Visualizing the anti-inflammatory cannabinoid Type-2 receptor

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23 March 2023

Medicinal chemists describe how small molecule probes allow for the detection of CB_2R, and thereby enable the discovery of novel anti-inflammatory treatments

The G-protein-coupled receptor (GPCR) CB_2R [1] is an essential constituent of the endocannabinoid system (ECS), a central lipid signalling system in all vertebrates [2]. Preclinical studies have shown that CB_2R activation is a general and robust principle to attenuate inflammation and associated tissue injury in a wide variety of pathological conditions.

Strong accumulating evidence suggests that diseases such as heart disorders, gastrointestinal, liver, kidney, lung, neurodegenerative or neuroinflammatory diseases, pain, skin pathologies, rheumatoid arthritis, endometriosis and eye diseases might be therapeutically addressable by CB_2R [3].

Type-2 Cannabinoid Receptor (CB_2R) – A key toward the treatment of inflammatory diseases

Although several promising therapeutic small molecule drugs are in advanced clinical trials, no selective CB_2R activators, so-called agonists, have reached the market to leverage the enormous therapeutic potential of CB_2R. The reason for this is challenges related to:

1. Determining the localization and functional expression of CB_2R;
2. Insufficient quality and selectivity of early tool compounds;
3. Overall characteristics of first drug development candidates;
4. Lack of translatability of promising preclinical efficacy data toward humans which has been discussed in a recent open access government contribution entitled 'Challenges bringing CB_2R medicine to bedside'[4].

Tools for detecting CB_2R protein

While sophisticated mRNA techniques reliably enable the measurement of CB_2R messenger RNA (mRNA) expression, the functional detection and localization of CB_2R protein remained challenging for the past three decades.

Generally, antibodies are used for such purposes but in the case of CB_2R no specific antibodies could be obtained despite extensive investigations. Only very recently, highly selective chemical tools, so-called labelled chemical probes, have been developed which now allow for the reliable detection of CB_2R protein [5].

A labelled chemical probe is a small molecule that is a specific ligand for the target protein of interest, in our case CB_2R, and bears a reporter unit that allows a spatiotemporal characterization of target ligand-protein interactions. Such probes can be used to verify e.g., target protein engagement and quantify target occupancy, identify biomarkers and thereby support preclinical studies and human trials with therapeutic CB_2R agonists (Figure 1).

While antibodies usually are limited to the detection of one animal species and require considerable effort and experimental adaptions for intracellular delivery or *in vivo* applications, a particular advantage is that a labelled chemical probe can be derived from small molecule ligands similar to the present drug molecule and can therefore be used throughout the entire translational path across species. Particularly fluorescently labelled and positron emission tomography (PET) tracers are of highest relevance as chemical probes for CB_2R.

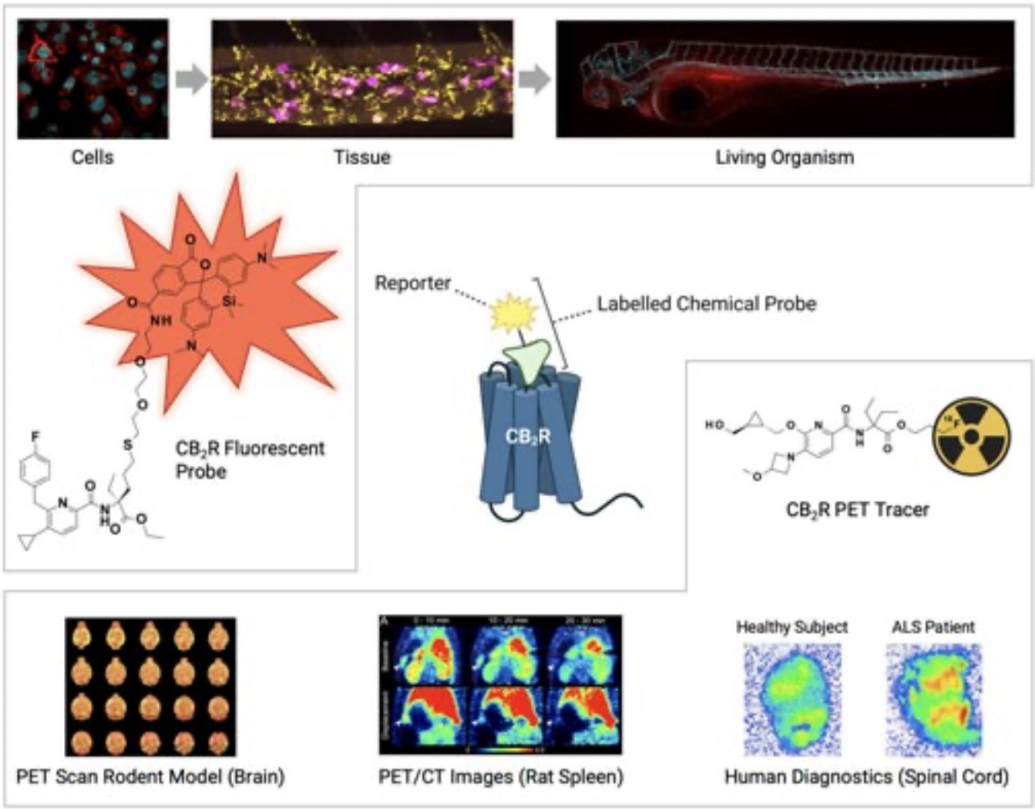

Figure 1: Labelled chemical probes are small molecules that are ligands for a molecular target such as the GPCR CB2R and bear a reporter unit that allows characterization of ligand- target interactions. Such probes address fundamental questions associated with the respective molecular target. They impact all stages of drug discovery programs starting from target identification and validation up to applications as target engagement biomarkers or diagnostics in clinical studies. Figure 1 was created with BioRender.com.

Fluorescently labelled CB_2R ligands – Illuminating CB_2R

Fluorescently labelled CB_2R probes carry a fluorescent dye serving as a light-based reporter group. When a specific wavelength of light is shone on them, the dye absorbs the light and then re-emits a different specific wavelength. This emitted light can then be

detected and analyzed in colorful images up to single molecule resolution. Because the fluorescent probes are attached to CB_2R, the fluorescence signal detected indicates the location of that receptor.

Highly specific novel CB_2R fluorescent probes have been successfully applied to detect and visualize the cellular trafficking of CB_2R in different living cellular systems and were used to study cell signalling mechanisms and expression throughout the body in living organisms (6). In clinical studies, such probes can be applied for detecting target occupancy levels of a development candidate e.g. in blood samples from healthy volunteers or patients thus supporting the identification of therapeutically relevant doses for anti-inflammatory treatments.

CB_2R PET tracers – Non- invasive 3D visualization of CB_2R in living organisms

PET tracers belong to the group of radiolabeled imaging agents. The introduction of the PET emitting atom is of particular advantage since depending on the used isotope there is no or only minimal one single atom changes for the introduction of the reporter necessary.

Therefore, no alteration of the pharmacological properties of the parent drug is observed.

Such chemical probes are applied for disease quantification, e.g. by comparing protein distribution and levels in the healthy and diseased state, for drug candidate selection and for facilitating their clinical development toward anti-inflammatory treatments.

A prime area of application is the therapeutic dose selection in clinics, which is decisive for successful enrollment of the clinical trial and challenging when like for CB_2R no predictive animal model or validated clinical biomarker is available – especially for targets in the central nervous system or organs that cannot be sampled easily. For these drugs, imaging agents are the only way to quantify target engagement in patients and predict a clinical dose that will achieve efficacy with minimal unwanted side effects in anti-inflammatory treatments.

Recently discovered CB_2R PET tracer [^{18}F]RoSMA-18-d_6 is based on the short-lived positron emitter [^{18}F] (7). It was successfully applied in vivo PET studies and allowed to detect CB2R upregulation on post-mortem human ALS spinal cord tissues thus holding great promise for using CB2R as a marker of neuroinflammation and for supporting clinical studies with therapeutic CB2R agonists for the treatment of neuroinflammatory diseases.

In summary, the recently discovered high-quality labelled CB_2R fluorescent and PET probes have significantly contributed to a better understanding of CB_2R expression in health and disease states across species and deepened the insights into the receptors function and mechanism of action.

Furthermore, these probes allow translational studies to an unprecedented extent supporting the clinical development of CB_2R agonists and ultimately unlocking the great potential of this receptor for anti-inflammatory treatments.

References

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Acknowledgements

We wish to thank all colleagues who have contributed over the years to our investigations on CB_2R.

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