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Cardio-, hepato- and pneumoprotective effects of autophagy checkpoint inhibition by targeting ACBP/DBI

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Abstract: Acyl-CoA binding protein (ACBP), also known as diazepam-binding inhibitor (DBI), acts as a paracrine inhibitor of autophagy. We characterized a monoclonal antibody neutralizing mouse ACBP/DBI (a-DBI) for its cytoprotective effects on several organs (heart, liver and lung) that were damaged by surgical procedures (ligation of coronary and hepatic arteries or bile duct ligation), a variety of different toxins (acetaminophen, bleomycin, carbon tetrachloride or concanavalin A) or a methionine/choline-deficient diet (MCD). In all these models of organ damage, a-DBI prevented cell loss, inflammation and fibrosis through pathways that were blocked by pharmacological or genetic inhibition of autophagy. The hepatoprotective effects of a-DBI against MCD were mimicked by three alternative strategies to block ACBP/DBI signaling, in particular (i) induction of ACBP/DBI-specific autoantibodies, (ii) tamoxifen-inducible knockout the *DBI* gene, and (iii) a point mutation in the gamma-2 subunit of the GABA_A receptor (*Gabrg2*^{F771}) that abolishes binding of ACBP/DBI. We conclude that a-DBI-mediated neutralization of extracellular ACBP/DBI mediates potent autophagy-dependent organ protection by on-target effects, hence unravelling a novel and potentially useful strategy for autophagy enhancement. “Autophagy checkpoint inhibition” (ACI) can be achieved by targeting ACBP/DBI.

Paper that is commented on:

ACBP/DBI neutralization confers autophagy-dependent organ protection through inhibition of cell loss, inflammation and fibrosis. Motiño O, Lambertucci F, Anagnostopoulos G, Li S, Nah J, Castoldi F, Senovilla L, Montégut L, Chen H, Durand S, Bourgin M, Aprahamian F, Nirmalathan N, Alvarez-Valadez K, Sauvat A, Carbonnier V, Djarvarheri-Mergny M, Pietrocola F, Sadoshima J, Maiuri MC, Martins I, Kroemer G. *Proc Natl Acad Sci U S A*. In press

Main text:

Acyl-CoA binding protein (ACBP) is a phylogenetically ancient intracellular protein that binds so-called activated fatty acids (acyl coenzyme A esters) to facilitate their transport and metabolism. ACBP is also known under the name of diazepam-binding inhibitor (DBI) because it interacts with benzodiazepine receptors including the $\alpha 2$ subunit of the gamma-aminobutyric acid (GABA) receptor of the A type (GABA_A). Importantly, ACBP/DBI is a leaderless protein that can be released through an autophagy-dependent pathway and then acts on GABA_A receptors to inhibit autophagy, thus participating to an inhibitory feedback loop that limits autophagy. This feedback loop or “autophagy checkpoint” can be interrupted by knockdown or knockout of the *DBI* gene coding for ACBP/DBI, by neutralizing ACBP/DBI by means of autoantibodies or externally supplied antibodies, or by mutating the $\alpha 2$ subunit of GABA_A receptor (GABRG) to abolish ACBP/DBI binding (*Gabrg2*^{F771}). Hence, genetic removal of ACBP/DBI expression, neutralization of ACBP/DBI or mutation of its receptor all similarly enhance autophagic flux. In other words, “autophagy checkpoint inhibition” (ACI) can be achieved by targeting ACBP/DBI. We have chosen this terminology, ACI, in analogy with immune checkpoint inhibition (ICI), which has become the backbone of most oncological treatments aiming to stimulate anticancer immune responses. ICI targets immunosuppressive ligand-receptor interactions (between PD-L1 and PD1 or between CTLA-4 and its receptors) by suitable monoclonal antibodies (against PD-L1, PD-1 or CTLA-4), thus releasing the break from the immune system, while ACI would target ACBP/DBI to release the break from autophagy.

One peculiarity of ACI targeting ACBP/DBI is the multiplicity of autophagy-inducing effects. Thus, beyond rapid autophagy induction achieved by neutralizing the interaction between ACBP/DBI and GABA_A expressed on multiple cell types, ACBP/DBI neutralization also reduces food intake and stimulates lipolysis with a consequent increase in free fatty acids and ketone bodies that can be expected to induce long-term effects favoring autophagy. We observed in the past that ACBP/DBI neutralization by means of a specific monoclonal antibody (a-DBI) prevented high-fat diet-induced obesity as well as adiposity, hepatosteatosis and type-2 diabetes (**Fig. 1A**). However, from these experiments it was not clear whether the liver-protective effects were secondary to the induction of autophagy or the reduction of caloric uptake induced by ACBP/DBI. Driven by this consideration, we decided to investigate the

capacity of a-DBI to prevent partially non-alcoholic steatohepatitis (NASH) induced by a protocol that usually causes weight loss (rather than weight gain) and that involves a hepatotoxic methionine/choline deficient diet (MCD). In this model, we observed that weekly a-DBI injections prevented NASH, as they partially antagonized the MCD-induced weight loss. Conversely, inhibition of autophagy by daily injections of 3-hydroxychloroquine or knockout of ATF4 abolished the anti-NASH effects of a-DBI. Hence the hepatosteatosis-suppressive effect of a-DBI could be uncoupled from its anti-obesity action but appeared to rely on autophagy induction. Accordingly, a-DBI induced the stigmata of autophagy in hepatocytes and reduced the local expression of pro-inflammatory and pro-fibrotic gene products, as well as the infiltration of the organ by macrophages (**Fig. 1B**).

Intrigued by these observations, we determined whether the effects of a-DBI would be truly on-target. For this, we used three alternative methods for blocking the ACBP/DBI system, namely (i) vaccination with adjuvanted ACBP/DBI to break self-tolerance and to induce ACBP/DBI-specific autoantibodies, (ii) and ubiquitous tamoxifen-inducible knockout of exon 2 of the *DBI* gene, and (iii) a point mutation in the gamma-2 subunit of the GABA_A receptor (*Gabrg2*^{F77I}) that abolishes binding of ACBP/DBI. All three alternative methods similarly protected against MCD-induced NASH, supporting the idea that a-DBI acts solely on ACBP/DBI (rather than on additional cross-reactive targets) to achieve hepatoprotection (**Fig. 1B**).

We also wondered whether a-DBI would have a broader hepatoprotective effect against mechanical insults (such as ischemia/reperfusion of the hepatic artery, which causes necrosis, and bile duct ligation, which leads to fibrosis) and toxins (such as acetaminophen and concanavalin A, which both trigger necrosis, and carbon tetrachloride which induces cell death and fibrosis). In all these conditions, a-DBI conferred significant hepatoprotection that was reversed by 3-hydroxychloroquine injections, suggesting that it required autophagy to be efficient. Moreover, a-DBI did not only mediate prophylactic effects but also helped to reverse manifest MCD-induced NASH and carbon tetrachloride-induced liver fibrosis (**Fig. 1C**).

In a final set of experiments, we determined the capacity of a-DBI to act on other organs than the liver. Indeed, we observed that a-DBI prevented the fibrosis of lungs induced by intranasal administration of bleomycin, coupled to a reduction in inflammation- and fibrosis-relevant genes. Moreover, a-DBI, which induces autophagy and mitophagy in the heart muscle, was

capable of reducing the infarcted area of mice subjected to ligation of the left coronary artery. This cardioprotective effect of a-DBI was lost after cardiomyocyte-specific knockout of the essential autophagic gene *ATG7*, meaning that it depended on autophagy (**Fig. 1D**).

In sum, in mouse models, ACI with a-DBI has broad cardio-, hepato- and pneumoprotective effects. Nonetheless, there are several challenges for the preclinical development of ACI. First of all, it remains to be determined whether other organs (e.g., gut, joints, kidney, retina...) or systemic diseases (e.g., cystic fibrosis, lupus erythematosus, septic shock...) that might be targeted by autophagy enhancers would profit from the cytoprotective and anti-inflammatory effects of a-DBI. Second, given that the a-DBI antibody used in our studies was mouse-specific (and hence did not recognize human ACBP/DBI), it will be important to develop a new series of monoclonal antibodies that target both mouse and human ACBP/DBI to increase the translational relevance of the studies.

Conflicts of interest: GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sotio, Tollys, Vascage and Vasculox/Tioma. GK has been consulting for Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. Among these, patents were licensed to Bayer (WO2014020041-A1, WO2014020043-A1), Bristoll-Myers Squibb (WO2008057863-A1), Osasuna Therapeutics (WO2019057742A1), PharmaMar (WO2022049270A1 and WO2022048775-A1), Raptor Pharmaceuticals (EP2664326-A1), Samsara Therapeutics (GB202017553D0), and Therafast Bio (EP3684471A1).

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Figure Legend

Fig. 1: Schematic effects of ACBP/DBI neutralization. A. Anti-obesity effects. B. Anti-steatohepatitis effects in the context of methionine/choline-deficient diet. C. Other hepatoprotective effects. D. Cardio- and pneumoprotective effects. Abbreviations: Acyl-CoA binding protein (ACBP), Actin alpha 2 (Acta2), Catalase (CAT), Cluster of Differentiation 68 (CD68), Concanavalin A (ConA), Carnitine palmitoyltransferase I (Cpt1a), Diazepam-binding inhibitor (DBI), EGF-like module-containing mucin-like hormone receptor-like 1 (F4/80), Heme oxygenase 1 (Hmox1), Glucose transporter (GLUT), Glutathione peroxidase (Gpx), Glutathione reductase (Gsr), Interleukin 1 beta (Il1b), Interleukin 6 (Il6), Nuclear factor erythroid 2-related factor 2 (Nrf2), Monocyte chemoattractant protein 1 (Mcp1), Non-alcoholic steatohepatitis (NASH), Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing 3 (Nrlp3), Platelet-derived growth factor (Pdgf), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Pgc1a), Peroxisome proliferator-activated receptor alpha (Ppara), Superoxide dismutase 1 (Sod1), Superoxide dismutase 2 (Sod2), Uncoupling protein 1 (UCP1), Tumor necrosis factor alpha (Tnf α).

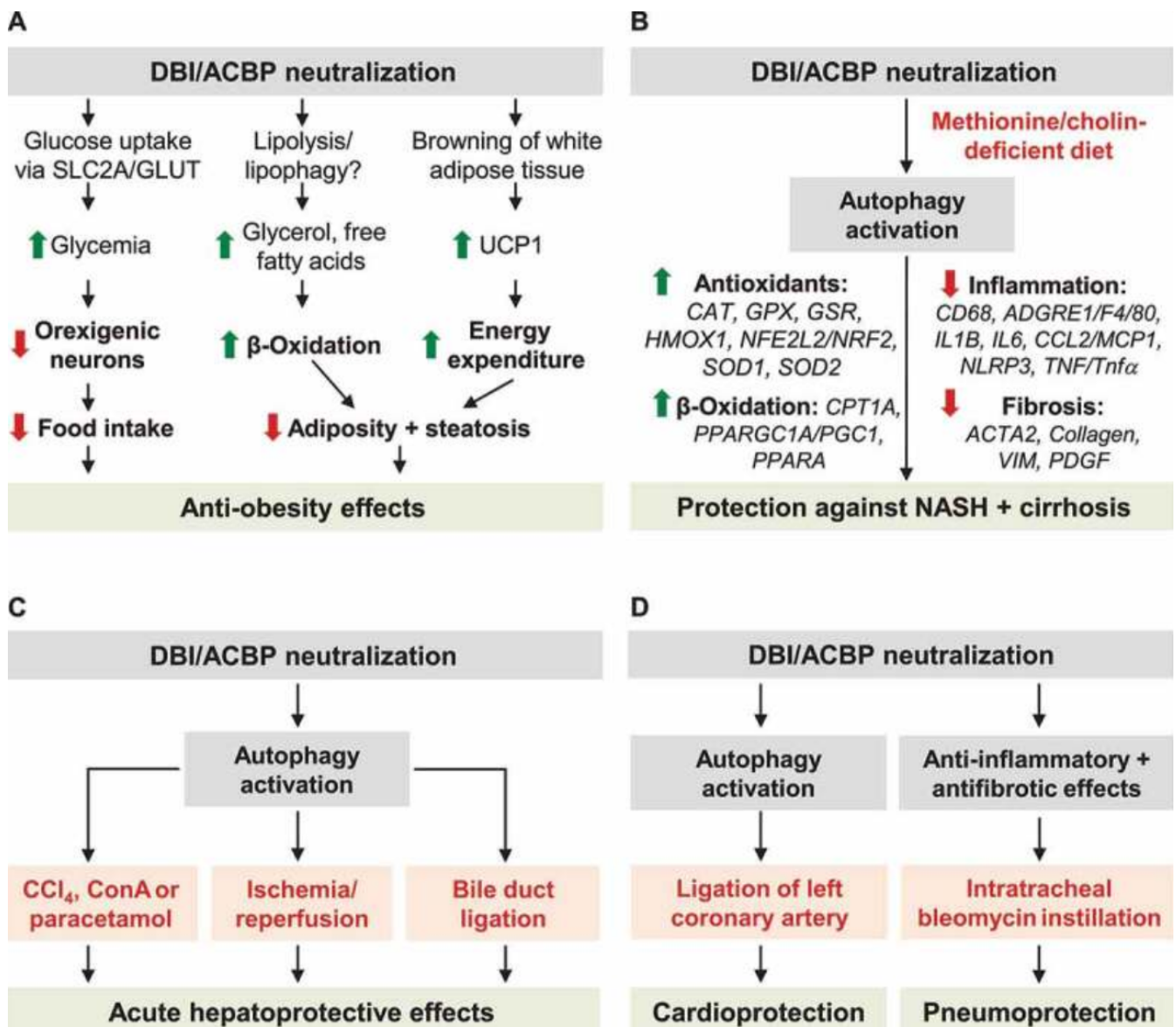


Figure 1