Development of immunoassays of catecholamines and their metabolites

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Received 9 October 2016
Accepted 23 November 2016

Journal of The Arab Society for Medical Research 2016, 11:37–42

Catecholamines take the role of neurotransmitters and hormones and are involved in many diseases. Sensitive immunoassays for catecholamines and their metabolites were required to study the diagnoses of diseases. First, we achieved success in producing specific antibodies for catecholamines and basic metabolites using each antigen. We applied a monoclonal antibody to 3-methoxy-4-hydroxyphenylglycol to measure the concentrations to diagnose depression. The acidic homovanillic acid (HVA) and d-3-methoxy-4-hydroxymandelic acid (VMA) were useful in mass screening of neuroblastoma using monoclonal antibodies. The paper reviews these developments for future use.An antigen is important to produce the specific antibody. The amino acid residue of each catecholamine and l-3,4-dihydroxyphenylalanine protected with *N*-maleyl group as well as basic metabolites was reacted with bovine serum albumin using Mannich reaction in the presence of formaldehyde. The *N*-maleyl group of the conjugate was moderately liberated to give rise to the antigen. The antigen was injected with Freund's Complete Adjuvant to the rabbit or mouse. The conventional or monoclonal antibody was used for radioimmunoassay or enzyme immunoassay.Each immunoassay showed high specificity in discriminating not only the fine structure of the hapten but also body ingredients. The kits of HVA and VMA were useful in screening the urine from the infant with neuroblastoma. The secret of specific antibody preparation is dependent on the synthetic method of the conjugation, which is chemically moderate. The preparation of antibody requires long time to increase the affinity. Thus, the methods of antibody preparation are established and repeatable for anyone.The mass screening of neuroblastoma with HVA and VMA was easily applied to infants. Our previous results had established immunoassays for all members of catecholamines and their metabolites for diagnosis and research.

Keywords:
3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol, epinephrine, homovanillic acid, immunoassay, metanephrine, norepinephrine

J Arab Soc Med Res 11:37–42

1687-4293

Background

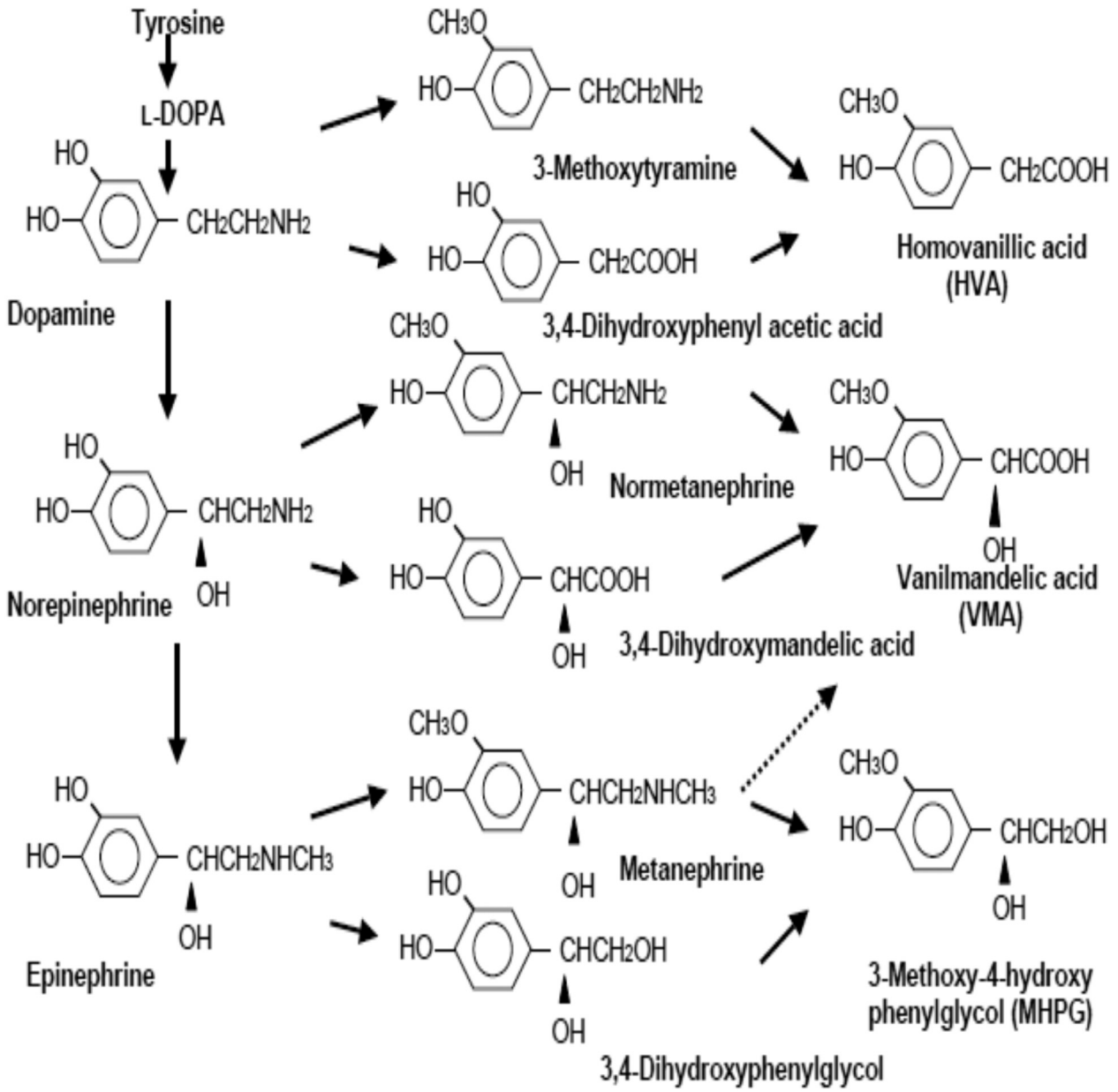
Catecholamines take the role of neurotransmitters and hormones. They are involved in many diseases, such as Parkinsonism, depression, cancer, and dementia. Julius Axelrod established the metabolism (Fig. 1) and working mechanism of catecholamines, and was awarded Nobel Prize as described in Nobel Lecture, 12 December 1970. Dopamine in the central nervous system decreases to give rise to Parkinsonism and a type of depression or dementia; hence, its precursor, l-3,4-dihydroxyphenylalanine (l-DOPA), is a good drug. Catecholamines and metabolites are found increased in catecholamine-producing tumors such as neuroblastoma and phaeochromcytoma.

Sensitive immunoassays of catecholamines and their metabolites were required to be studied for the diagnoses of various related diseases. They are unstable and are present at very low concentrations in body fluids. Thus, we tried to develop the sensitive immunoassays. First, we achieved success in producing specific antibodies to epinephrine, norepinephrine, and dopamine and its precursor, l-DOPA, using each antigen coupled the hapten with albumin using Mannich reaction [1–7].

In a similar manner, the antibody for the basic metabolite such as 3-methoxy-4-hydroxyphenyl-2-methylamino ethanol (metanephrine) was produced [8,9]. With advancement in antibody production, the hybridoma technique for the monoclonal antibody, developed by Cesar Milstein, was awarded Nobel Prize in 1984. We also applied a monoclonal antibody to neutral metabolite and the concentration was measured to diagnose depression [10–13].

The acidic metabolites 3-methoxy-4-hydroxyphenylacetic acid [homovanillic acid (HVA)] and d-3-methoxy-4-

Figure 1



Metabolism of catecholamines. Modifying from Miwa *et al*. [1].

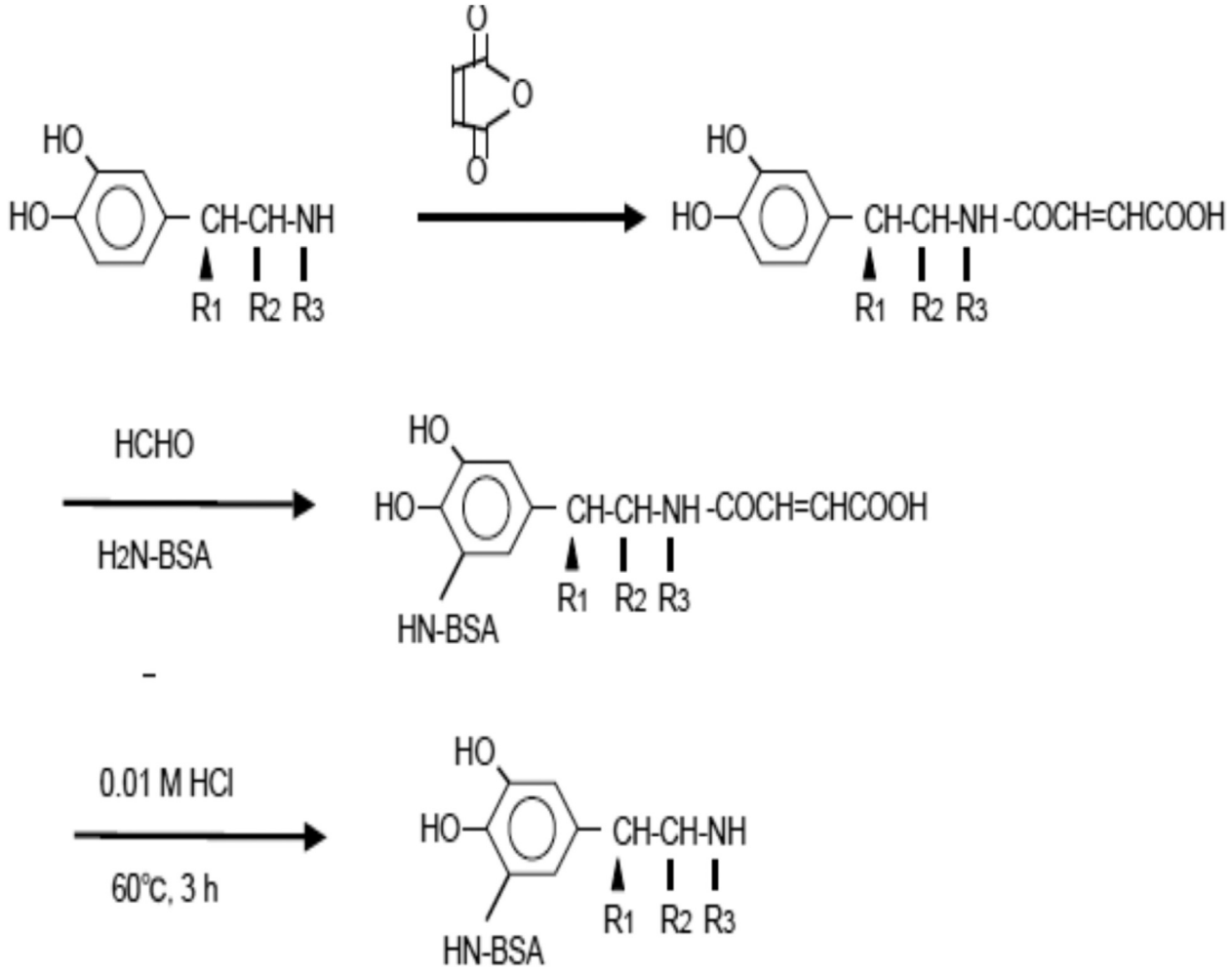
hydroxymandelic acid (VMA) were useful in mass screening of neuroblastoma using monoclonal antibodies with techniques approved by the Food and Drug Administration in October 1989, and by Japanese government in November 1990 [14–16]. The paper reviews these developments for future use [7].

Methods

To give a hapten antigenecity, the catecolamine or the metabolite each must be coupled with a carrier protein. There are many ways to conjugate the hapten of fine structure. The side chain of the hapten was easy to couple with the amino groups of the carrier; the antibodies were not specific to discriminate the phenol derivatives of catecholamine or metabolite previously published [1–7]. The Ethical Committee on human research at Setsunan University, Faculty of Pharmaceutical Sciences, Osaka, Japan has been approved the protocol for all our previous research including in that review, and the medical consent has been approved from the parents of patients.

An antigen is important to produce the specific antibody. The amino acid residue of each catecholamine and l-DOPA protected with *N*-maleyl group was reacted with bovine serum albumin using Mannich reaction in the presence of formaldehyde. The *N*-maleyl group of the conjugate was moderately liberated to give rise to the antigen, as shown in Fig. 2 [1]. The metabolites were not

Figure 2



Synthesis of antigens of catecholamines. Modifying from Miwa *et al*. [1].

required to protect with the maleyl group. Catecholamine or metabolite was displaced in the neighbor position of phenol with aminomethyl-lysine residue of albumin. The antigen was injected with Freund's Complete Adjuvant to the rabbit or mouse. The conventional or monoclonal antibody was used for radioimmunoassay or enzyme immunoassay.

Summary

Each immunoassay showed high specificity in discriminating not only the fine structure of the hapten but also body ingredients. The antibody to natural l-epinephrine binds with l-form but not d-form, as shown in Table 1. It is important to bear in mind that the true conformation of the asymetric carbon of the side chain is d-form from d-mandeliic acid; however, it is conventionally denoted as l-form, because of the levorotatory property of the natural epinephrine. The provability of such high specific antibody is so low that it took 1 year and only one rabbit produced among 40 rabbits. The antibody to l-norepinephrine was also selected high specific, as shown

Table 1 Relative cross-reactivity of the antibody to l-epinephrine to analogs by means of radioimmunoassay

Analog	Relative amount for 50% inhibition
l-Epinephrine	1
d-Epinephrine	10
d-l-Epinephrine-3-*O*-sulfate	2×10^2
d-l-Epinephrine-4-*O*-sulfate	2×10^2
l-Norepinephrine	10×10^4
Dopamine	$>10\times10^4$
l-DOPA	10×10^3
d-l-metanephrine	3×10^2
d-l-Normetanephrine	$>10\times10^4$
Epinine	7×10^2
d-l-Synephrine	2×10^2
d-l-Isoprenaline	2×10^3
Adrenalone	1×10^2
Adrenochrome	$>1\times10^3$
Trans-2-methylamino-1,5,6-trihydroxy-1,2,3,4-tetrahydronaphthalene	$>1\times10^3$

Modifying from Yoshioka *et al.* [6]. Relative cross-reactivity=relative amount for 50% inhibition of ^{3}H-epinephrine/amount for 50% inhibition of the analog. l-DOPA, l-3,4-dihydroxyphenylalanine.

Table 2 Cross-reactivity of the antibody to l-norepinephrine to analogs by means of radioimmunoassay

Analog	Cross-reactivity (%)
l-Norepinephrine	100
d-Norepinephrine	1.6
l-Epinephrine	1.3
Dopamine	0.013
l-DOPA	<0.01
d-l-Octopamine	2.0
d-l-Normetanephrine	1.0
3,4-Dihydroxyphenyl acetic acid	<0.01
3,4-Dihydroxymandelic acid	<0.01
Homovanillic acid	<0.01
Vanilmandelic acid	<0.01
d-l-1,2,3,4-Tetrahydro-4,6,7-trihydroxy-2-methylisoquinoline · HCl	<0.1
d-l-1,2,3,4-tetrahydro-4,7,8-trihydroxy-2-methylisoquinoline · HCl	<0.1

Modifying from Yoshioka, *et al.* [5]. Cross-reactivity=amount for 50% inhibition of ^{3}H-d-norepinephrine/amount for 50% inhibition of the analog. l-DOPA, l-3,4-dihydroxyphenylalanine.

Table 3 Cross-reactivity of the antibody to d-l-metanephrine to analogs by radioimmunoassay

Analog	Cross-reactivity (%)
d-l-Metanephrine	100
d-l-Normetanephrine	1
3-Methoxytyramine	<0.02
l-Epinephrine	0.1
d-Epinephrine	0.1
l-Norepinephrine	<0.02
Dopamine	<0.02
d-l-Synephrine	1.0
Vanilmandelic acid	<0.02
Homovanillic acid	<0.02
5-Hydoroxytryptamine	<0.02
Mescaline	<0.02

Modifying from Shirahata *et al.* [8]. Cross-reactivity=amount for 50% inhibition of ^{3}H-d-l-metanephrine/amount for 50% inhibition of the analog.

in Table 2. The antibody to the basic metabolite, metanephrine, also discriminated the fine structure of the analogs (Table 3). The monoclonal antibody to 3-methoxy-4-hydroxyphenylglycol was specific (Table 4) and is applied in the diagnosis of depression. The antibody for VMA was selected specifically (Table 5), as well as the one for HVA. The kits for HVA and VMA were useful in screening the urine from infants with neuroblastoma. Apparently, a healthy infant was found with neuroblastoma (Fig. 3) [16].

We had been involved in the development of gas chromatography with mass spectrometry and high-performance liquid chromatography, which were not so convenient and needed high technology and were time consuming [17]. Immunoassay is very simple and easy to measure many samples at once. It was very

Table 4 Cross-reactivity of the monoclonal antibody to d-3-methoxy-4-hydroxyphenylglycol to analogs using EIA

Analog	Cross-reactivity (%)
d-3-Methoxy-4-hydroxyphenylglycol	100
l-3-Methoxy-4-hydroxyphenylglycol	8
d-l-3-Methoxy-4-hydroxyphenylglycol	66.7
d-l-3-Methoxy-4-hydroxyphenylglycol-4-*O*-sulfate	20
d-l-3,4-Dihydroxyphenylglycol	<4
d-l-Normetanephrine	<4
d-l-Metanephrine	<4
Dopamine	<4
l-DOPA	<4
d-l-Vanilmandelic acid	<4
Homovanillic acid	<4
d-l-Norepinephrine	<4
l-Epinephrine	<4
3-Methoxytyramine	<4
3-Methoxytyrosine	<4
3,4-Dihydroxymandelic acid	<4
3,4-Dihydroxyphenyl acetic acid	<4
l-Phenylalanie	<4
Tyramine	<4
Vanillic acid	<4
d-Mandelic acid	<4
Homocatechol	<4
d-l-Octopamine	<4
5-Hydroxytryptamine	4
5-Hydroxydopamine	10

Modifying from Hirose *et al.* [13]. l-DOPA, l-3,4-dihydroxyphenylalanine.

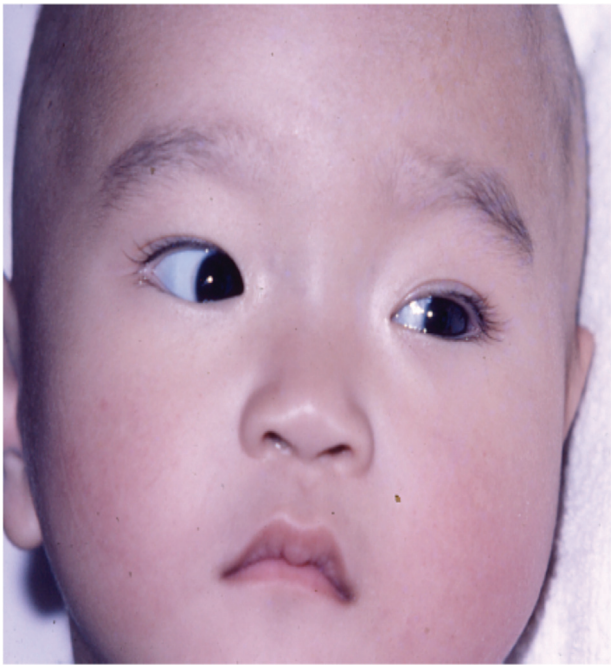
Table 5 Cross-reactivity of the monoclonal antibody to d-vanilmandelic acid to analogs using enzyme immunoassay

Analog	Cross-reactivity (%)
d-Vanilmandelic acid	100
d-l-Vanilmandelic acid	50
Homovanillic acid	<0.01
3,4-Dihydroxymandelic acid	4
3,4-Dihydroxyphenyl acetic acid	<0.01
Metanephrine	<0.01
Vanilpyruvic acid	4
Vanillic acid	<0.01
Dopamine	<0.01
5-Hydroxyindoleacetic acid	<0.01
Vanillactic acid	<0.01
3-Methoxy-4-hydroxyphenyglycol	<0.01

Modifying from Yoshioka *et al.* [14].

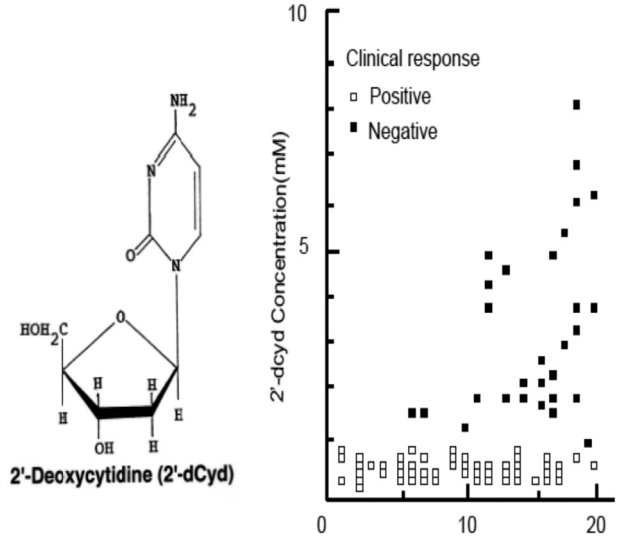
difficult to make specific antibodies for three endogenous catecholamines and their metabolites of fine structure, before our success. The secret of specific antibody preparation depended on the synthetic method of the conjugation, which was chemically moderate. The preparation of antibody took long time and many animals to increase the affinity. Once the antibodies of high affinity are obtained (Tables 1–5), it is easy to make specific and sensitive immunoassays for practical measurements described in the results. Thus, the

Figure 3



A patient with neuroblastoma found by enzyme immunoassay (EIA). Original picture of the patient modifying from Yokomori *et al*. [16].

Figure 4



2'-deoxycytidine levels in bloods of individual breast cancer patients under chemotherapy. Modifying from Yoshioka *et al*. [20].

methods of antibody production are established and repeatable for anyone.

When the specific antibody was, however, not obtained with another conjugation of a hapten with a carrier protein, some pretreatment of sample was possible rough to separate catecholamines and metabolites, which were recognized with not so much specific, but common antibody [18].

The mass screening of neuroblastoma with HVA and VMA was easily applied to infants. Positive infants (Fig. 3) were operated [16]. This method was used in the USA and is commercially available worldwide [19]. It was, however, not yet defined to estimate good or bad prognosis of the cancer. In that case, I recommend the other prognostic biomarkers, 2'-deoxycytidine, which was increased in breast cancer (Fig. 4) [20], bladder cancer, hepatoma, and acute leukemia in our joint project with Prof. M. El-Merzabani, National Cancer Institute, Cairo University, for more than 30 years. Immunoassay of 2'-deoxycytidine was established for the future use [21–23].

Conclusion

We have established immunoassays of all members of catecholamines and their metabolites for diagnosis and research [7]. To the best of our knowledge, such basic methods for catecholamines and their metabolites have been rarely developed. This review is renewal to evaluate the significance.

Acknowledgements

I am grateful to Professor Karam Mahdy of National Research Center, Cairo, who gave me a chance to present this review at the 5th International conference of the Arab Society for Medical Research, Sharm El Sheikh, 28–31 October in 2016.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Miwa A, Yoshioka M, Shirahata A, Nakagawa Y, Tamura Z. Preparation of a specific antibody to each one of catecholamines and L-DOPA. Chem Pharm Bull 1976; 24:1422–1424.

2 Miwa A, Yoshioka M, Shirahata A, Tamura Z. Preparation of specific antibodies to catecholamines and L-3, 4-dihydroxyphenylalanine. I. Preparation of the conjugates. Chem Pharm Bull 1977; 25:1904–1910.

3 Miwa A, Yoshioka M, Tamura Z. Preparation of specific antibodies to catecholamines and L-3, 4-dihydroxyphenylalanine. II. The site of attachment of catechol moiety in the conjugates. Chem Pharm Bull 1978; 26:2903–2905.

4 Miwa A, Yoshioka M, Tamura Z. Preparation of specific antibodies to catecholamines and L-3, 4-dihydroxyphenylalanine. III. Preparation of antibodies to epinephrine for radioimmunoassay. Chem Pharm Bull 1978; 26:3347–3352.

5 Yoshioka M, Iwai-Yamasaki M, Kuakazu-Wada E, Shirahata A, Tamura Z. Radioimmunoassay of L-norepinephrine. Biogenic Amines 1987; 43:211–217.

6 Yoshioka M, Kobayashi-Iwase Y, Suga K, Shirahata A, Tamura Z. Radioimmunoassay of L-epinephrine. Biogenic Amines 1987; 43: 219–227.

7 Yoshioka M. Immunoassays of catecholamines and their metabolites. In: Parvez H, Naoi M, Nagatsu T, Parvez S, editors. Techniques in behavioral and neural sciences, 11: methods in neurotransmitter and neuropeptide research. Part 2. Amsterdam: Elsevier; 1993:1–25.

8 Shirahata A, Yoshioka M, Tamura Z. Studies on radioimmunoassay of metanephrine. Chem Pharm Bull 1980; 28:2994–3001.

9 Shirahata A, Yoshioka M, Tamura Z. Unexpected conversion of epinephrine into tetrahydroisoquinolines in solution containing ascorbic acid. Chem Pharm Bull 1982; 30:1325–1357.

10 Yoshioka M, Negoro Y, Kanemoto K, Parvez H. Preparation of antigen and antibody to 3-methoxy-4-hydroxyphenylglycol (MHPG). In: Nagatsu T, Fisher A, Yoshida M, editor. Basic, clinical, and therapeutic aspects of Alzheimer's and Parkinson's diseases, Vol. 1. New York:Plenum Press; 1990: pp. 499–502.

11 Hirose K, Asada K, Darwish I, Akizawa T, Yoshioka M. Chiral analysis of 3-methoxy-4-hydroxyphenylglycol in human urine. J Pharm Biomed Anal 1997; 9/10:1241–1247.

12 Hirose K, Akizawa T, Yoshioka M. Preparation of monoclonal antibody against D-3-methoxy-4-hydroxy-phenylglycol. Anal Chim Acta 1998; 365:129–135.

13 Hirose K, Akizawa T, Asada K, Tanaka Y, Negoro Y, Yoshioka M. Syntheses of antigens conjugated with 3-methoxy-4-hydroy-phenylglycol by Mannich reaction for enzyme immunoassay. Anal Clin Acta 1998; 365:137–145.

14 Yoshioka M, Aso C, Amano J, Tamura Z, Sugi M, Kuroda M. Preparation of monoclonal antibodies to vanilmandelic acid and homovanillic acid. Biogenic Amines 1987; 43:229–235.

15 Kuroda M, Sugi M, Ikeda T, Fujimoto M, Yokomori K, Amano J, Yoshioka M. Enzyme immunoassay of vanilmandelic acid (VMA) in urine. Biogenic Amines 1990; 7:257–263.

16 Yokomori K, Horii Y, Tsuchida M, Kuroda M, Yoshioka M. A new urinary mass screening system for neuroblastoma in infancy by using monoclonal antibodies against VMA and HVA. J Pediatric Surgery 1989; 24:391–394.

17 Yoshida A, Yoshioka M, Parvez H. Review: high performance liquid chromatography of metabolites of catecholamines and serotonin in urine, plasma, cerebrospinal fluid and brain tissue. I. Analytical methodology. In: Yoshioka M, Parvez S, Miyszaki T, Parvez H, editors Progress in HPLC, 4: supercritical fluid chromatography and micro-HPLC. Utrecht: VSP BV; 1989:229–271.

18 Ater JL, Gardner KL, Foxhall LE, Therrell BL, Bleyer WA. Neuroblastoma screening in the United States. Cancer 1998; 82:1593–1602.

19 Precopiou M, Finney H, Akker SA, Chew SL, Drake WM, Burrin J, Grossman AB. Evaluation of an enzyme immunoassay for plasma-free metanephrines in the diagnosis of catecholamine-secreting tumors. Eur J Endocrinol 2009; 161:131–140.

20 Masanori Y, Abu-Zeid M, Kubo T, El-Merzabani M. Identification of a previous unknown compound as 2'-deoxycytidine found in the plasma of breast cancer patients under chemotherapy. Biol Pharm Bull 1994; 17:169–172.

21 Omura K, Hirose K, Itoh M, Akizwa T, Yoshioka M. Development of enzyme immunoassay of 2'-deoxycytidine. J Pharm Biomed Anal 1997; 15:1249–1256.

22 Ahmed WA, Abu-Zeid M, Yoshioka M, Kahled H, El-Merzabani M. Elevation 2'-deoxycytidine plasma level in Egyptian breast cancer patients subjected to chemotherapy. J Egypt Ger Soc Zool 2001; 35A:249–263.

23 Iwazaki A, Imai K, Nakanishi K, Yoshioka M. Changes in 2'-deoxycytidine levels in various tissues of tumor-bearing mice. Oncol Letters 2010; 1:999–1004.