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REVIEW ARTICLE: AN OVERVIEW ON THE METHODS OF SCREENING ANTIOBESITY ACTIVITY

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ABSTRACT

Purpose: The incidence of obesity around the world continues to increase and is considered a major cause of morbidity and mortality through various diseases. Various animal models can be used for the study of various compounds for the discovery of a novel treatment for the obesity. It can range from food intake studies in lean rodents to studies in obese animals due to high fat diet. The use of these animals helps us to determine whether the weight loss is due to fat loss or due to changes in other parameters. Various behavioral models can be used to study the food intake pattern and the effect of the drug on that pattern. These animal models have very good predictive validity as the effect of the drug on the animal models form the basis for the effect of the drug in humans. The drug induced weight loss in humans can be predicted to an extent from the studies carried out on these animal models.

Approach: The published articles from Pubmed and other standard resources are used to study, review and compile the overview on screening methods used in Anti-obesity activity.

Findings: The animal models of obesity can be used to study about the effects of parameters like insulin level, resistance and leptin levels in obesity. Acute and chronic feeding studies can be carried out. While studying about anti-obesity drugs the factors that lead to weight gain has to be considered.

Conclusion: The obesity can be genetic or may be due to some other reason. The common cause for obesity is food intake with high calories and fat. So diet induced obese animal models are an important part of the studies. The animal models for the drug induced weight gain helps in the study of the effect of anti-obesity drugs on the drug induced weight gain. All these models have been found to have utility in predicting the weight loss in obesity in man.

Keywords: *Obesity, Animal models, High Fat Diet, Drug induced obesity, genetically modified models.*

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INTRODUCTION

Obesity is a medical condition in which excess fat accumulates in the body that have a negative effect on a person's health, which leads to decreased life expectancy and increased health problems. In other terms, it is defined as the body mass index (BMI) greater than 30 kg/m^2, with the range 25-30 kg/m^2 is referred as overweight. Among the various health care experts around the world, the global incidence of obesity is considered as one of the leading causes for morbidity and mortality in the current as well as the future generations[1]. The consequences of obesity are the risk factors of various life threatening diseases and disorders, including heart disease, obstructive sleep apnea, certain types of cancer, osteoarthritis, dyslipidemia, HTN, atherogenesis and type-2 DM, muscle weakness, respiratory failure. Various animal models are used for the study of factors causing obesity and for the discovery of various treatments for obesity[2]. The study may range from effect of food intake in lean rodents to long term studies in animals exhibiting obesity due to long term high fat diets. These animal models can be utilized to study about the beneficial changes in key plasma parameters like insulin and be used as behavioral models[3]. Animal models have excellent predictive validity. So it can be used for the study of drug induced weight loss in man. The present article aims to

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provide an overview of the various models used in the screening of antiobesity activity.

ANIMAL MODELS USED IN THE DISCOVERY OF TREATMENT OF OBESITY

The regulation of the body weight is mainly dependent on two factors ie, food intake and energy expenditure. The interaction between food intake and energy expenditure determines weight gain and weight loss. An increase in the daily intake of energy accompanied by a sedentary life style leads to the development of obesity. This relationship between food intake, energy expenditure and body weight leads to the different mechanism by which a drug may cause weight loss and is also of relevance in the selection and development of appropriate animal models for evaluating the anti-obesity potential[4].

The various animal models are:

1. Acute models of food intake
2. Chronic models of food intake and body weight

ACUTE MODELS OF FOOD INTAKE

Although not closely related to man phylogenetically, rats and mice are used as the predominant models of drug discovery for human obesity. Rats and mice are omnivores like men and they have a complex taste and digestive system for the identification and consuming of variety of food items. They even there are some similarities neurologically and anatomically in the brain areas that are thought to play a role in the control of food intake. It is also established that there are similarities between the effect produced by various neuro-transmitters and peptides on the food intake and energy homeostasis in rodents and human[5]. Many pharmacological approaches for the treatment of obesity have focused on drugs that target in reducing food intake. The investigation of the acute effect of the test drug on the food intake of a lean rodent act as a rapid screening technique and as well provide information regarding the relative potency of the drug to inhibit food intake and also the duration of action of compounds in vivo[6].

Though the methods used for detecting the drug induced changes in the acute intake of food vary inter laboratory, it mainly involves stimulating food intake in rodents in to some extent and then subsequently evaluating the effect of a treatment in which the baseline of food intake is elevated. That is acute food intake need not be done in an obese animal. Mostly the male animals are used so as to avoid the effect of menstrual cycle on food intake. The study is conducted during dark periods when the rodents exhibit high levels of food intake[7].

The disadvantages of acute food intake tests are that it is insensitive to drugs with delayed onset of action. In such cases it may be required to give a repeated initial dose. Another drawback is that it is not relevant to all mechanism of drug action. The acute food intake is of little value when used for the identification of drugs that will increase energy expenditure or that act by any other mechanism[8].

CHRONIC MODELS OF FOOD INTAKE AND BODY WEIGHT

Acute studies are mostly undertaken in lean animals to learn about the drugs profile more rapidly and get information like potency, efficacy, duration of action and side effects in vivo. Such models are used for selecting compound for chronic studying. Sub chronic or chronic food intake studies can be carried out in lean rodents as well as obese animal. The choice of the sex of animal model plays an important role. Female rat has more subcutaneous fat than male and they are more sensitive to the inhibitory action of leptin on food intake. Male rodents have more visceral fat and more sensitive to the inhibitory action of insulin on food intake. So the effect of drugs on body weight should be studied on both male and female models[9,10].

Obese animal models can be broadly divided into two subcategories:

1. Diet induced models of obesity
2. Genetic models of obesity

DIET INDUCED OBESITY MODELS

For this test the normal lean rats or mice are made accessible to high fat diet for about 3-4 months. So these animals exhibit increases in body weight with time characterized mainly by the increase in body fat. In addition, theseanimals may exhibit resistance to insulin, intolerance to glucose, elevated plasma leptin level and mild dyslipidemia with elevated plasma cholesterol and TG level and they may sometimes there is an alteration in the blood pressure level. All these changes in diet induced obese rodent mimic the changes seen in obese patients[11,12].

There are many differences in the methods used for the study with some lab using commercially available high fat diets while others using cafeteria diets[13]. The two main drug application of these models is the study of 5-HT_{2C}agonist[14], Lorcaserin and the study of the combination of Topiramateand Phentermine[15].The study showed that the combination was more effective in reducing body weight than a single drug used alone[16]. The diet induced models have an excellent predictive validity and they are good models for the study of obesity in humans.

CAFETERIA DIET

In diet induced obesity studies, the models under cafeteria diet are allowed to select themselves highly palatable and very much readily available food like cookies, meat, candy, etc. These food items contain a good amount of salt, sugar and fat and they simulate the common diet pattern seen nowadays among the people. But the nutritional and non-nutritional values of these diets are not well defined. Along with that the animal model selects different food every time and there is a difficulty in carrying out further studies. Due to these drawbacks the cafeteria diet is a poor choice of diet for scientific research[17].

HIGH FAT DIET

The research laboratories use high-fat diet, which contains 32-60% of the calories derived from fat itself. The 60%Kcal fat diet in humans is considered extreme. But these 60% Kcal fat diets are used to induce obesity in rodents which gain more weight more quickly as it is allowing the researchers to screen the compounds and its effects in a very short period[13].

One of the factors that should be considered while choosing the high-fat diet is the type of fat it contains. The many high fat diet used in animal models contains saturated fat like beef, coconut oil, etc. and these diets induce obesity in certain strains of animal very easily. But fats like omega-3 fatty acid is studied for their beneficial effects. Studies have proved that animal models on fish oil diet gained less weight compared to those models on saturated fat diet and they were more insulin sensitive too. It is known that the fatty acids affect the phenotype by various mechanisms so it is very important to include the data about the amount and type of fat used in the study so that the data can be compared[18].

Even though most of the rodents tend to become obese on the high fat diet, there are chances of variability in the extent of weight gain and other factors like glucose tolerance, insulin resistance and TG depending on the strain of the animal model[19]. Some strains of the animal models are found to be more susceptible to weight when on high fat diet compared to other stains while some strains which showed a similarity in weight gain varied in other parameters like insulin resistance and glucose tolerance etc. Some strains of the model are found to be resistant to obesity[20]. Some of the animal model strains that tends to gain weight areC51B16 and AKR mouse. The animal models like SWR/J and A/J are simply resistant to obesity. Even within the same strain some variability was observed in weight gain when the animal models were bred in different facilities. Some animal readily gained weight, whereas other stains gained weight as in a normal low fat diet. This enabled the researchers to study the animal models that are prone to obesity and that are resistant to it.

ATHEROGENIC DIET

The diet which is used to induce atherosclerosis is known as atherogenic diet. The key features of the diet used to induce atherosclerosis in rodents in animal models may vary according to the feeding length, desired end point and the research model. There are many types of atherogenic diet varying in just key ingredients used and some of the atherogenic diet are[21];

- Western purified atherogenic diet
- Western purified atherogenic diet with added cholesterol and cholate source
- Hybrid high fat diet with added cholesterol and cholate source
- A standard diet with added cholesterol

All these diets are used to induce hypercholesterolemia and mild atherosclerosis. Among them only western purified atherogenic diet promotes obesity, all others induce hypercholesterolemia without promoting obesity. The cholate source used in some of the diets help in the absorption of fat and cholesterol and it also reduces the disposal of cholesterol by bile acid synthesis[22].

The western purified atherogenic diet, whichpromotes obesity along with inducing atherosclerosis mainly contains[23];

- High Fat Diet (20-23% by weight; 40 - 45% kcal from fat)
- Saturated Fatty Acids (SFA >60% of total fatty acids)
- Milkfat/Butterfat
- Sucrose (34% by weight)
- Cholesterol (0.2% total)

GENETICALLY MODIFIED MODELS OF OBESITY

Genetically obese animals which have characteristic single gene mutations are widely used as the models for the study of genetically induced obesity[24]. These models commonly include ob/ob mouse, db/db mouse and Zucker fa/fa rat. The obesity in these genetically modified animals becomes visible within several weeks of birth and they continue to put on weight with about 50% of their body weight accounted for body fat[25-27].

The db/db mice have elevated glucose levels and are commonly used as the models for diabetes[31]. The ob/ob mice have mild to moderate hyperglycemia ant the Zucker fa/fa rats do not have elevated glucose level, but they are resistant to insulin[25,26]. The db/db mouse are found to be hypertensive but are not consistent[27].

The mutation in the ob/ob mice prevented the production of leptin, the protein of obese gene, where as Zucker fa/fa rats have elevated circulating leptin but are deficient in leptin receptors. The levels of leptin correlates with the degree of adiposity of mammals[26]. So the ob/ob mice were not used as the model for genetically induced obesity as the leptin was prevented from being produced[25]. The Zucker fa/fa rats and db/db mouse are the commonly used models.

These long term studies can be used not only to assess body weight and food intake but it can also be used to find out if the treatment have led to any changes in water intake, or if there is any improvement in glucose control or insulin sensitivity or it can be used as a measure to confirm that the changes in body weight is due to loss of fat. The blood samples can be also be taken to study about the changes in various key hormones and markers in chronic dosing.

DRUG INDUCED OBESITY MODLES

Weight gain is one of the common side effect of the most widely used drugs like certain antidiabetic, tricyclic anti-depressants, anti- psychotics etc. The use of these drugs may lead to obesity and other co-morbid problems. This weight gain can be viewed as problematic clinically and it affects patient adherence to the medication also. The exact mechanism for this weight gain is not clearly understood[28]. These drugs have been found to interfere with the central appetite-regulating neurotransmitters

and it may also produce certain sedative and anticholinergic effect which may lead to changes in the expenditure of energy. Different studies were carried out to find out the possible mechanisms by which these drugs led to weight gain and research were done to find out an effective measure to avoid this and treat it.

Animal models can be used to study about the potential of certain drugs to cause weight gain. If antidiabetic drugs like pioglitazone caused weight gain it may lead to many other problems as most diabetic patients tend to be obese and it may further complicate and lead to comorbid diseases.

Some of the categories of drugs that lead to weight gain and in which studies have been carried out are glucocorticoids, antipsychotics, antidepressants, anti-convulsants, anti-migraine and antihistamine drugs, antidiabetic drugs, non-specific beta blockers, sex hormones like estrogen. Some of these drugs have been found to affect the leptin levels and which can be co-related to weight gain. Leptin, which is synthesized and secreted from fat cells and muscle cells play a key role in signaling the size of the adipose tissue and various metabolic pathways of glucose and lipids.It is found that leptin integrated along with insulin is a key factor in the homeostasis of fat stores[29]. Most of the obese animals, especially mammals have been found to have elevated leptin and insulin levels. The insulin helps in the expression of the leptin gene in rodents and may lead to weight gain. As the body weight increased the level of serum leptin and insulin levels were found to be enhanced in the adipose tissue[30].

The animal models like the rats of Wistar strain are used for the study of the effect of anti-obesity drugs on drug induced weight gain. These studies is to find out whether the anti-obesity drugs have the potential to prevent weight gain due to drug intake[31].

MODELS TO ASSESS BEHAVIOURAL SPECIFICITY

The various factors that affect the intake of food in rats and mice include stress, sickness and drug induced toxicity. So it is necessary to determine in the beginning stage of the research itself that the regulation of the body weight and food intake is due to the specific action of the drug and not due to unwanted side effects. A drug that is found to reduce the intake of food would be ideally evaluated to examine if the decrease in food intake is due the specific regulatory mechanism or due to non-specific disruption of normal feeding patterns or drug induced malaise[32]. The models used for this study include:

1. Models for behavioral satiety sequence
2. Models of drug induced malaise and aversion

MODELS OF BEHAVIORAL SATIETY SEQUENCE

The animals should always be observed for any behavioral changes when the food is measured in all the feeding studies. These models helped in the establishment that the anti-obesity drugs like d-fenfluramine and sibutramine inhibit intake of food in a physiological manner by advancing the natural satiety rather than disrupting the normal feeding behavior[33]. The behavior profiling helped in revealing that reduction in intake of food produced by the CB_1 antagonist rimonabant, in a part may be due to behavioral change of compulsive scratching and grooming[34,35].

MODELS OF DRUG INDUCED MALAISE

Some drugs are found to reduce food intake by causing gastrointestinal malaise rather than by normal specific regulatory mechanism. But these are very difficult to detect in animals as they may have normal behavior. The persistent eating of inert substance by the rat can be used as a means to measure and this behavior is called as pica[36]. The substance commonly used for the study of pica is kaolin or china clay. These substances provide relief to the gastrointestinal stress by adsorbing the toxins on toit and not allowing them to get absorbed into the circulation. This technique was used for the establishment that the potential anti-obesity drug, E-6837, exenatide and devalinitide do not induce kaolin intake at doses that reduce food intake.

MOLECULAR APPROCHES TO OBESITY

Genomics and Genetics

The human genome as a whole, its interactions with each other and with environment is the basis of genomics[37]. Some hormones and neurotransmitters (such as leptin, cocaine- and amphetamine-regulated transcript (CART), and ghrelin) are involved in regulation of appetite and energy expenditure. These hormones effect on specific centers in the brain that control the sensations of satiety[38]. This disorder is polygenic and its genetic role in regular obesity is estimated to be 40-70%. The studies into genome level has led to the discovery of many genetic loci linked to body mass index and risk of obesity[39]. By ELISA technique, it is clear that the gene expression specific to adipose cells (apM1) produce a kind of soluble matrix protein called adiponectin in obese subjects is less when compared with normal subjects[40]. Another study revealed that an established gene ZFP36 is responsible for obesity related metabolic disorders[41]. It was revealed in a recent study that new novel genes that are associated with obesity are driven by HFD and the mRNA levels of KCTD15 is related to the nutritional condition[42]. In the last decade, Genome Wide Association Studies (GWAS), led to the discovery of more or less 32 genetic loci that are associated with body mass and obesity[43].

Metabolomics

Metabolomics is a systematic study of metabolites; (the small molecules are produced by the process of metabolism), and plays a major role in understanding the pathways underlying obesity-associated co-morbidities. Metabolomics is a promising approach for elucidating further molecular mechanisms[44]. Recent metabolomic studies, in addition, contribute to advanced biomarker discovery in which metabolic markers and pathways of

disease-associated intermediate phenotypes is the main scope of this discipline. Novel therapeutic targets as biomarker agents would be identified by the application of diagnostic techniques in a personalized healthcare setting[45]. In one study, GC × GC-TOF led to the detection of 1200 compounds with purity better than 0.2, compared to 500 compounds with purity up to 2.5 in one-dimensional GC-TOF. The compounds identified include many of the compounds previously reported in NMR studies. Spleen samples of several obese NZO mice and lean C57BL/6 control strains were analyzed into exhibit the prominent role of GC × GC-TOF for biomarker detection[46]. By the use of spectrometry-based metabolomics in one study, it was revealed that xanthohumol (XN), a prenylated flavonoid from hops could possibly reduce weight gain[47]. A mass spectrometry-based metabolomics study targeting 163 metabolites of serum samples revealed the metabolic determinant of weight loss during intervenes. 80 obese children aged 6–15 years having completed the one-year lifestyle intervention program 'Obeldicks', 40 that achieved a substantial reduction of their body mass index standard deviation score (BMI-SDS) duringthis intervention, and 40 that did not improve their overweight status phosphatidylcholine metabolism and abdominal has a major role in obesity in body weight regulation[48].

Proteomics

Obesity-associated disorders are resulted from obesity-induced changes in adipokine profiles. Adipokines are adipocyte-secreted proteins that dysfunctional adipose tissue can be detected from their evaluations during weight gain and weight loss[49]. Recent advances in spectrometry-based proteomics has been helped to understand the molecular mechanisms and omental fat function in the pathogenesis of obesity-associated diseases[50,51]. One study showed that plasma ceruloplasmin serves as biomarker[52]. Two-dimensional electrophoresis study showed that weight-loss program would change the proteome of the serum of Beagle dogs before and after weight loss, considered potential markers of obesity and obesity-related disease processes in dogs via mass spectrometric were identified. These differentially regulated spots corresponded to retinol-binding protein 4, clusterin precursor, and a-1 antitrypsin, respectively[53]. In one recent study, chemoproteomic Cell Surface Capture (CSC) technology was applied for surfaceome maps of primary adipocytes derived from different mouse models for metabolic disorders. A set of cell surface glycoproteins with modulated location specific abundance levels was revealed by relative quantitative comparison between these surfaceomemaps. Functional evidence of obesity modulated cell surface glycoproteins in adiponectin secretion and the lipolytic activity of adipocytes were revealed for its contribution in adipocyte malfunction in obesity. Adipocyte function in obesity can be improved by the regulation of concerted activities of this factor[54].

CONCLUSION

The global incidence of obesity is a major cause of morbidity and mortality. Various animal models can be particularly used to investigate the anti-obesity potential of various drugs and their combinations. The commonly used animal model is rats and mice. They show some similarity to men in their complex taste and digestive system. They have good predictive validity. But nevertheless it is to be remembered that rodents are not humans and there can occur species wise difference in pharmacokinetics, efficacy and tolerance factors. The utility of these animal models can be used to determine if the weight loss is due to fat loss or other factors and can also be used to assess the key plasma parameters. The animal models have excellent predictive validity whereby drug induced weight loss in rodents subsequently means the weight loss in man.

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