RELATIONSHIPS BETWEEN OFF-TARGET INHIBITION PROFILES AND ADVERSE EFFECTS BY ANTI-
CANCER PROTEIN KINASE INHIBITORS
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[Purpose] Recently, anti-cancer protein kinase inhibitors are shown to interact with various kinases which
are not intended as primary targets. However, the medical consequences of off-target inhibitions are poorly
understood. In this study, we aimed to clarify the relationships between off-target inhibition profiles and drug
adverse effects, comparing erlotinib and gefitinib, which are EGFR inhibitors, and sunitinib and sorafenib, which
are multi-kinase inhibitors.

[Methods] Firstly, we compared kinase occupancy profiles at the clinical doses based on reported Kd values
against 290 distinct human protein kinases, and picked out candidate kinases which might be involved in the
erlotinib-related skin toxicity or the sunitinib-related liver toxicity. We measured IC50 values using recombinant
protein kinase assay and confirmed the inhibition at the clinical concentration. As for the erlotinib-related skin
toxicity, effects on lymphocytes responses are analyzed in vitro. We also confirmed the effect on skin toxicity
in vivo using irritant contact dermatitis model. As for the sunitinib-related liver toxicity, the inhibitory effect on
liver glycogenolysis was analyzed in vivo.

[Results and Discussion] LOK, which negatively regulates T cell function, is a candidate causative kinase
for erlotinib-related skin toxicity. T-cell migration and IL-2 secretion response were enhanced by treatment
with erlotinib or siRNA against LOK. Additionally, ear swelling was significantly severe in mice treated with
erlotinib comparing to gefitinib. PHKG2, which catalyzes liver glycogenolysis, is a candidate causative kinase for
sunitinib-related liver toxicity. Glycogenolysis was significantly inhibited in mice treated with sunitinib, which
might lead to liver disorder.

[Conclusions] Off-target inhibitions are in part involved in the development of unexpected drug adverse effects.

GLUTATHIONE DECREASE IS ONE OF KEY FACTORS FOR DRUG-INDUCED LIVER INJURY IN RATS INDUCED
BY LIPOPOLYSACCHARIDE AND DICLOFENAC CO-ADMINISTRATION
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[Purpose] We previously analyzed 60 serum samples of drug-induced liver injury (DILI) patients using capillary
electrophoresis mass spectrometry and observed that the ophthalmic acid-related peptides concentration
tends to increase with the severity of DILI. This suggested that the decrease of hepatic glutathione may be
a common feature in DILI, however the involvement of glutathione in DILI has not been extensively studied,
yet. The aim of the present study was to investigate the role of glutathione in the development of DILI in rat
model.

[Methods] DILI model was established by pre-administration of lipopolysaccharide (LPS) (1mg/kg, i.p.) at
2hr before diclofenac administration (100 mg/kg, i.v.) (LPS+diclofenac model). In some experiments, reduced
glutathione (GSH) or N-acetylcysteine (100 mg/kg each, s.c.) was administered at -1hr, 0hr, 1hr, and 2hr after
LPS administration. Hepatic glutathione, serum alanine aminotransferase (ALT) and bilirubin levels were
monitored.

[Results and Discussion] Hepatic glutathione level was reduced to 65% of control at 2hr after LPS
administration. Diclofenac administration induced mixed-type liver injury only when LPS was pre-administrated.
GSH or N-acetylcysteine treatment recovered hepatic glutathione level and significantly suppressed the
elevation of serum ALT and bilirubin. Administration of diclofenac to rats pre-administered with diethyl
maleate, a GSH depletor, induced elevation of serum markers, although the extent of elevation was lesser than
that induced by LPS+diclofenac model.

[Conclusions] Decrease of hepatic glutathione may be one of the essential factors to induce DILI. It is possible
that glutathione and other unidentified factors may synergistically aggravate DILI induced by LPS and
diclofenac.

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