ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1339–1342

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

Identification of the presence of gliadin in drugs using the dot–blot assay[1]

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Received for review 13 September 1995; revised manuscript received 24 January 1996

Keywords: Coeliac disease; Dot–blot assay; Gliadin; Prolamins

1. Introduction

Coeliac disease is a specific hypersensitivity to dietary gliadins and related prolamins from selected cereals (rye, barley and oats), resulting in small intestinal villous atrophy followed by absorptive abnormalities in patients with the disease and the presence of a high level of sera antiprolamin antibodies [1]. Prolamins from maize and rice are considered non-toxic for coeliac patients [2]. Gliadins are wheat prolamins and they are major storage proteins of wheat kernel endosperm.

A gluten-free diet (i.e. a diet without toxic prolamins) is a lifelong treatment for coeliac patients. Total avoidance of toxic prolamins is not so simple because of their presence in unexpected sources. Previous work of the current authors [3] showed the presence of gliadins as impurities in industrial starch commonly used in the preparation of pharmaceutical products as diluent, binder or filler. Most drugs are not accompanied by a lable indicating the presence of prolamins [4] and there is no accurate information or standardized methodology for gliadin detection in pharmaceuticals.

The goal of this work was to investigate the presence of gliadin in drugs most commonly used in Yugoslavia using the dot–blot assay as a sensitive semiquantitative technique.

2. Experimental

2.1. Materials and reagents

Table 1 lists 47 drugs commonly used in Yugoslavia which were tested for the presence of gliadin in this study. Commercial integral cereal flour of wheat, rye, barley, oats and maize was from Macrobiotics Center Trim (Yugoslavia) and rice was from Euritio (Italy).

Gliadin was obtained from Sigma (USA), rabbit antigliadin antisera (polyclonal) was from Behring (Germany), a BCA protein assay kit was from Pierce (USA) and all other reagents—

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[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(96)01785-2

Table 1
Presence of gliadin[a] in drugs frequently used in Yugoslavia

No.	Commercial name	Generic name	Source	Form	Result
1.	Acetysal pH 8	Acetysalicylic acid	ICN Galenika	Tablets	–
2.	Aciklovir	Aciklovir	Zdravlje	Tablets	–
3.	Alfacet	Cefaklor	ICN Galenika	Capsules	–
4.	Alfogel	Aluminium phosphate	ICN Galenika	Gel	+
5.	Analgin	Metamizol	Panfarma	Tablets	–
6.	Anbol	Acetylsalicylic acid + buffer	ICN Galenika	Tablets	–
7.	Astemisan	Astemizole	Zdravlje	Tablets	+
8.	Atenolol	Atenolol	Farmakos DD	Tablets	–
9.	Bactrim	Sulfamethoksazol + trimethoprim	ICN Galenika	Tablets	–
10.	Bedoxin	Pyridoxin	ICN Galenika	Tablets	–
11.	Bensedin	Diazepam	ICN Galenika	Tablets	–
12.	Beviplex	Vitamins of B group	ICN Galenika	Coated Tablets	–
13.	Bisolvon	Bromheksin	Zdravlje	Tablets	–
14.	Bronal	Terfenadine	ICN Galenika	Tablets	–
15.	Buscopan	Scopolamine + butyl bromide	Zdravlje	Coated Tablets	–
16.	Chymociclar	Tetracycline hydrochloride + trypsin + chymotrypsin	ICN Galenika	Capsules	–
17.	Chymoral 100 forte	Trypsin + chymotrypsin	ICN Galenika	Coated Tablets	–
18.	Cliacil	Phenoxymethyl penicillin kalium	Jugoremedija	Tablets	+
19.	Digestal	Amylase + lipase + protease + bile	ICN Galenika	Coated Tablets	–
20.	Diclofenac	Diclofenac sodium	Panfarma	Talbets	+
21.	Dulcolax	Bisacodil	Zdravlje	Coated Tablets	+
22.	Febricet	Paracetamol	Panfarma	Tablets	+
23.	Festal	Amylase + lipase + protease + bile	Jugormedija	Coated Tablets	–
24.	Flonivin BS	Bacillus IP 5832	ICN Galenika	Capsules	+
25.	Gelusil lac	Magnesium aluminium silicahydrate	Hemofarm DD	Tablets	+
26.	Izopamil	Verapamil chloride	ICN Galenika	Tablets	–
27.	Lincocin	Lincomycin	Hemofarm DD	Capsules	–
28.	Midol	Acetysalicylic acid + buffer	Panfarma	Tablets	+
29.	Mucodine	Carbocistein	Zorka	Tablets	–
30.	Nifelat	Nifedipin	Zdravlje	Tablets	+
31.	Nirypan	Methyl prednisolone	Jugoremedija	Tablets	+
32.	Nystatin	Nystatin	Hemofarm DD	Coated Tablets	+
33.	Novalgetol	Metamizol	ICN Galenika	Tablets	–
34.	Oligogal Se	Vitamins A, C and E + Se	ICN Galenika	Capsules	+
35.	Paracetamol	Paracetamol	Jugoremedija	Tablets	–
36.	Palitrex	Cephalexin	ICN Galenika	Capsules	–
37.	Pentrexyl	Ampicillin	ICN Galenika	Capsules	–
38.	Prilazid	Cilazapril	ICN Galenika	Tablets	–
39.	Ranisan	Ranitidine	Zdravlje	Tablets	–
41.	Salbutamol	Salbutamol	Jugroemedija	Tablets	–
42.	Saridon	Paracetamol + propyphenazon + caffeine	ICN Galenika	Talbets	+
43.	Selvigon	Pipazetat	ICN Galenika	Coated Tablets	–
44.	Trental	Pentoksifiline	Jugoremedija	Coated Tablets	–
45.	Trimosul	Sufamethoksazole + trimethoprim	Panfarma	Tablets	–
46.	Verapamil	Verapamil	Zdravlje	Coated Tablets	–
47.	Vitamin C	Vitamin C	Farmakos DD	Tablets	–

[a] Assay sensitivity is 6 ng of gliadin in 2 μl dot.

sodium dodecyl sulfate (SDS), gelatin, Tween 20, Tris, goat antirabbit horseradish peroxidase-labeled antibody (HRP), 4-chloro-1-naphtol and nitrocellulose strips — were from Bio-Rad (USA).

2.2. Methods

Extraction of prolamins from dry defatted cereal flour was performed with 1% SDS in water after the albumin and globulin fractions had been removed by 0.34 mol l^{-1} sodium chloride extraction. Extraction with 1% SDS is more quantitative than the standard ethanol-based extraction procedure [3] and problems such as dissolving nitrocellulose with ethanol in the assay are avoided. Total proteins were determined by BCA assay with commercial gliadin as a standard. Extracted cereal prolamins and gliadin were used as references in the dot–blot assay.

Extraction of prolamins from drugs (tablets, coated tablets, capsules and gel) was performed by directly dissolving the drugs in 1% SDS as described by Miletic et al. [3]. A dot–blot assay was performed using a slightly modified procedure of the same authors. Optimal concentrations of antigen and primary and secondary antibodies were estimated performing the reaction with serial dilutions of them. Dots of 2 μl of SDS extract of pharmaceutical products were applied to nitrocellulose strips. Dots of commercial gliadin and prolamins extracted from wheat, rye, barley and oats (1 mg ml^{-1} in 1% SDS) were also applied as a positive control and dots of extracts from maize and rice at the same concentration were applied as a negative control. Unoccupied sites on the strips were blocked with a 3% solution of gelatin in 0.05% Tween 20/Tris–HCl (0.02 M)/Nacl (pH 7.4; 0.5 M) (TTBS). Strips were washed three times with TTBS and 1 ml of rabbit antigliadin antisera 1:200 in TTBS was applied. After washing the strips with TTBS, 1 ml of goat anti-rabbit antisera labeled with HRP 1:200 in TTBS was added. Strips were again washed with TTBS and 4-chloro-1-naphtol solution was used as a peroxidase-specific substrate. The assay sensitivity, defined as the lowest detectable level of gliadin and extracted cereal prolamins, was detected by dilution of antigen: 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003 and 0.0001 mg ml^{-1}. 2 μl samples of each dilution were applied to the nitrocellulose strip.

3. Results

Table 1 gives the commercial and generic names of the investigated drugs frequently used in Yugoslavia, the name of the company that manufactures the drug, the form of the drug tested and the result of the dot–blot assay for the presence of gliadin determined by the aforementioned method. 47 prescription and non-prescription drugs manufactured by seven Yugoslav pharmaceutical companies were tested and 31.91% (15 out of 47) gave positive reactions to gliadin.

The assay sensitivity, defined as the lowest detectable concentration of gliadin, was approximately 0.003 mg ml^{-1}. The sensitivity of the assay for other extracted prolamins was: rye, 0.1 mg ml^{-1}; barley, 0.03 mg ml^{-1}; and oats, 1 mg ml^{-1}; while the reaction with coeliacly non-toxic prolamins from maize and rice was negative at all investigated concentrations.

4. Discussion and conclusions

Gliadin was found in 31.91% of investigated drugs. Comparing this result with the study of 59 drugs commonly used in the USA [3], where gliadin was found in 71.18% of drugs examined by assay with a similar sensitivity, it can be concluded that either the purity of starch used in the Yugoslav pharmaceutical industry is higher, or that its origin is predominantly from coeliacly non-toxic sources such as maize, rice and potato.

The use of monoclonal mouse antibodies by other investigators [5–7], which react with a single epitope, is too limited for identification of all toxic prolamins. The advantage of using polyclonal antibodies in this assay is that they react with all prolamins (from wheat, rye, barley and oats) that can provoke relapse in coeliac patients.

There is no consensus about the maximum non-toxic intake of gliadin per day. Some researches suggested that 5–13 mg gliadin per day may cause no effects detectable by control biopsy

[8]. In some countries the official limit for gluten-free dietary products is 0.3 g of gluten in 100 g on a dry weight basis [9]. The sensitivity of this assay was found to be 0.003 mg ml^{-1}. Using 2 μl of sample for the dot in the assay, 6 ng of gliadin is detectable. When used frequently and in large amounts, some gliadin-containing drugs can be dangerous to coeliac patients. The dot–blot assay can be used as a simple, sensitive and quick method for detection of gliadin in drugs and dietary products.

References

[1] R. Troncone and A. Ferguson, J. Pediatr. Gastroenterol. Nutr., 12 (1991) 150–158.
[2] G. N. Ferstenstein, F. C. Hay, B. J. Miflin and P. R. Shewry, Planta, 162 (1984) 524–531.
[3] I. Dj. Miletic, V. D. Miletic, E. A. Sattely-Miller and S. S. Schiffman, J. Pediatr. Gastroenterol. Nutr., 19 (1994) 27–33.
[4] R. G. Challen and R. M. O'Shannassey, Med. J. Aust., 146 (1986) 91–93.
[5] A. R. Freedman, G. Galfre, E. Gal and P. J. Ciclitira, J. Immunol. Methods, 98 (1987) 123–127.
[6] A. R. Freedman, G. Galfre, E. Gal and P. J. Ciclitira, Clin. Chim. Acta, 166 (1987) 323–328.
[7] H. J. Ellis, A. R. Freedman and P. J. Ciclitira, J. Immunol. Methods, 120 (1989) 17–22.
[8] P. J. Ciclitira and H. H. Ellis, Panminerva Med., 33 (1991) 75–82.
[9] H. Mobacken, H. Andersson and R. Gilberg, Clin. Dermatol., 9 (1992) 415–41.