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THE COMPARISON OF THE BIOLOGICAL BEHAVIOR OF MORPHINE AND ITS N-OXIDES, IN RATS

E. GÜRKAN - B. DEMİREL - K. ÖZKER

Çekmece Nuclear Research and Training Center,
P.O. Box 1. Havaalanı - Istanbul

ABSTRACT

^{14}C labelled morphine and morphine N-oxide solutions are injected into rats for the in vivo detection and comparison of morphine N-oxides, the natural presence of which in Papaver somniferum L. was proved by us in an earlier work /1/ and morphine itself and the distributions of these two substances in the kidneys, liver, blood and urine of the injected animals are investigated, in various time intervals.

ÖZET

Daha önceki bir çalışmamızda Papaver somniferum L. bitkisinde doğal olarak var olduğunu kanıtladığımız /1/ morfin N-oksitlerinin ve morfinin vücuttaki etki mekanizmasını saptamak için sıçanlara ^{14}C ile etiketli belirli miktar morfin ve morfin N-oksit çözeltileri ayrı ayrı zerkedilmiş ve bu iki maddenin, deney hayvanlarından alınan böbrek, karaciğer ve kan, idrar örneklerindeki dağılımları incelenmiştir.

INTRODUCTION

The aim of this work is to investigate the long-term biological behavior of morphine, the most important alkaloid of Papaver somniferum L. and its N-oxides and to compare the in vivo distributions of these two substances. The presence of morphine N-oxides in the latex of the plant, which are proved to be natural products /2-6/ and which are formed by oxidation from morphine itself, is proved by us through radioactive and chemical methods in an earlier work /1/.

Since we know that morphine is metabolized in the liver /7/ and excreted in urine, the radioactivities of the samples taken from liver, kidneys, blood and

urine are counted beginning from fifteen minutes after the IV injection to six hours intervals. The experiments made with N-oxide solutions were repeated with morphine samples as well.

EXPERIMENTAL

|N-methyl-^{14}C| morphine HCl is used as a tracer. For this reason, a solution of this radioisotope, which was bought in the crystal form from the Amersham Radiochemical Center (57 mCi/m mol, 2.1 GBq/m mol) is prepared as indicated below:

|N-methyl-^{14}C| morphine HCl (crystal)

↓

Dissolved in H_2O

↓

Made alkaline with NH_4OH

↓

Extracted three times with $CHCl_3$/IPA (3/1)v/v

↓

Activity measured

↓

The organic part is evaporated to dryness

↓

The residue is treated with 0.5 ml 1 % acetic acid

↓

Made up to known volume with mannitol buffer

↓

Activity measured /1/.

A known amount from the above mentioned morphine HCl solution is oxidized with H_2O_2 and MnO_2, thus morphine N-oxides are obtained and the presence of them are proved with thin-layer chromatography.

0.1 ml (0.005 gr/100 gr rat) N-oxide sample is injected to the rats (Rattus norvegicus) through the tail veins. The activity of this amount is measured to be 44,526,59 dpm/0.1 ml. The same procedure is repeated with 0.05 ml (0.00625 gr/100 gr rat) morphine HCl solution, the activity of which is measured to be 488,483.5 dpm/0.05 ml.

The rats are killed in various time intervals after the morphine and morphine N-oxide solutions are injected. Liver and kidney samples taken from the animals are solubilized in NCS, whereas the blood samples are decolorized with benzoyl poroxide after they are solubilized in NCS, /8/ and both these organs and the blood samples and the 24 hours urine samples are counted in a scintillator which contains PPO, POPOP and toluene. The results are shown in Table-1.

The Packard Tricarb 3003 Liquid Scintillation Counter is used throughout this work. The counting efficiency is determined from standard quench calibration curves by the "Channels Ratio Method" /9-12/.

RESULTS AND DISCUSSION

According to the works made by several investigators /13-15/, it is obvious to come to the conclusion that morphine turns into some substances which show alkaloidal specialities, but which in reality are not alkaloids themselves and that morphine is not the end product of the metabolic changes in the plant, as it was thought before. For this reason, we wanted to compare the distribution of morphine and its N-oxides in rats.

An important decrease of radioactivity can be seen in the percent injected dose of the samples taken from the rats injected with morphine solution, beginning from fifteen minutes to four hours, post-injection.

An increase beginning from the second hour is seen in the radioactivity of the liver samples, in the experiments which are made with N-oxides and which are carried out beginning from fifteen minutes to six hours (Figs.1,2) reaching to a peak at the fourth hour. This can be explained with the fact that morphine N-oxides may rapidly demethylize and accumulate in the liver, thus morphine being changed into nor-morphine.

The known amount of decrease in the morphine experiments (Figs.1,2) can be explained with the slow rate of demethylization of morphine in comparison with its N-oxides.

It is obvious that the blood samples taken from the rats injected with morphine N-oxides remain stable beginning from fifteen minutes. This again can be explained with the demethylization of morphine N-oxides.

TABLE-1. The in vivo distribution of the radioactivities of morphine and its N-oxides. (The radioactivity of the injected morphine solution is 488,483 dpm/0.05 ml/100 gr. rat. The radioactivity of the injected N-oxide solution is 44,526 dpm/0.1 ml/100 gr.rat. The results obtained are the averages of 3 experiments. In addition to this table, the amounts of excreted morphine and N-oxide solutions are 31 ± 0.51 % of the injected dose in the N-oxide sample, and 67 ± 0.34 % of the injected dose in the morphine sample.)

N-oxide
The average values of the amounts distributed to the organs, from the injected dose.

Morphine
The average values of the amounts distributed to the organs, from the injected dose.

	dpm %/ml.	dose %/organ		dpm %/ml.	dose %/organ	
Time	Blood	Liver	Kidney	Blood	Liver	Kidney
15 Min.	3.30 ± 0.26	10.40 ± 0.91	7.41 ± 1.04	0.41 - 0.03	8.20 - 1.10	1.81 - 0.51
30 "	2.78 ± 0.16	10.65 ± 0.35	5.24 ± 0.88	0.58 - 0.05	5.62 - 0.98	1.64 - 0.08
60 "	3.00 ± 0.36	9.96 ± 0.09	3.47 ± 0.52	0.27 - 0.03	3.30 - 0.81	0.96 - 0.10
120 "	2.95 ± 0.21	10.25 ± 0.91	2.86 ± 0.57	0.023- 0.01	2.34 - 0.75	0.52 - 0.16
240 "	2.84 ± 0.15	16.60 ± 1.43	2.02 ± 0.28	0.023- 0.01	0.55 - 0.09	0.039- 0.01
360 "	3.06 ± 0.20	5.70 ± 0.75	5.36 ± 0.83			

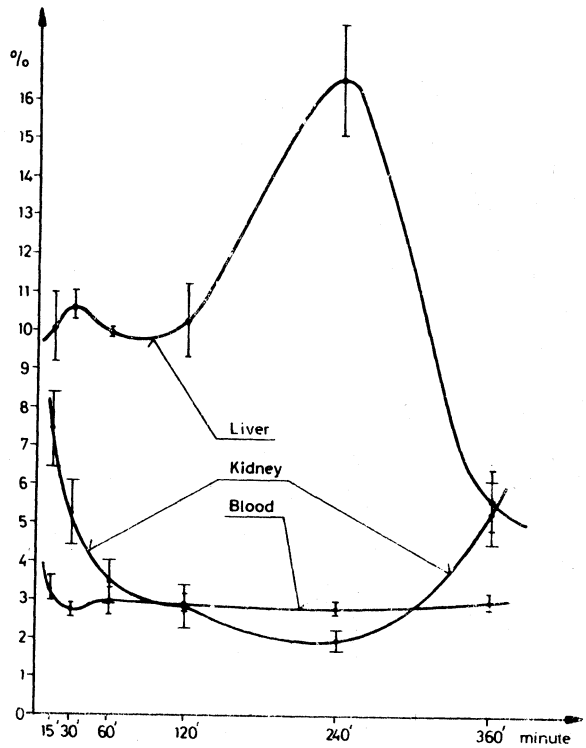


Fig. 1. The average N-oxide values of the amounts distributed to the organs, from the injected dose.

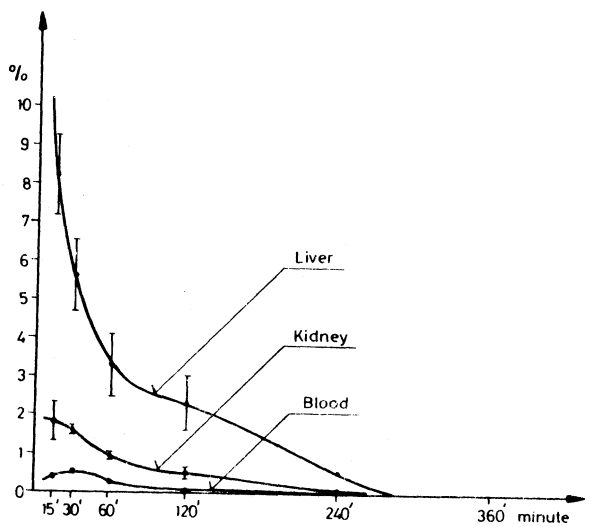


Fig. 2. The average morphine values of the amounts distributed to the organs, from the injected dose.

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