Health Effects of Coumestrol

Subjects: Others

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Coumestrol is a phytoestrogen produced in pulses including alfalfa, clover or soy. Its production is mainly associated to fungal attacks. It has been involved in the ewes' infertility syndrome discovered in the late 1940s in Australia and New-Zealand. Mainly considered as a toxic compound, it can be used to relief symptoms of estrogen deficiencies and therefore exhibits both adverse and beneficial effects at least in animal models.

Coumestrol

1. Human Exposure and Bioavailability

1.1. Exposure According to Diet

The coumestrol exposure in human is hardly known. Some authors reported that it could be present as traces in human food [1]. Coumestrol can also occur as methylated substances (4'-O-methyl and 7-O-methyl derivatives) which can be found in alfalfa [1]. When they reach the liver, the methylated forms can be demethylated into coumestrol by phase I enzymes. Coumestrol estrogenic potency is by far one of the highest *in vitro*, especially through ERα [2]. However, its bioavailability appears to be lower than that of isoflavones in rat [3]. No data could be found on human pharmacokinetics since this compound is considered toxic, therefore, bioavailability studies have not been published yet in humans.

1.2. Blood Concentrations

As mentioned above, this research aims at citing plausible health effects caused by relevant doses that can lead to efficient plasma levels. This means that blood concentrations obtained in dietary relevant situations should be given as a prerequisite for analyses of the effects. Coumestrol can be found at low concentrations in consumers' plasma. According to [4] the mean plasma level in Chinese volunteers was 2.7 ng/mL (2.2 – 3 ng/mL) which correspond to 10 nM. In Mexican women [5], coumestrol serum levels of 3 nM were described. As far as we know, there is no data on current serum measurements in European populations. The levels being below the quantification limits of the analytical techniques, might be an explanation. Again, coumestrol can be conjugated to sulfate or glucuronide residues and the free form may even be lower than the total aglycone forms generated by the extraction procedures used in [4] and in [5].

2. Beneficial Effects

2.1. Hormonal Effects

2.1.1. Menopausal symptoms

Coumestrol being considered as a toxic compound, no data were found on its potential effect on climacteric symptoms of menopausal and peri-menopausal women in interventional studies. However, the market of food supplements offers preparations based on soy, clover, alfalfa, kudzu, linseed and hop. Because the food supplement suppliers not always analyze their ingredients with careful attention the presence of coumestrol in clover, soy or alfalfa extracts used for food supplement cannot be excluded.

2.1.2. Bone health

No data seem to exist on the effect of coumestrol on menopausal women bone health. Only one study tested the effect of this compound in rodents [6], showing a preventive effect against bone loss in an ovariectomized rodent model at 10 mg/kg/day. The *in vivo* estrogenic activity of coumestrol on bone cells has also been shown to prevent osteoclast differentiation [7] and to enhance osteoblast formation [8]. However, the doses required were 5 to 10 µM. Such plasma concentrations can be achieved in rodent on contaminated chow diet but not in humans.

2.1.3. Estrogen responsive tissues

In Western developed countries, coumestrol is only anecdotally present in human food, and therefore, its effects on estrogen-dependent cancers was only scarcely examined. There is no strictly controlled RCT and only a few observational studies involving coumestrol are available since this compound is frequently under assays detection limits in biological samples. In [9], coumestrol combined to precise Snp (Single Nucleotide Polymorphism) present in the promoter region of the ERb subtype, was shown to be strongly correlated to a decreased risk of prostate cancer. But this correlation was weakened since coumestrol urine levels measured in this research were close to the detection limit.

2.2. Metabolic Beneficial Effects

2.2.1. Effect on cholesterol

There is no human data on the effect of coumestrol on cholesterol blood levels

2.2.2. Effect on metabolic syndrome

There are few data on the effect of coumestrol on metabolic syndrome and lipid associated disorders. They were essentially obtained on animals and express contradictory opinions. On the one hand, some studies consider coumestrol as a beneficial agent for lipidic and glycemic profiles such as [10] which examined the effect of different extracts of alfalfa sprouts in rats. In this research, 10-hydroxycoumestrol was the only polyphenol potentially involved in a beneficial effect. On the other hand, some authors considered that coumestrol, as a natural endocrine disruptor, was able to impair liver function and consequently lipid and glucose blood levels in rat [11]. Besides, Liu *et*

al. [4] associated plasma phytoestrogens, including coumestrol, to a lower risk of developing metabolic syndrome. However, the phytoestrogens measured in plasma could be considered as biomarkers of a vegetable-based diet which is known to reduce the risk of metabolic syndrome. Therefore, the link between coumestrol and either lipid or glucose impairments in humans is not direct and should be further demonstrated.

2.2.3. Effects on diabetes

Few data can be collected when data bases are interrogated associating coumestrol and diabetes. Studies were usually performed in rodents or *in vitro*. In animals, they were more related to the adipocyte physiology. *In vitro,* the doses used in [12] between 20 and 50 µM are far from being in line with plasma doses corresponding to human dietary exposure. Therefore, an effect of coumestrol on diabetes diseases is far from being proven.

3. Adverse Effects

Based on the discovery of phytoestrogens, the reproductive issues will be carefully scrutinized. In addition, in 1977, Farnsworth and co-workers, reported a large list of plants that were long used in Western countries as anti-fertility agents [13][14]. Among them, 60% contained isoflavones or coumestrol. This sustained their estrogenic effects in humans.

3.1. Reference Doses

According to [15] a reference dose exist for coumestrol and it is of 0.33 mg/kg bw/d. It is based on a LOAEL of 100mg/kg/day obtained on a mice model. Currently, in Europe, the human exposure to coumestrol is equal or below 0.016 µg/kg bw/d [16].

3.2. Hormonal Based Effects

3.2.1. Pituitary interactions

The effect of coumestrol on GnRH and LH secretion has been documented in the late ‘80s [17]. In ovariectomized rats, pre-treated intravenously with estradiol-17β or coumestrol, a GnRH challenge (50 ng/kg bodyweight, *i.v.*) was performed to check its effect on LH release. It was shown that estradiol low-dose pre-treatment (10 ng/kg bodyweight) significantly enhanced GnRH-induced LH release while pre-treatment with higher dose of estradiol (1000 ng/kg bodyweight) blocked the GnRH-induced rise. Coumestrol pre-treatment at all doses tested (10, 100, and 1000 ng/kg bodyweight) reduced without abrogating GnRH-induced LH release. Such an experiment showed that Coumestrol *in vivo* can be as active as estradiol when administrated intravenously. However, in a normal situation, coumestrol should be absorbed orally and therefore its poor bioavailability would probably reduce its efficacy.

3.2.2. Estrogen based toxic effects

There is hardly any data on the effect of coumestrol on human reproduction because the common exposure is low and when correlation of reproductive parameters was attempted with coumestrol in biological fluids, it was unsignificant [18]. Conversely, in animals, many effects were recorded in rats after plausible dietary administration. In [19], a review summarizing the effects of phytoestrogens neonatally administered in rodent models, it was reported that coumestrol in rat can induce: early vaginal opening, increased initial uterine wet weight and later decreased adult uterine weight. Such effects were also observed in mice neonatally treated with Diethylstilboestrol. When administered to rats during postnatal days 10–14, coumestrol reduced the number of endometrial glands observed in adults and reduced the expression of estrogen receptors. Mice treated neonatally with coumestrol also showed squamous metaplasia and an abnormal collagen deposition in the uterine wall. In adult rats, Whitten and co-workers reported various deleterious effects of coumestrol orally administered at a dose of 100 µg/g [20]. These effects were typically estrogenic and depended on the time of administration. They induced: a decrease in LH production, aberrant cycles, reduced cyclicity, persistent oestrus and uterotrophy in females. They also induced uterine cell proliferation, progestin receptor induction, estrogen receptor activation, decreased age of uterus canalization and early age at first oestrus. Coumestrol also affected male reproductive characters including behaviour by increasing the time to react to receptive females. Coumestrol also decreased testosterone serum levels and testicular size of rat exposed during adulthood, most probably via a pituitary interaction. The actual human exposure which is generally low, most probably avoid such endocrine disruptions.

3.2.3. Thyroid based toxic effects

Possibly due to structural similarities with thyroid hormones and to genotoxic effects, coumestrol at doses over 40µg/day was associated with higher risk of thyroid microcarcinomas in Connecticut patients taking part in a case control study [21]. More precisely, coumestrol exposure was stratified into 5 levels: very low <40 µg/day; low=40 to 80 µg/day; medium=80 to 130 µg/day; high= 130 to 200 µg/day and very high >200 µg/day. The OR = 2.48, 95% confidence interval (CI), was 1.39–4.43 for low exposure; OR = 2.41, 95% CI, 1.32–4.40 for medium exposure; and OR = 2.38, 95% CI, 1.26–4.50 for very high exposure when compared with very low exposure. Interestingly the risk of thyroid cancer was not significant with exposure doses between 130 and 200 µg/day probably due to a low number of subjects. In this studies isoflavones that were in the few mg range were not effective or protective.

4. Conclusions

Coumestrol, exhibit estrogenic activities at relevant dietary intakes in animal models and farmed animals. As such, it shown beneficial effects in cases of estrogen deficiency but also potential deleterious effects when estrogens are not required. Coumestrol is considered as a toxic compound, and carefully monitored in human food. Its presence and action in food supplements based on clover or alfalfa extract cannot be excluded since the ingredients used for such foodstuff are not always fully scrutinized.

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