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Phenotypic screening of cannabinoid receptor 2 ligands shows different sensitivity to genotype

Julia M. Hillger [a], Beau le Roy [a], Zi Wang [d], Thea Mulder-Krieger [a], Dorret I. Boomsma [b], P. Eline Slagboom [c], Erik H.J. Danen [d], Adriaan P. IJzerman [a], Laura H. Heitman [a,*]

[a] Division of Medicinal Chemistry, LACDR, Leiden University, The Netherlands
[b] Department of Biological Psychology, Vrije Universiteit Amsterdam, The Netherlands
[c] Section of Molecular Epidemiology, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, The Netherlands
[d] Division of Toxicology, LACDR, Leiden University, The Netherlands

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CP55940 (PubMed CID: 10479060)
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ABSTRACT

The Cannabinoid Receptor 2 (CB_2R) is a G protein-coupled receptor (GPCR) investigated intensively as therapeutic target, however no drug has reached the market yet. We investigated personal differences in CB_2R drug responses using a label-free whole-cell assay (xCELLigence) combined with cell lines (Lymphoblastoid Cell Lines) from individuals with varying CB_2R genotypes. Responses to agonists, partial agonists and antagonists of various chemical classes were characterized. Endogenous cannabinoids such as 2-AG induced cellular effects vastly different from all synthetic cannabinoids, especially in their time-profile.

Secondly, the Q63R polymorphism affected CB_2R responses in general. Agonists and especially partial agonists showed higher efficacy in a Q63R minor homozygote versus other genotypes. Non-classical cannabinoid CP55940 showed the most pronounced personal effects with highly reduced potency and efficacy in this genotype. Contrarily, aminoalkylindole compounds showed less individual differences.

In conclusion, a label-free whole-cell assay combined with personal cell lines is a promising vehicle to investigate personal differences in drug response originating from genetic variation in GPCRs. Such phenotypic screening allows early identification of compounds prone to personal differences ('precision medicine') or more suited as drugs for the general population.

1. Introduction

The Cannabinoid Receptor 2 (CB_2R) is a class A G Protein-Coupled Receptor (GPCR) which has been investigated intensively, for instance as therapeutic target for novel immunomodulators [1]. The Cannabinoid receptor family consists of CB_1R, CB_2R and as of late, the former orphan receptors GPR55 and GPR18. Together with their endogenous ligands, they form part of the endocannabinoid system which is involved in many physiological processes. CB_2R is a (predominantly) $G\alpha_i$-coupled receptor which is expressed mainly in cells of the immune system, such as T- and B-lymphocytes, as well as the central and peripheral nervous system and the gastrointestinal tract [1–3]. As such, the CB_2R is involved in a wide range of pathological conditions ranging from atherosclerosis [4], neuropathic pain [5], neurodegenerative diseases [6], osteoporosis [7] and autoimmune diseases [8] to cancer [9–11]. Hence, the CB_2R has been in the focus of drug development efforts for over a decade. However, no selective drug targeting the CB_2R has made it to the market as of yet. There can be several reasons as to why drugs fail in clinical trials, one of which is

Abbreviations: 2-AG, 2-Arachidonoylglycerol; ACN, Acetonitrile; AEA, Anandamide; cAMP, cyclic adenosine 5′-monophosphate; CB_1R, Cannabinoid Receptor 1; CB_2R, Cannabinoid Receptor 2; CI, Cell Index; CNR1, Cannabinoid Receptor 1 gene; CNR2, Cannabinoid Receptor 2 gene; Δ CI, Δ Cell Index or Delta Cell Index; DMSO, dimethylsulfoxide; EBV, Epstein-Barr Virus; EC_{50}, half maximal effective concentration; EC_{80}, 80% maximal effective concentration; E_{max}, maximum effect; FCS, Fetal calf serum; GoNL, Genomes of the Netherlands consortium; GPCR, G Protein-Coupled Receptor; Ind., Individual; K_i, equilibrium inhibition constant; LCL, Lymphoblastoid cell line; NTR, Netherlands Twin Register; PTX, Pertussis Toxin; RTCA, real-time cell analyzer; SNP, Single Nucleotide Polymorphism.

* Corresponding author.
E-mail address: l.h.heitman@lacdr.leidenuniv.nl (L.H. Heitman).

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differences in individuals' responses to the drug. In fact, even the most widely prescribed and sold drugs, the so-called big 'blockbuster' drugs, only work in 35–75% of all patients [12], as individual drug response varies due to differences in genetics, lifestyle and environment. Therefore, personalized or precision medicine aims to personalize drug prescriptions based on a patient's individual characteristics, e.g. genetic information, and thereby decreases risks of ineffective dosing or side-effects [13,14]. An abundant source of genetic variation in humans is Single Nucleotide Polymorphism (SNP), which can lead to an alteration in the amino acid sequence of a protein [15]. Many polymorphisms have been documented in the CB_2R, including three that change the amino acid sequence and occur highly frequently in the population, namely Q63R, Q66R and H316Y [16]. Of these, both Q63R and H316Y have been linked to various pathological conditions. Q63R is special, as it can be caused by a SNP (rs2501432) as well as a dinucleotide polymorphism (rs35761398). Q63R has been shown to be involved in schizophrenia and depression [17–19], alcoholism [20], eating disorders [21], early menarche in obesity [22] and various immune system related disorders [23–25], while H316Y has been associated with lowered bone mineral density [26].

We investigated personal differences in CB_2R drug responses using a sensitive *in vitro* assay, i.e. a label-free cellular assay using the xCELLigence system, in combination with personal cell lines. With the xCELLigence, whole-cell responses are measured non-invasively allowing for the investigation of drug responses in an unbiased way, i.e. without selecting one signaling pathway or effect. The personal cell lines used in this study were Lymphoblastoid Cell Lines (LCLs) obtained from participants of the Netherlands Twin Register (NTR), which are derived from B-lymphocytes and thus endogenously express the CB_2R [27,28]. Using LCLs from individuals with different CB_2R genotypes, we tested a number of ligands ranging from agonists and partial agonists to antagonists (Fig. 1), which have potential use in different pathological indications. Firstly, endogenous cannabinoids are fatty acid derivatives such as the eicosanoids 2-AG (2-Arachidonoylglycerol), the main endogenous ligand for CB_2R, and AEA (anandamide) [29,30]. Synthetic cannabinoids can be divided into classical and non-classical, such as JWH133 and CP55940, respectively. Another large class of synthetic cannabinoid receptor ligands are the aminoalkylindoles, of which WIN55212-2 is the most studied agonist and AM630 is one of the most utilized CB_2R antagonists [1,31]. Several classes also contain partial agonists, such as aminoalkylindole GW405833 or BAY59-3074, which belongs to a separate chemical class.

In this study, we show that the xCELLigence in combination with these personal cell lines can be successfully applied to investigate personal differences in drug response originating from, for instance, genetic variation in GPCRs. We furthermore demonstrate that while certain classes of CB_2R ligands show individual differences, others deliver consistent effects independent of genotype. Thus while taking personal medical effects into account, it is still possible to identify potential 'blockbuster' drugs by using such phenotypic screening methods with personal cell lines.

2. Material and methods

2.1. Chemicals and reagents

Fibronectin from bovine plasma, Roswell Park Memorial Institute (RPMI) 1640 cell culture medium (25 mM HEPES and $NaHCO_3$) and Pertussis Toxin (PTX) were purchased from Sigma Aldrich (Zwijndrecht, NL). CB_2R ligands AM630, GW405833 and CP55940 were purchased from Sigma Aldrich, BAY59-3074, WIN55212-2 mesylate, JWH133 and AEA from Tocris Bioscience (Bristol, UK) and 2-AG from Cayman Chemicals (Ann Arbor, MI, USA). All other chemicals and reagents were of analytical grade and obtained from commercial sources, unless stated otherwise.

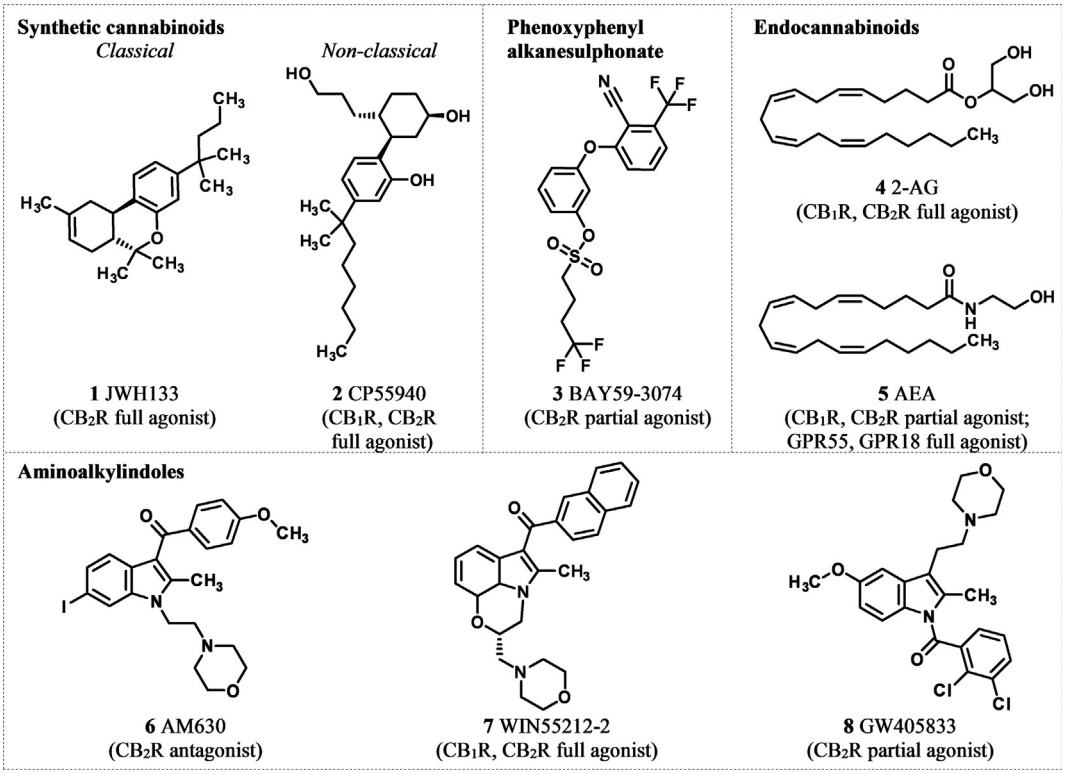

Fig. 1. Chemical structures of CB_2R ligands characterized in this manuscript.