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A Non-Cannabinoid Immunogen Used to Elicit Antibodies with Broad Cross-Reactivity to Cannabinoid Metabolites

REFERENCE: Salamone SJ, Bender E, Hui RA, Rosen S. A non-cannabinoid immunogen used to elicit antibodies with broad cross-reactivity to cannabinoid metabolites. J Forensic Sci 1998;43(4): 821–826.

ABSTRACT: A benzpyran derivative was linked to the lysines of bovine thyroglobulin (BTG) where 69% of the available lysines were modified. This derivative was designed to elicit antibodies that were directed towards the conserved epitopes of cannabinoid metabolites that appear in urine. Polyclonal antibodies from sheep and goats and murine monoclonal antibodies were generated using this immunogen. The cross-reactivity of the antibodies was compared with antibodies generated from the more traditional phenolic-linked or 9-linked immunogens. An ELISA assay was developed using Δ^9-11-nor-9-carboxy-tetrahydrocannabinol (COOH-THC) to obtain a standard curve. The antibodies generated using the benzpyran immunogen showed an average of two to three times higher cross-reactivity towards 11-OH-Δ^9-THC, 8β-OH-Δ^9-THC, 8α-OH-Δ^9-THC, 11-OH-Δ^8-THC, and 8β,11-di-OH-Δ^9-THC than antibodies that were generated by traditional cannabinoid immunogens. The selectivity of the benzpyran-elicited antibodies was also compared with antibodies derived from traditional immunogens using clinical urine samples that were confirmed for cannabinoids by GC/MS. The total cross-reactive cannabinoid values obtained with the benzpyran-elicited antibodies were 49% higher than the values obtained using the traditional immunogen structures. The benzpyran immunogen-induced antibodies exhibited the same low cross-reactivity for non-structurally related compounds as antibodies derived from traditional immunogens. The novel benzpyran immunogen used in this study is the first non-cannabinoid immunogen used to generate cannabinoid-selective antibodies and demonstrates the usefulness of such a structure in developing broadly cross-reactive cannabinoid antibodies.

KEYWORDS: forensic science, non-cannabinoid immunogen, cannabinoid antibodies, cannabinoid immunogens, cannabinoid metabolites, substance abuse detection

When a cannabinoid compound is conjugated to a carrier protein for the purposes of making an immunogen, the site of linkage on the cannabinoid molecule to the carrier protein will determine the selectivity of the resulting antibodies. Common sites of linkage of cannabinoid haptens to a carrier protein have typically been either out of the 1-position (1–4), the 2-position (5,6), the 9-position (7–9) and the 5′-position (10) (see Fig. 1) of Δ^9-11-nor-9-carboxy-tetrahydrocannabinol (COOH-THC) or very closely related compounds. Studies in the past have shown that when cannabinoid haptens are linked to a protein through the 1 or 5′-positions the corresponding antisera display high selectivity to the unconjugated form of COOH-THC, the primary tetrahydrocannabinol (THC) metabolite found in human urine. The cross-reactivity for the 8 and 11-hydroxylated metabolites as well as for the glucuronides is lower for this antiserum due to the high recognition of the antibodies for this part of the molecule (11–14).

Antibodies generated by the 9-position linked immunogens are not as selective for the end of the molecule that is attached to the protein carrier. These antibodies display better binding to COOH-THC, the 8, 9, and 11-hydroxylated cannabinoids and their corresponding glucuronides than antibodies generated from the 1 and 5′-position immunogens (12,14,15). While the cross-reactivity for antibodies generated by the 9-position linked immunogens is less selective for the cannabinoid nucleus at the point of attachment to the protein, it does exhibit some selectivity for the cannabinoid nucleus in this region.

The THC molecule is extensively metabolized at the 8 and 11-positions and is subsequently glucuronidated before excretion in the urine (16–18). In order to increase the sensitivity in a urine-based immunoassay it would be of benefit to have antibodies that are indifferent to the cyclohexyl portion (9-position end) of the cannabinoid nucleus. An immunogen was synthesized that did not possess the antigenic determinants of the cyclohexyl ring and contained only the structural features of the benzpyran core (Fig. 2). This bicyclic immunogen (Fig. 3, compound **2**) was prepared from a hapten (Fig. 3, compound **9**) containing the corresponding benzpyran nucleus of the THC molecule. The characteristics of antibodies generated from this non-cannabinoid nucleus immunogen are described and assay results with clinical samples are given.

Materials and Methods

All solvents and starting raw materials were purchased from Aldrich Chemical Company (Milwaukee, WI). All biochemical materials such as bovine thyroglobulin (BTG) and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (St. Louis, MO). The glucuronide of Δ^9-11-nor-9-carboxytetrahydrocannabinol (COOH-THC-Glu) was purchased from Alltech (State College, PA). The cannabinoid metabolites Δ^9-11-nor-9-carboxytetrahydrocannabinol (COOH-THC), 8β-hydroxytetrahydrocannabinol (8β-OH-THC), 8α-hydroxytetrahydrocannabinol (8α-OH-THC), 11-hydroxy-Δ^8-tetrahydrocannabinol (11-OH-Δ^8-THC),11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC) and 8β,11-dihydroxy-Δ^9-tetrahydrocannabinol (8β,11-diOH-THC) were purchased from El Sohly Laboratories (Oxford, MS). Cannabinoid standards (COOH-THC) in urine at concentrations of 0, 25, 50, and 100 ng/mL were supplied from Roche Diagnostic Systems

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Received 12 Sept. 1997; and in revised form 4 Nov. 1997; accepted 1 Dec. 1997.

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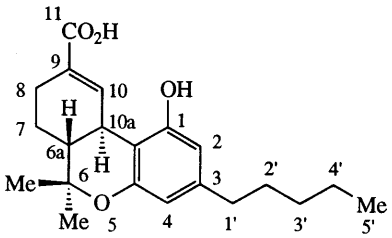

(1)

FIG. 1—*Δ^9-11-nor-9-carboxytetrahydrocannabinol.*

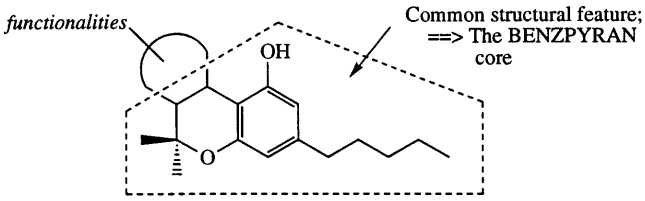

FIG. 2—*Benzpyran core of cannabinoid metabolites.*

(Somerville, NJ). Clinical negative urine samples and cannabinoid positive urine samples were obtained from a drug testing laboratory. The negative samples were screened negative by the OnLine Assay for Cannabinoids (Roche Diagnostic Systems) and the cannabinoid positive samples were screened positive by OnLine using a 50 ng/mL cutoff (COOH-THC) and confirmed by GC/MS.

The samples obtained from the clinical laboratory were stored at −20°C for six months before use and were tested on the same day for all the antibodies evaluated. Enzyme Linked Immunosorbent Assay (ELISA) plates were purchased from Coster (Cambridge, MA) and Alkaline Phosphatase was purchased from Zymed (South San Francisco, CA).

Immunogen Preparation

9-Position Immunogen—To a solution of 1.10 g of (BTG) in 22 mL of 50 mM $NaHCO_3$ pH 8.0 and 66 mL of dimethylsulfoxide (DMSO) was added at room temperature 6.8 mL of a solution of 1.00 g of the cannabinoid derivative [9R,-S-(6aα,10aβ)]-1-[5-(6a,7,8,9,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]pyran-9-yl)-1-oxopentyloxy]-2,5-pyrrolidinedione (19,20) in 14 mL of DMSO. The solution was stirred at room temperature overnight. The resulting solution was transferred to dialysis tubing and dialyzed sequentially against six changes of DMSO-50 mM potassium phosphate (KPi) pH 7.5 with gradually decreasing amounts of DMSO before dialyzing against five changes of 50 mM KPi pH 7.5. The BTG control was treated in a similar manner. The retentate from dialysis of the conjugate was then centrifuged to remove a small amount of solid material and the supernatant decanted off to give a solution of the 9-position immunogen in 50 mM KPi pH 7.5. The protein concentration was determined (Bio-Rad Coomassie Blue protein assay) to be about 4.7 mg/mL. The extent of modification of available lysines was determined by the Trinitrobenzene-sulfonic acid method (TNBS method) (21) to be about 98%, as measured against a BTG control.

Benzpyran Immunogen—The hapten (**9**) used in this immunogen was prepared according to Fig. 3 where the starting compound (**3**) was prepared according to a previous report (20). To a solution of 478 mg of purified bovine thyroglobulin (BTG) in 10.0 mL of 50 mM potassium phosphate buffer (KPi) pH 7.5 cooled in an ice bath was slowly added (dropping funnel) with constant stirring 30 mL of dimethyl sulfoxide (DMSO) over about 30 to 40 min, to give a solution of protein in 75% DMSO-50 mM phosphate buffer. From the resulting solution, 3.2 mL of solution was removed and kept as the control sample. To the remaining solution, containing about 440 mg BTG, was added in one lot a solution of 44 mg of 1-[3-(5-Hydroxy-2,2,4-trimethyl-7-pentyl-2H-1-benzopyran-3-yl)-1-oxopropoxy]-2,5-pyrrolidinedione (**9**) in about 3 mL of DMSO. The reaction was allowed to warm up to RT overnight with stirring. The slightly cloudy reaction solution was transferred to dialysis tubing (SpectraPor 7; molecular weight cut-off 50,000). The BTG control was also transferred to dialysis tubing. Both solutions were dialyzed at RT sequentially against 2 L each of 75% DMSO-50 mM KPi pH 7.5, 50% DMSO-50 mM KPi pH

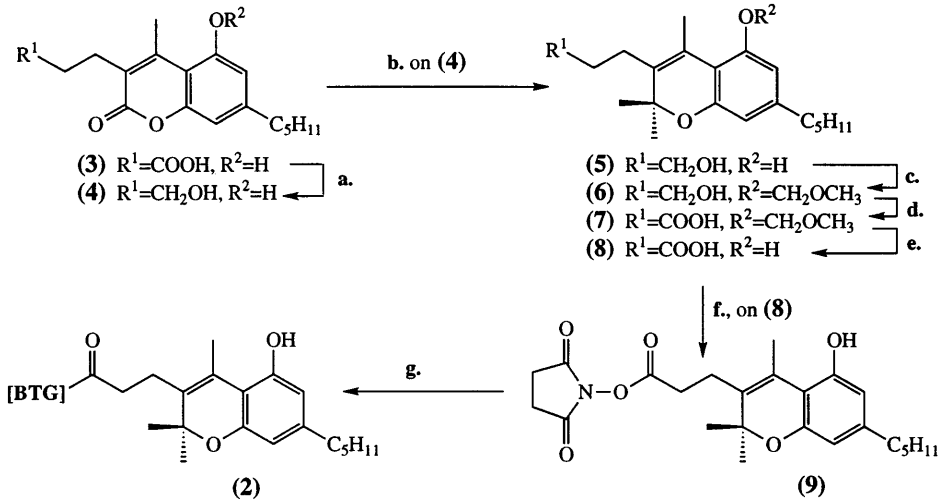

FIG. 3—*C_5H_{11} = n-pentyl;* **a.** *borane-tetrahydrofuran complex, in tetrahydrofuran (THF), −20°C −10°C, 65%;* **b.** *excess (xs.) methyl magnesium bromide, diethyl ether-THF (3:2), inverse addition at reflux. ~23%;* **c.** (i) *1 equivalent (eq) sodium hydride, dimethylformamide (DMF), room temperature (RT);* (ii) *1 eq methoxymethyl chloride, −94%;* **d.** *xs. pyridinium dichromate, DMF, RT, 24%;* **e.** *xs. pyridinium paratoluenesulfonic acid, tert-butanol, reflux, 26%;* **f.** *N-hydroxysuccinimide, 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride, methylene chloride, RT, 65%;* **g.** *bovine thyroglobulin (BTG), 75% dimethylsulfoxide (DMSO)-50 mM phosphate pH 7.5.*

7.5, 25% DMSO-50 mM KPi pH 7.5, and 50 mM KPi pH 7.5, before dialyzing against 6 × 4 L of 50 mM KPi pH 7.5 at 4°C. The resulting retentates were separately filtered through 0.8 μ filter units. Seventy-five mL of the benzpyran immunogen was obtained as a solution in 50 mM KPi pH 7.5. The protein concentration was determined (Coomassie Blue) to be 4.6 mg protein/mL, using the BTG control as the standard. The extent of available lysine modification was determined by the TNBS method to be about 69%, as measured against the BTG control.

Microtiter Plate Coating (THC-BSA)

The hapten used to prepare the 9-position immunogen was also used to prepare the THC-BSA conjugate by the following procedure: To a solution of 250 mg of Bovine Serum Albumin (BSA) in 5 mL of [50 mM KPi pH 7.5] and 14 mL of DMSO cooled in an ice water bath was added a solution of 3.6 mg of [9R,S-(6aα,10aβ)]-1-[5-(6a,7,8,9,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]pyran-9-yl)-1-oxopentyloxy]-2,5-pyrrolidinedione (19,20) in 1 mL of DMSO. The solution was stirred overnight at room temperature and then transferred to dialysis tubing (with a molecular weight cutoff of 10,000) and dialyzed in a manner similar to that described in the immunogen preparation. Filtration of the final conjugate retentate then gave 45 mL of a solution of conjugate in 50 mM KPi pH 7.5. The protein concentration was determined (Coomassie Blue protein assay) to be 4.9 mg/mL as measured against a standard sample of BSA.

Polyclonal and Monoclonal Antibody Preparation

Polyclonal Antibodies—Six-month to one-year-old goats and sheep were immunized with 3 mg of immunogen conjugate on day 0 emulsified in Complete Freunds Adjuvant. Subsequent immunizations were with 1 to 3 mg of immunogen conjugate emulsified in Incomplete Freund's Adjuvant given every four weeks. Blood was then taken from the animals and antisera prepared according to methods previously described (22).

Monoclonal Antibodies—Eight- to ten-week-old Balb/C mice (Jackson Laboratories) were injected with the 1-position (1–4), 9-position (7–9), or benzpyran immunogen. Mice immunized with the benzpyran immunogen were primed with the 1-position and 9-position immunogens on day 0 and day 25, respectively. On day 0, mice were injected with 100 μg of the immunogen emulsified in Complete Freund's Adjuvant (CFA) in a 1:1 ratio. On day 25, mice were boosted with 100 μg of the immunogen emulsified in Incomplete Freund's Adjuvant in a 1:1 ratio. A final boost series was administered using 400 μg, 200 μg, and 200 μg of the immunogen diluted in PBS and given at 72 h, 48 h, and 24 h, respectively, prior to cell fusion.

Splenocytes from an immunized mouse were isolated and fused to NSO myeloma cells in a 4:1 ratio using 50% polyethylene glycol, as described previously (23,24). NSO cells were plated at 250,000 cells/mL in 96-well microtiter plates and incubated at 37°C in a 9% CO_2 incubator until the clones were of sufficient size to screen.

Ascites Generation—Eight- to ten-week-old Balb/C female mice were primed with 0.5 mL pristane 7 to 14 days prior to injection of the cells for ascites. Ascites fluid was recovered as per methods well known in the art.

ELISA Plate Analysis

Ninety-six-well microtiter plates were coated with 50 μL of 5 μg/mL of the THC-BSA conjugate diluted in PBS and incubated for 2 h at room temperature. The liquid was removed from the plates by flicking them into a sink and blotting the plates onto absorbent paper. One hundred microlitres of 1% BSA in {PBS/azide} was dispensed into each well and the plates were incubated for 1 h at room temperature. Following the incubation, the plates were washed 3X with {PBS/.01% Tween 20}. Twenty-five microlitres of 1% BSA was added to the wells of each plate, followed by 25 μL of cell supernatant from each of the wells of the cell fusion. The plates were covered and incubated at 37°C for 1 h. The plates were washed on the plate washer three times with {PBS/ Tween 20} and 50 μL of anti-mouse antibody conjugated to alkaline phosphatase were added to each well. The plates were incubated at 37°C for 1 h and were then washed as described above. The assay was developed by the addition of 1 mg/mL paranitrophenyl phosphate dissolved in diethanolamine buffer at pH 9.8. The substrate-containing plates were incubated at room temperature for 30 min. Fifty μL of 3M NaOH were added to the wells to stop the enzyme reaction. The plates were read immediately at 405 nm.

Competition Assay and Analysis of Cross-Reactivity

The competition assays were set up as above except that the cross-reactant (25 μL) or urine specimen was added to the plate in the presence of antibody-containing cell supernatants. The cross-reactivity was calculated using the equation provided below. All calculations were based upon binding and displacement at the 50% of maximum O.D. (optical density) binding point.

$$\%\ \mathrm{CR} = \frac{(\text{O.D. } \textit{without} \text{ cross-reactant} - \text{O.D. } \textit{with} \text{ cross-reactant}) \times (100/\text{C.F.})}{\text{O.D. } \textit{without} \text{ COOH-THC} - \text{O.D. } \textit{with} \text{ COOH-THC})}$$

where C.F. is a correction factor used to account for the different levels of cross-reactant that may be used in an assay, and C.F. = ng of cross-reactant/ng Δ^9-THC acid.

Results

Cross-Reactivity Studies

Polyclonal antibodies from sheep and goats and murine monoclonal antibodies were generated through the use of both the benzpyran and 9-position immunogens. Using COOH-THC as a standard, the cross-reactivity of the antibodies towards COOH-THC-Glu, 8β-OH-THC, 8α-OH-THC, 11-OH-Δ^8-THC, 11-OH-Δ^9-THC, and 8β,11-diOH-Δ^9-THC were determined by ELISA. The most common metabolites found in human urine are COOH-THC and its glucuronide (COOH-THC-Glu) and 11-OH-Δ^9-THC. Lesser amounts of 8β,11-di-OH-Δ^9-THC are found while 8 α-OH and 8β-OH THC are not detected in the majority of samples (29). These compounds contain modifications on the cyclohexyl portion of the cannabinoid nucleus a portion which is not present in the benzpyran immunogen. The compounds were selected in order to probe the antibodies for their ability to cross-react with the part of the cannabinoid molecule that is extensively oxidized. The 9-position generated antibodies should be capable of recognizing changes on the cyclohexyl ring and would then display lower cross-reactivity towards metabolites possessing significant changes in

the cyclohexyl ring, such as is the case with the major metabolites. Conversely, the benzpyran generated antibodies should be insensitive to these changes and hence display higher cross-reactivity towards the cannabinoid metabolites wherein the cyclohexyl ring has been modified. All of the metabolites tested have been previously identified in human urine samples (11–13,16,29), and high cross-reactivity to these metabolites is relevant for developing clinically sensitive antibodies.

The plate coating contained BSA conjugated to a 9-position THC hapten. The cross-reactivity was determined by the difference in competition of the antibody for the plate hapten *versus* each THC metabolite. The results (Table 1) of these experiments show that the polyclonal antisera produced from the benzpyran immunogen show two to four times higher cross-reactivity to the various THC metabolites than polyclonal antisera produced from the 9-position immunogen. The one exception to this difference in cross-reactivity is seen with COOH-THC-Glu. In this case the cross-reactivities shown by the benzpyran generated antibodies are only slightly higher than that shown by antibodies generated by the 9-position immunogens. This is not unexpected since all cross-reactivity is measured relative to the COOH-THC standard, which has the same structure at the cannabinoid nucleus as COOH-THC-Glu. Since antibodies generated out of the 9-position immunogen are selected on the basis of preferential binding to a THC derivative possessing a carboxy functionality and a double bond at the 9-position (i.e., COOH-THC), the corresponding 9-position glucuronide possessing the same carboxy and double-bond functionalities should not affect the binding of the antibody to the cannabinoid nucleus. There may be some steric inhibition of binding to the COOH-THC-Glu by the antibody but as the data in Table 1 indicate, the effect is small and the cross-reactivity of the antibodies generated by either immunogen for COOH-THC-Glu is high. The number of animals shown in Table 1 is representative of a larger animal population and the results are typical of what the cross-reactivity differences are between antibodies generated by benzpyran and 9-position immunogens. The cross-reactivity difference is also independent of the animal species (goat or sheep) tested.

Generation of monoclonal antibodies using the benzpyran and 9-position immunogens yielded similar results as in the case of the polyclonal antibodies. The benzpyran immunogens produced antibodies that consistently showed higher cross-reactivity with all of the THC metabolites than antibodies produced from the 9-position immunogen (Table 1). The one exception to this is with the COOH-THC-Glu where the cross-reactivity differences were not as dramatic (see discussion in preceding paragraph) and were high in both types of antibodies. The cross-reactivity differences among the antibodies generated by the two immunogens were not as great as in the case of the polyclonal antibodies and generally ranged from two to three times more for antibodies produced by the benzpyran immunogen. Other monoclonal antibodies were tested and the characteristics listed in Table 1 are consistent and representative for the respective immunogens.

Clinical Performance

A monoclonal antibody produced from each of the two immunogens was selected for further characterization with human urine samples obtained from a urine drug testing laboratory. Samples that were positive for COOH-THC by gas chromatography/mass spectrometry (GC/MS) were evaluated with monoclonal 11A6 (benzpyran immunogen) and monoclonal 11E (9-position immunogen). Consistent with the metabolite cross-reactivity, antibody 11A6 gave higher total values for cross-reactive cannabinoids than did antibody 11E (Table 2). The difference in the value given for cross-reactive cannabinoids between both antibodies was greater than 49% since many samples showed greater than 100 ng/mL cannabinoid equivalents with antibody 11A6. One hundred negative samples were also tested with both antibodies. With these

TABLE 2—*Value (ng/mL) of cross-reactive cannabinoids in clinical specimens using monoclonal antibodies 11E and 11A6.*

	MoAb 11E* (9-Substituted)	MoAb 11A6* (Benzpyran)	GC/MS Value†
	40	82	33
	24	49	37
	93	>100	68
	63	>100	59
	44	79	42
	62	96	34
	65	>100	89
	85	>100	73
	95	>100	53
	57	>100	33
Average	62	90	52

* ng/mL COOH-THC equivalents.
† ng/mL COOH-THC.

TABLE 1—*Comparison of antibody cross-reactivity with cannabinoids using ELISA.*

		% Cross-Reactivity						
Immunogen Structure	Antibody	COOH-THC	8β-OH-THC	11-OH-Δ^8-THC	11-OH-Δ^9-THC	8α-OH-THC	8,11-diOH-THC	COOH-THC-Glu
Benzpyran	goat polyclonal 257	100	131	121	116	136	131	95
Benzpyran	sheep polyclonal 1172	100	112	109	96	125	114	ND
Benzpyran*	goat polyclonal 120	100	153	126	130	155	151	ND
Benzpyran*	sheep polyclonal 1212	100	142	135	129	157	135	ND
9-Position	goat polyclonal 421	100	30	40	32	38	44	85
Benzpyran	murine monoclonal 11A6	100	85	100	98	91	98	100
Benzpyran	murine monoclonal 25D	100	99	100	100	103	101	103
9-Position	murine monoclonal 4F	100	4.4	36	28	89	0	82
9-Position	murine monoclonal 5G	100	34	54	53	91	26	85
9-Position	murine monoclonal 11E	100	28	85	28	36	44	86

* NOTE: The immunogen used for these animals was prepared from the saturated benzpyran analog of compound 9.
ND = Not determined.

samples there were no false positive results, and the use of the 11A6 antibody did not give a higher background when compared with the use of the 11E antibody.

Discussion and Conclusion

Previous studies have shown that antibodies generated from immunogens based on COOH-THC in which the protein is linked to the cannabinoid nucleus through the 1, 2, or 5′-positions of the molecule are more selective for the cyclohexyl ring. These antibodies in turn display lower cross-reactivates to the 8, 9, and 11-substituted metabolites. In addition, clinical samples tested with antisera generated from these immunogens give lower values of total cross-reactive cannabinoids (10,12–14).

Other studies have shown that immunogens prepared from a protein linked to a cannabinoid derivatized at the 9-position elicit antibodies that are less selective for the cyclohexyl ring. These antibodies cross-react more broadly with the 8, 9, and 11-substituted metabolites and give higher total values for cross-reactive cannabinoids in clinical samples than antibodies generated by the 1, 2, or 5′-position immunogens (12,14,15). Antibodies generated from the 9-position immunogens can be selected for either high or low cross-reactivity towards the 8, 9, and 11-substituted metabolites, and the differences can range from 10% to 100%. It is, however, difficult to find a particular antibody or antiserum generated from a 9-position immunogen that has close to 100% cross-reactivity towards all of the 8, 9, and 11-substituted metabolites at once (9,26–28). Since the 9-position immunogen contains the cyclohexyl ring, finding antibodies that do not recognize or are indifferent to all of the antigenic determinants in this portion of the molecule is extremely difficult.

In this study an immunogen was prepared that did not contain any of the antigenic determinants of the cyclohexyl ring. This was accomplished by using a non-cannabinoid benzpyran structure that contains the epitopes of the more conserved benzpyran core (Fig. 2) of the cannabinoid molecule. The antibodies generated using this type of benzpyran immunogen display very broad cross-reactivity to the 8, 9, and 11-substituted metabolites (Table 1). The cross-reactivity observed can range from 85% to greater than 150% and is consistently high for all of the metabolites tested with a particular antibody or antiserum. This broad cross-reactivity manifests itself in higher values for cross-reactive cannabinoids in clinical samples (Table 2), while at the same time a low background is maintained with negative urine samples. Since antibodies generated by the benzpyran immunogen display higher values of COOH-THC equivalents with urine samples, the clinical sensitivity using such antibodies would be higher than that of antibodies generated by traditional immunogens. This increase in assay sensitivity has the disadvantage of producing more specimens that are positive in a screen, but which cannot be confirmed using the same GC/MS cutoff that is used with more traditional antibodies. Therefore, in order to maintain the same confirmation rate with the benzpyran generated antibodies, a lower GC/MS cutoff would be needed.

The benzpyran immunogen gave a similar response in all the species tested. The benzpyran polyclonal antisera showed broader cross-reactivity than the benzpyran monoclonal antibodies. This may be due to the nature of the polyclonal mixture where the cross-reactivity is representative of a wide range of antibody selectivity and affinities. In the case of monoclonal antibodies the selectivity is greater due to the single-binding nature of the antibody. There was a greater difference in cross-reactivity between the 9-position and benzpyran generated polyclonal antisera than for the corresponding monoclonal antibodies. This is not unexpected since the 9-position generated monoclonal antibodies were selected for their broader cross-reactivity. With the polyclonal antisera it was not possible to select certain antibodies and the composition of the antibody mixture represents a wide range of selectivity.

In summary, this paper demonstrates the ability of a non-cannabinoid immunogen to generate anti-cannabinoid polyclonal and monoclonal antibodies. By using a drug derivative that contains antigenic determinants of the conserved portions of the cannabinoid nucleus it is possible to elicit antibodies that broadly cross-react with many of the most common cannabinoid metabolites. This broad cross-reactivity translates into an immunoassay that yields higher total values for cross-reactive cannabinoids with urine samples shown by GC/MS to be positive for cannabinoids. At the same time, urine samples shown to be negative for cannabinoids yield low background values which are comparable to antibodies that have been generated from immunogens made with intact cannabinoid derivatives. This concept can be applied to other drug assays where the portion of the molecule that undergoes extensive metabolism can be eliminated from the immunogen structure so long as there is a core structure that is preserved. The results of such an immunogen would be to elicit antibodies which yield higher cross-reactive values with positive clinical samples.

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