery %[a]	Recovery Range
Aqueous soln.	99.4	(99.1–100.2)
Rabbit urine	89.4	(87.6– 92.4)
Rabbit feces	80.4	(77.6– 82.3)
Rat blood	88.0	(86.0– 90.6)
Rat intestines	99.5	(99.0–100.0)
Rat brain	95.0	(94.1– 96.0)
Rat skeletal muscle	85.0	(82.3– 87.7)
Rat kidney	90.1	(88.4– 91.6)
Rat lung	94.6	(93.2– 96.0)
Rat liver	75.0	(71.2– 79.0)

[a] Average values based on three animals.

TABLE III.—PARTITION RATIO OF AUTHENTIC NOSCAPINE AND POSTADMINISTRATION NOSCAPINE AT DIFFERENT PH VALUES[a]

Buffer, pH	Concn. in Org. Phase/Total Concn. Authentic Noscapine	Concn. in Org. Phase/Total Concn. Postadministration Noscapine
3.0	0.03	0.03
4.0	0.22	0.23
4.5	0.58	0.54
5.0	0.76	0.80
6.0	0.96	0.94
8.0	1.02	1.00

[a] Concentration in organic phase/total concentration.

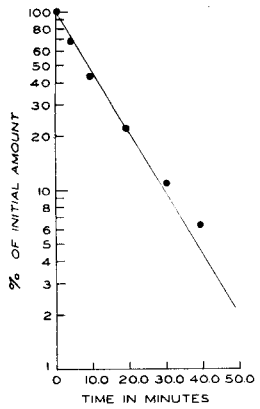

Fig. 3.—First-order plot for the disappearance of injected noscapine in mice.

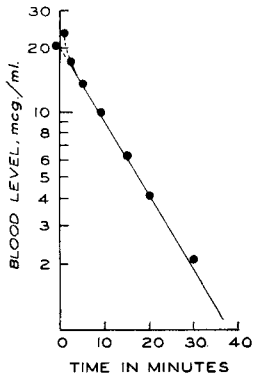

Fig. 4.—First-order plot for the disappearance of noscapine from the blood of rabbits.

solutions of different pH values, and the relative amounts of noscapine left in the organic layers were calculated. The same procedure was repeated with an aqueous solution of noscapine hydrochloride. Table III shows that there is good agreement between the partition ratios for authentic noscapine and for the drug isolated after injection in mice. This indicates that the method estimates noscapine with a high degree of specificity.

Kinetics of Metabolism.—The rate of metabolism of noscapine was studied in intact mice. Noscapine hydrochloride, 50 mg./Kg., was injected into the tail vein of albino mice (Webster strain) weighing 20 ± 4 Gm. (This dose killed none of our mice in preliminary trials but caused convulsions in all of them.) The drug was administered in isotonic saline containing 2 mg./ml. of the alkaloid. At fixed time intervals, a mouse was homogenized in a Waring Blendor; 1 *N* hydrochloric acid was used to make a 1:10 w/v dilution of the mouse. The amount of noscapine in the homogenate was determined on 1.0-ml. aliquots as described.

When the results were plotted on semilogarithmic paper (Fig. 3), a straight line was obtained for at least 20 minutes after administration. Over this period, the disappearance of noscapine appears to follow first-order kinetics with a half-life of 8.8 minutes and a rate constant equal to 7.9×10^{-2} minutes^{-1}.

These studies indicate that noscapine has a relatively short life in the body and should not cumulate in body depots. An experiment to test this latter postulate was carried out as follows.

Mice weighing 18 to 22 Gm., were given approximately 100 mg./Kg. intraperitoneally at 4-hour intervals for 12 hours for a total of 8 mg. After 12 hours of rest, the mice were homogenized, and the amount of free noscapine was determined. Less than 1% (0.6 to 0.8) of the total dose was recovered.

Rate of Disappearance from the Blood.—Rabbits, weighing 3 to 4 Kg., were injected with heparin sodium, 500 I.U./Kg. through the marginal vein of the left ear; after 5 minutes, noscapine hydrochloride, 15 mg./Kg. dissolved in saline, was administered through the same ear. An intravenous catheter (Abbot Venocath. 18 gauge) was introduced in the right ear vein and held in position with adhesive tape. Almost immediately after administration of the alkaloid, the animals showed a brief period of excitation. Blood samples were withdrawn through the catheter at regular intervals and analyzed for noscapine. The results for one animal are illustrated graphically in Fig. 4. The drug disappeared from the blood by first-order kinetics, the half-life being 9.0 minutes. A second animal gave a half-life of 9.2 minutes.

Urinary and Fecal Excretion.—Rabbits, weighing about 3 Kg., were given approximately 15 mg./Kg. of noscapine hydrochloride intravenously. Total urine and feces were collected for 24 and 48 hours and analyzed for free noscapine. Fecal levels of the drug were too low to be detected (<0.1 mcg./Gm.). The urinary excretion is given in Table IV, which shows that less than 1% of the injected dose is excreted by this route as the unchanged drug. The excreted substance was identified as noscapine by countercurrent distribution (7) and by thin-layer chromatography. In the countercurrent distribution experiment, 2.0 ml. of postadministration rabbit urine was made alkaline with sodium bicarbonate and extracted with 10.0 ml. of cyclohexane–benzene (4:1). After centrifugation, 5.0 ml. of the organic layer was subjected to an eight-tube transfer

TABLE IV.—URINARY EXCRETION OF FREE NOSCAPINE IN THE RABBIT AFTER INTRAVENOUS ADMINISTRATION

Wt. of Rabbit, Kg.	Amt. of Noscapine Injected, mg.	Noscapine in Urine, mg. 0–24 Hr.	24–48 Hr.	Dose Excreted, %
3.5	50	0.20	0.0	0.4
3.0	45	0.14	0.0	0.3
3.4	45	0.40	0.0	0.9

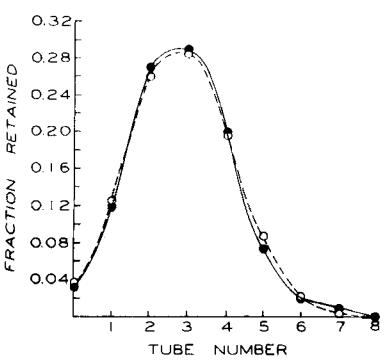

Fig. 5.—Countercurrent distribution. Key:——, authentic noscapine; — — —, recovered noscapine from rabbit urine.

TABLE V.—TISSUE DISTRIBUTION OF NOSCAPINE AFTER INTRAVENOUS ADMINISTRATION OF 25 mg./Kg. IN RATS[a]

Tissue	mcg./Gm. 1 Min.	2 Min.	8 Min.	15 Min.	30 Min.	60 Min.
Blood	35.0	23.6	11.7	6.4	3.2	0.4
Brain	140.3	51.3	15.0	8.2	0.0	0.0
Kidneys	220.4	83.3	23.5	16.7	7.0	1.9
Lungs	82.5	56.9	27.5	21.2	15.0	4.0
Liver	70.0	42.8	40.8	33.0	14.8	5.6
Skeletal muscle	32.0	20.9	12.3	9.6	4.6	<0.1
Small intestine	...	28.8	10.5	21.9	22.2	7.3

[a] The results are average values based on two rats.

with an equal volume of pH 4.5 McIlvaine buffer as the stationary lower phase. At the end of the operation, each tube was analyzed for total noscapine, and the fraction contained in each tube was calculated. The experimental curve was fitted to the theoretical countercurrent distribution curve for noscapine according to the method of Way and Bennett (8). Figure 5 illustrates the countercurrent distribution of the recovered substance between cyclohexane–benzene (4:1) and McIlvaine buffer of pH 4.5. The distribution behavior of the excreted material is consistent for that of a single substance and is practically identical with the theoretical curve for authentic noscapine. Thin-layer chromatography of the urine extract on silica gel G with a solvent system consisting of ethanol and benzene (1:4) (9) gave, on spraying with Dragendorff's reagent, an alkaloidal spot having the same R_f value as that of noscapine.

Organ and Tissue Distribution.—Long Evans rats, weighing 300 to 400 Gm., were used for studying the distribution of noscapine in various organs and tissues of the body. An intravenous dose of 25 mg./Kg. was administered to each animal. At fixed time intervals, the rats were decapitated, and the blood was collected immediately in a beaker containing 1.0 ml. of heparinized saline. The various organs and tissues were removed quickly and homogenized in 1 *N* hydrochloric acid, and aliquots were analyzed for free noscapine. The results are given in Table V and show that noscapine appeared in all the tissues soon after administration. High concentrations were found in the brain and the kidneys within 1 minute, but the drug disappeared at a rapid rate. A somewhat slower rate of disappearance was observed for the organs and tissues; in the intestine, the level appeared to be relatively constant over a 30-minute period. The high initial level in the brain is interesting in view of the brief period of convulsion or hyperexcitation produced in the animals after administration. The transient nature of this CNS stimulation is consistent with the rapid disappearance of the drug from the brain.

DISCUSSION

The disposition of noscapine in general follows the distribution pattern described for most organic bases (10) because there is a rapid disappearance of the compound from the blood concomitant with a rapid tissue uptake by organs perfused by a rich vascular supply. The ability of certain organs to concentrate noscapine rapidly is indicated by the fact that at 1 minute the noscapine level in the kidneys of the rat exceeded that in the blood by more than sixfold. Brain levels at 1 minute were surprisingly high, exceeding those in all organs and tissues other than the kidneys. However, the drug concentration in the CNS fell off rapidly; by 30 minutes, it was not detectable, whereas other organs and tissues still exhibited appreciable concentrations of noscapine. At the end of 1 hour, however, low or no levels were detectable in the other organs and tissues.

These findings are not in harmony with the proposition suggested by Vedsö (5) that the rapid disappearance of noscapine in the blood is due to rapid and cumulative tissue uptake rather than a rapid rate of biotransformation. Our data indicate that, while organ uptake is indeed rapid, cumulation of the drug does not occur. Since renal and fecal excretion are not important factors, it would appear that the substance is metabolized in the body at a fast rate. The studies to date have shown that the biotransformation may go by several pathways and involve complex changes in the structure of the molecule. So far, there is evidence for the presence of at least two noscapine metabolites in the urine.

The results on the mouse, rabbit, and rat differ with respect to findings on humans (5). The rate of decay of noscapine in the plasma of humans is considerably slower than in the three species studied. Whether this is related to a slower rate of degradation of the compound awaits further studies.

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