Arh. hig. rada toksikol., *34* (1983) 201—207

Original Scientific Paper
UDC 613.84:616-078

INVESTIGATION OF THE CONTENT AND RATIO OF COTININE AND NICOTINE-1'-N-OXIDE IN THE URINE OF SMOKERS

B. Nikolin, M. Lekić, M. A. Rashid Mahmud and O. Višnjevac
Faculty of Pharmacy, Institute for Pharmacy and Medicinal Chemistry, University of Sarajevo

(Received for publication June 18, 1982)

By metabolic degradation of nicotine in the organism of smokers are formed nicotine metabolites such as cotinine, nicotine-1'-N-Oxide, nicotine-1, 1'-N-dioxide and others. Recently, special attention has been drawn to the ratios of these metabolites in the biological fluids of diseased smokers.

In this work the urinary content of nicotine, cotinine and nicotine-1'-N-oxide was analysed and their ratios were determined. This and other studies suggest that the ratios of these components in urine extracts may play a role in the early diagnosis of the cancer of some organs.

The alkaloid nicotine constitutes the chief active principle of tobacco. Its amount in various types of tobacco varies widely. Besides nicotine, other alkaloids like nornicotine, anatabine, nicotyrine and anabasine are also present in tobacco.

In the process of smoking, nicotine enters the organism and is eliminated in urine partly unchanged and partly in the form of metabolites.

The determination of nicotine and its metabolites in the biological fluids has been subject of many investigations. Cotinine (1) and nicotine-1'-N-oxide (2, 3, 4) are known to possess carcinogenic properties.

Beckett and co-workers (5) reported the urinary excretion of nicotine-1'-N-oxide in smokers under normal conditions to be about half that of cotinine. The same authors (6) examined the proportion of nicotine and cotinine in smokers and non-smokers after intravenous administration of nicotine in the form of nicotine hydrogen tartarate.

Gorrod and co-workers (7) determined the concentrations of cotinine and nicotine-1'-N-oxide by gas chromatography in the urine of smokers with cancer of the urinary bladder, and compared their ratio with the

ratio of the same metabolites in the urine of healthy smokers. In 80% of the smokers with cancer of the urinary bladder, the cotinine/nicotine-l'-N-oxide ratio increased 3—10 times. These results give rise to the question whether, in patients with liver and lung cancer, the cotinine/nicotine-l'-N-oxide ratio is also altered and in which direction.

Many analytical methods have been developed for the isolation, identification and estimation of nicotine and its metabolites in the biological fluids of humans and in various animal tissues. At present gas chromatography is also frequently used as a very sensitive method.

McNiven and co-workers (8) determined the contents of nicotine and cotinine in the urine of smokers by gas chromatography on a 14.5% SE—30 column with a strontium—90 argon detector, using 3-methyl-3 phenylpiperidine as internal standard. In their determination of the nicotine urinary content *Beckett and Triggs* (9) used a column of 2% Carbowax 20M mixed with 2% KOH, and chlorphentermine as internal standard.

MATERIAL AND METHODS

Nicotine, A. R., was of E. Merck, Germany provenience. Cotinine and nicotine-l'-N-oxide were synthesized from nicotine by the authors with the methods described in literature (10, 11).

Urine was collected from the patients with a diagnosis of liver and lung cancer, stationed in the Surgical and Internal Clinic, University Medical Centre, Sarajevo, and in the Hospital for Pulmonary and Internal Diseases, Kasindol, Sarajevo.

Chromatography was carried out on a Perkin Elmer F—17 gas chromatograph with a flame ionization detector. The chromatographic conditions were tested with the following liquid phases and columns: 3% SE—30 Chromosorb W HP 100—120 mesh, metallic, 1 m x 3 mm; 2% Carbowax 20M, 5% KOH Chromosorb W NAW 80—100 mesh, metallic, 1 m x 3 mm; 10% SE—30, Chromosorb W NAW 80—100 mesh, glass, 2 m x 6 mm; and 3% OV—17, Chromosorb GAW TMCS 80—100 mesh, metallic, 1 m x 6 mm.

In order to find out suitable internal standards, the following substances were tested: amphetamine sulphate, ephedrine sulphate, ethylbutylamide, propylbutylamide, nikethamide, quinoline, codeine phosphate, diphenhydramine hydrochloride, 4-methylpiperidine, 4-methylpiperazine and pyridine.

For the actual determination of nicotine and its metabolites in the extracts, two columns, viz. 10% SE—30 and 3% OV—17, which proved satisfactory for the analysis, were used. As internal standard for analysis of nicotine, freshly distilled quinoline was used, and for analysis of cotinine the most suitable internal standard was codeine.

Table 1.

Urinary content of nicotine, cotinine and nicotine-1'N-oxide in healthy smokers

Subjects		Number of cigarettes smoked per day	Urine		Content (mg/24 h) of			Cotinine/nicotine-1'-N-oxide
Sex	Age		Volume (ml)	pH	Nicotine	Cotinine	Nicotine-1'-N-oxide	
m	27	30	920	6.5	0.44	2.90	4.54	0.64
m	27	20	2500	6.7	3.21	6.38	7.50	0.85
m	36	40	850	6.5	3.21	4.68	6.16	0.76
m	21	60	970	6.4	0.81	1.65	2.19	0.75
m	35	40	940	6.3	0.52	1.41	1.65	0.85
m	24	30	960	6.1	0.98	5.31	2.74	1.94
m	28	25	950	6.0	1.64	1.35	1.10	1.23
f	23	25	1000	6.0	1.35	2.11	1.80	1.17
m	30	20	580	6.3	2.68	3.90	2.25	1.73
m	32	20	1270	6.2	5.38	3.84	9.49	0.40
m	22	20	730	6.3	0.99	2.65	2.65	0.37
f	25	20	900	6.4	0.99	0.85	0.59	1.44
m	50	30	2050	5.5	2.38	0.38	0.95	0.40
m	30	20	980	5.6	4.16	0.64	3.76	0.17
m	40	30	1210	5.0	6.41	0.31	5.99	0.05
f	45	30	890	5.9	7.48	0.77	6.27	0.12
m	45	20	1050	5.5	4.17	0.29	2.52	0.11
m	50	20	1410	5.5	2.58	0.53	1.07	0.50
Mean	—	—	1120	6.1	—	—	—	0.75
S. D.	—	—	470	0.5	—	—	—	0.56

Table 2.

Urinary content of nicotine, cotinine and nicotine-1'-N-oxide in liver cancer patients

Subjects		Number of cigarettes smoked per day	Urine		Content (mg/24 h) of			Cotinine/nicotine-1'-N-oxide
Sex	Age		Volume (ml)	pH	Nicotine	Cotinine	Nicotine-1'-N-oxide	
m	75	20	90	6.3	0.22	0.19	0.07	2.7
m	62	25	1120	6.5	0.28	1.72	0.35	4.9
f	55	40	1090	5.9	1.34	3.30	0.57	5.8
m	68	30	1550	5.0	1.75	4.30	0.66	6.5
m	71	20	1430	5.5	0.98	2.40	0.33	7.2
m	35	60	1260	6.0	3.49	7.90	0.99	7.9
Mean	—	—	1090	5.9	—	—	—	5.8
S. D.	—	—	520	0.5	—	—	—	1.9

Table 3.

Urinary content of nicotine, cotinine and nicotine-1'-N-oxide in liver cancer patients

Subjects		Number of cigarettes smoked per day	Urine		Content (mg/24 h) of			Cotinine/nicotine-1'-N-oxide
Sex	Age		Volume (ml)	pH	Nicotine	Cotinine	Nicotine-1'-N-oxide	
m	48	10	1500	6.4	0.34	0.75	0.25	3.0
f	36	20	1240	6.2	1.77	4.46	0.46	9.7
m	55	60	1860	6.1	11.30	1.58	0.51	3.1
f	60	5	830	6.1	0.25	0.25	0.05	5.0
f	72	5	300	6.7	0.08	0.15	0.02	7.5
m	45	25	1460	6.7	3.28	11.07	1.04	10.6
m	55	10	1420	6.7	0.62	10.90	0.82	13.3
m	65	10	200	6.5	0.92	0.14	0.02	7.0
m	59	20	990	5.5	3.92	8.40	0.96	8.8
m	35	15	1100	5.5	1.69	3.60	0.48	7.5
m	40	25	1310	5.0	5.55	10.00	1.44	7.0
Mean	—	—	1110	6.1	—	—	—	7.5
S. D.	—	—	500	0.6	—	—	—	3.1

The volume of 24-hour urine samples and pH values of the samples were measured, and the concentrations of nicotine, cotinine and nicotine-l'-N-oxide were determined.

Nicotine and cotinine in urine were extracted by the method of *Beckett and Triggs* (9), while nicotine-l'-N-oxide was extracted by the method of *Beckett and co-workers* (5).

For the extraction of nicotine in urine, the internal marker quinoline (50 μg/ml) was added to 50 ml of urine. It was then acidified with 5M HCl and extracted with ether. The acidic ether extract was discarded, and the urine was made alkaline with 5M NaOH and extracted again with ether. The combined alkaline ether extracts were evaporated to a small volume at about 40 °C and analysed by gas chromatography.

Cotinine was extracted from urine in an alkaline medium with dichloromethane. Codeine was used as internal standard. Nicotine-l'-N-oxide was determined in the form of nicotine. Each sample was reduced with $TiCl_3$ in an acidic medium, and thus nicotine-l'-N-oxide was converted to nicotine, which was then extracted as described above.

RESULTS AND DISCUSSION

Analytical data for nicotine, cotinine and nicotine-l'-N-oxide in the samples of urine, and the cotinine/nicotine-l'-N-oxide ratios are shown in Tables 1, 2 and 3.

From the results of this study which we carried out with the aim of finding the most satisfactory liquid phase and chromatographic conditions for the estimation and identification of nicotine and its metabolites, and on the basis of the results obtained by urine analysis we may conclude that column 10% SE—30 Chromosorb W NAW 80/100 mesh, 2 m x 6 mm, is applicable for the analysis of nicotine at 150 °C and for the analysis of cotinine at 250 °C but that column 3% OV—17 Chromosorb G 80/100 mesh, 1 m x 6 mm, is far more satisfactory in this respect as it gives a much better resolution.

The results obtained with a very limited number of urine samples from healthy smokers and those of liver and lung cancer patients show the values of the cotinine/nicotine-l'-N-oxide ratio to be 7—10 times higher in cancer patients than in healthy persons.

According to *Gorrod and co-workers* (7) the cotinine/nicotine-l'-N-oxide ratio in the urine of smokers with cancer of the urinary bladder is 3—10 times higher than in the urine of healthy smokers.

It is uncertain whether the increased metabolite ratio in the urine of liver and lung cancer patients indicates a possible causative mechanism in the etiology of cancer or simply exhibits changes in the oxidative metabolism of nicotine caused by the disease. However, it may be concluded that the study of this ratio in a larger population of smokers with liver and lung cancer could establish whether the increased ratio can be used as an early indicator of the development of cancer.

ACKNOWLEDGEMENT

This work has been financially supported by the Republican Research Fund of the Socialist Republic of Bosnia and Herzegovina.

References

1. *Truhaut, R., de Clercq, M., Loisillier, F.:* Pathol. Biol. (Paris), *12* (1964) 39.
2. *Brown, G. B., Suguira, K., Cresswell, R. M.:* Cancer Res., *25* (1965) 986.
3. *Teller, M., Suguira, K., Parham, J. C., Brown, G. B.:* Proc. Am. Assoc. Cancer Res., *9* (1968) 70.
4. *Brown, G. B.:* Prog. Nucleic Acid Res. Mol. Biol., *8* (1968) 209.
5. *Beckett, A. H., Gorrod, J. W., Jenner, P.:* J. Pharm. Pharmacol., *23* (1971) Suppl. 55S—61S.
6. *Beckett, A. H., Gorrod, J. W., Jenner, P.:* J. Pharm. Pharmacol., *23* (1971) Suppl. 62S—67S.
7. *Gorrod, J. W., Jenner, P., Keysell, G. R., Mikhael, B. R.:* J. Natl. Cancer Inst., *52* (1974) 1421.
8. *McNiven, N. L., Raisinghani, K. H., Patashnik, S., Dorfman, R. I.:* Nature, *203* (1965) 788.
9. *Beckett, A. H., Triggs, E. J.:* Nature, *211* (1966) 1415.
10. *McKennis, H., Jr., Turnbull, L. B., Bowman, E. R., Wada, E.:* J. Am. Chem. Soc., *81* (1959) 3951.
11. *Taylor, E. C., Boyer, M. E.:* J. Org. Chem., *24* (1959) 275.

Sažetak

ISPITIVANJE KOLIČINE I ODNOSA KOTININA I NIKOTIN-1'-N-OKSIDA U URINU PUŠAČA

Metabolizmom nikotina u organizmu pušača nastaje ceo niz različitih metabolita, kao: kotinin, nikotin-1'-N-oksid, nikotin-1,1'-N-dioksid i drugi. U ekstraktima urina zdravih osoba i pacijenata obolelih od karcinoma pluća i jetre praćena je količina nepromenjenog nikotina i metabolita. Za ova određivanja primenjena je gasna hromatografija na koloni 3% OV-17 i nikotin je praćen na temperaturi od 150 °C uz interni standard hinolin, a kotinin na temperaturi od 250 °C uz interni standard kodein. Posebno je praćen odnos kotinina i nikotin-1'-N-oksida. Iz dobivenih rezultata može se zaključiti da je odnos navedenih metabolita veći 7—10 puta kod pacijenata obolelih od karcinoma pluća i jetre nego kod pušača zdravih osoba.

Na temelju dobijenih rezultata može se pretpostaviti da bi praćenje odnosa metabolita nikotina kod pušača moglo poslužiti i kao jedan od pokazatelja u ranoj dijagnostici razvoja karcinoma.

Farmaceutski fakultet,
Institut za farmaciju i medicinsku hemiju,
Univerzitet u Sarajevu, Sarajevo

Primljeno 18. VI 1982.