CASE STUDY OF CARCINOGENICITY BY INITIATION-PROMOTION MODEL

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According to the ICH safety topic S1.B, “Testing for Carcinogenicity of Pharmaceuticals” (step 2 Draft Guideline), the initiation-promotion (IP) model in rodents is situated on one of the additional tests for detecting carcinogenic activity in vivo.

In this report, we examine Spironolactone (SPL), an anti-aldosterone diuretic agent and non-genotoxic carcinogen (IARC ; Lumb et al.), and simulate an evaluation process for carcinogenic activity using IP models, from the selection of study models to the evaluation of results and mechanistic studies concerning the action of SPL. Finally, we point out some problems associated with the IP model for assessing the carcinogenicity of novel pharmaceuticals.

1. Profile of SPL toxicity

A 4-week subacute dietary study of SPL (doses of 0, 200, 600 and 2000 ppm) using male F344/DuCrj rats was conducted to assess the maximum tolerated dose and potential target organs or tissues of carcinogenicity of SPL. Dose-dependent decreases in body weight gain were observed, and liver and thyroid weights of animals given 2000 ppm were higher than controls. Histopathologically, hepatocyte hypertrophy and thyroid follicular epithelial cell hypertrophy were observed at doses of 200 and 600 ppm. No other organs or tissues had histopathological changes which would suggest that SPL has carcinogenic potential.

2. Selection of assay model

The spectrum of organs and tumor types for each IP model depends on the initiator chemical(s) which were initially administered to the animals. Therefore, it is very important to predict likely target organs or tissues from other toxicology data, and to choose appropriate initiators and test systems.

A 4-weeks study suggested that the liver (hepatocytes) and thyroid (follicular epithelial cells) were potential target organs for SPL carcinogenicity, so we selected the medium-term liver bioassay for carcinogens and the thyroid IP model to assess the carcinogenic potential of SPL.

The medium-term liver bioassay for carcinogens is a well-validated test system for predicting the hepatocarcinogenicity of test substances using diethylnitrosamine-induced rat liver preneoplastic glutathione S-transferase (GST-P) positive foci (Ito et al., 1988 ; Ito et al., 1989). Another thyroid IP model is a typical thyroid 2-stage model which uses dihydroxypropylisothiourea (DHPN) as an initiator. In many experiments, DHPN were used as an initiator for thyroid carcinogenesis (Hiasa et al. ; Moore et al.). In this model, carcinogenic activity is evaluated by...
estimating preneoplastic hyperplasia, adenoma and carcinoma of thyroid follicles.

A small scale full-range carcinogenicity study was also carried out to validate the results of these IP models.

All three studies were performed using male F344/DuCrj rats. The experimental designs and SPL doses are shown in Fig. 1.

3. Dose selection of IP models

The same dose levels of as the full-range carcinogenicity study can be used in IP models, because the same rodent species and strains as the repeated dose toxicity study were used. The above preliminary study indicated that the maximum tolerated dietary dose of SPL for male F344 rats was 2000 ppm. A dose of 2000 ppm was selected as the highest doses of the medium-term liver bioassay for carcinogens and the thyroid IP model.

4. Results of SPL carcinogenicity using IP models

Morphometrical analysis of GST-P positive foci per liver section (Fig. 2) revealed that the area of GST-P positive foci was significantly increased by SPL treatment at a dose of 2000 ppm (0.65 ± 0.39) compared to controls (0.36 ± 0.22). No significant increases were observed in the number of area of GST-P positive foci in animals given 1000 ppm or in the number of lesions at 2000 ppm. These data indicate that SPL potentially enhances the preneoplastic liver lesion development in rats at a dose of 2000 ppm. In the full-range study, hepatocellular adenomas were induced at 2000 ppm (4/24, 17% vs. 0/24 of control). Equivocal induction was seen in animals given 670 ppm (2/24, 8%). A Comparison of the results of the above liver IP model with those of the full-range study indicates that the degree of induction of GST-P positive foci in the IP model corresponds to the incidences of liver tumors in the long-term study.

The results of the thyroid IP model and the full-range study are shown in Fig. 3. Follicular hyperplasias were induced in all animals given 600 or 2000 ppm of SPL. The incidences of follicular adenomas/carcinomas were significantly higher in animals given 2000 ppm than controls. In animals given 200 ppm, no thyroid tumors were observed and the incidence of follicular hyperplasia was not significantly different from controls. These data suggest that 600 and 2000 ppm doses of SPL have the potential to induce thyroid tumors, and that 200 ppm is the putative threshold level of follicular proliferative lesions for SPL in this test system. In the full-range study, follicular adenomas/carcinomas were induced in animals given 2000 ppm of SPL. The incidences of follicular hyperplasias for 670 and 2000 ppm were significantly higher than the controls and were dose-dependent. The results of the thyroid IP model, like for liver, correspond to those of the full-range study.

In the full-range study in male rats, no other SPL-induced tumors were observed in animals given 670 and 2000 ppm.

5. Mechanistic studies for SPL liver and thyroid carcinogenicity

To clarify the mechanisms of liver and thy-
and 2000 ppm (Fig. 4). The serum T4 levels of SPL treated animals were slightly higher than those of the controls (Fig. 4). Based on these results, we believe that SPL induced an increase in T4 excretion from liver, following which serum thyroid hormone levels probably decreased at the early phase of SPL treatment. These sequential changes caused a negative feedback mechanism via pituitary TSH release, leading to an over-stimulation of the thyroid follicles. Despite the decrease in serum T4 levels, our data reveal a slight increase in T4 levels. This is because serum samples in which T4 levels were measured, were collected at Week 12 when thyroid hyper-function had already been induced by SPL.

6. Conclusions and problems with IP models for assessing pharmaceuticals

The data from IP models generally have higher conformity with the full-range study, as shown above. IP models are useful for assessing carcinogenic potential in putative target organs or tissues suspected from the results of toxicology studies. The study size (number of animals, study period etc.) of IP models is relatively small compared with a full-range study. As was the case for the thyroid carcinogenicity of SPL, this models enable us to examine the dose-response relationship using multiple doses and the relationships between trigger changes of carcinogenicity and the induction of histopathological proliferative changes. Based on these merits, IP models may be not only valuable as an additional test, but also for assessing the carcinogenic potential of phar-
maceuticals at early stages of the development process.

The evaluation of the carcinogenicity of novel pharmaceuticals using IP models for submission to regulatory authorities is not without drawbacks. Some concerns relate to the selection and justification of IP models and an appropriate initiator, and also to the selection of a positive control which validates the results of experimental data. There are no standards for these important points. When the test substance has no suspected target organs or tissues, it is extremely important to evaluate the experimental data to justify the selection of the experimental model and the positive control substance. Others include an appropriate study design (duration of experiment and number of animals per group), the need for both sexes or not, diagnostic criteria of preneoplastic proliferative lesions etc. To use IP models to a series of carcinogenicity studies for pharmaceuticals, it is necessary to build consensuses on the above problems among ICH participants, in parallel with progress in the ICH S1.B draft guideline.

REFERENCES


